



ENCYCLOPEDIA OF BEHAVIORAL NEUROSCIENCE

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Acute Dependence

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Glossary

Acute dependence – A state of dependence on a substance revealed through signs of spontaneous or precipitated withdrawal after acute exposure to a drug or alcohol.

Chronic dependence – A state of dependence on a substance revealed through signs of spontaneous or precipitated withdrawal following long-term exposure to a drug or alcohol.

Conditioned withdrawal – Signs of withdrawal that are elicited or exacerbated by cues that have been paired with withdrawal through a process of associative conditioning.

Precipitated withdrawal – Signs of withdrawal elicited abruptly by administration of a competitive antagonist in a drug-dependent organism.

Spontaneous withdrawal – Time-dependent emergence of withdrawal symptoms upon cessation of drug or alcohol intake in a dependent organism.

Prolonged use of many substances of abuse including alcohol, opioids, stimulants such as cocaine, amphetamines, nicotine, and cannabinoids can lead to a state of dependence that results from the adaptation of organ systems, in particular the central nervous system (CNS; so-called neuroadaptation), to the continued presence of the substance in the system. Dependence on a substance is measured by the emergence of drug-specific clusters of withdrawal symptoms upon cessation of use; these withdrawal symptoms reveal the underlying adaptive responses that have formed, and are typically opposite in nature to the direct effects of the substances themselves.

Animal models utilizing passive exposure to drugs of abuse, and less frequently active exposure by allowing animals to self-administer to the point of dependence, have yielded significant insights into the neurobiological mechanisms mediating dependence on and withdrawal from chronic drug administration – in effect examining substrates mediating the maintenance of an established state of dependence. However, it has been known since the pioneering work of Wikler and Carter, in the 1950s, and Martin and Eades, in the 1960s, that organisms can show withdrawal signs after a single treatment with opioid agonists such as morphine, and that the symptoms of

withdrawal from single treatment qualitatively resemble the symptoms of withdrawal from chronic opioid exposure. This state of acute dependence, defined as “a state in which abstinence [withdrawal] can be demonstrated or precipitated following either a single dose or a short-term infusion of morphine” (see Martin and Eades, 1964, under Further Reading), may provide a means to understanding the neural basis of initial neuroadaptive responses to opioids and other abused substances.

Historical Evolution of the Concept of Acute Dependence

The early work of Wikler and Carter, in 1953, and Martin and Eades, in 1961 and 1964, measured acute dependence on morphine in dogs with a chronic transection of the spinal cord to produce hindlimb paralysis. In this model, spinally mediated reflex activity recovers after a period of time, and is suppressed by morphine treatment, but when acute, high, bolus doses or short-term intravenous infusion of morphine were followed by an opioid antagonist, the antagonist produced hindlimb reflex hyperactivity that is qualitatively similar to what is seen in antagonist-precipitated withdrawal from chronic morphine. Martin and Eades noted, however, that while the spinal reflex hyperactivity was qualitatively similar between dogs treated acutely versus chronically with morphine, both the duration and intensity of reaction to a fixed high dose of the antagonist nalorphine were much greater after chronic morphine administration.

Further work on the topic of acute opioid dependence in the early 1970s employed primarily antagonist-induced jumping behavior in mice to demonstrate that opioid antagonists such as naloxone could elicit increased escape jump attempts when administered 15 min to several hours after high doses of morphine or several other opioid agonists. These studies made significant advances in characterizing the acute dependence phenomenon by:

1. extending the range of agonists studied for their ability to support acute dependence;
2. establishing the time-course between agonist pretreatment and antagonist precipitation that supported maximal withdrawal behavior; and
3. demonstrating through dose-response analysis of naloxone potency that higher doses of naloxone were required to elicit withdrawal jumping after acute

single-dose agonist pretreatment than were required after more chronic agonist exposure. This latter observation was consistent with the earlier demonstrations of Way and colleagues that naloxone's potency to elicit opioid withdrawal signs following different chronic regimens of opioid exposure is inversely related to the severity of dependence.

Reports on withdrawal jumping and spinal reflex hyperactivity following acute morphine treatment were dismissed by some as an epiphenomenon. It was suggested that these findings were most parsimoniously explained as largely species-specific artifactual motoric responses to high doses of agonist and antagonist, but were not indicative of a rapid induction of opioid dependence. Indeed, the doses of morphine employed in these studies were often five- to 10-fold (or more) higher than typical analgesic doses in the studied species.

Despite these early criticisms, interest in acute dependence as a potentially valid and valuable model of the initial adaptive response to drugs of abuse has grown in the past 30 years as subsequent work revealed that acute dependence:

1. could be observed in the human with clinically relevant doses of morphine;
2. could be observed in a variety of species beyond dogs and mice, including rats, gerbils, hamsters, guinea pigs, and monkeys;
3. could be produced by lower doses of opioid agonist that fell within the typical analgesic dose range for a given species;
4. was associated with a much broader subset of the full constellation of opioid withdrawal signs than was initially appreciated; and
5. could be demonstrated with other drugs, including sedatives such as alcohol, benzodiazepines, and barbiturates.

Moreover, as the important distinction between aversive emotional components of opioid withdrawal (e.g., anxiety, depression/dysphoria) and somatic/physiological components (e.g., weight loss, diarrhea, autonomic hyperactivity) became better appreciated, it was discovered that brain systems mediating the negative emotional consequences of withdrawal may be much more sensitive than circuits mediating somatic withdrawal signs to rapid neuroadaptation after initial drug exposure. Finally, demonstrations that repeated intermittent treatment with drugs such as opioids or alcohol at daily, weekly, or even longer intervals led to a progressive increase in the severity of withdrawal signs, and the recruitment of additional signs not observed after single treatments, provided empirical evidence in favor of the notion that acute dependence may indeed represent the initial point on a continuum towards the development of chronic

dependence. **Table 1** provides a summary of key findings, to date, emerging from studies of acute dependence on opioids, alcohol, benzodiazepines, and barbiturates, the details of which are described more fully in the sections that follow.

Acute Opioid Dependence

To date, the majority of studies on acute dependence have employed opioid agonists such as morphine to induce the acute adaptive response. Indeed, opioid dependence – whether acute or chronic – is by far the most thoroughly characterized form of adaptation to substances of abuse. Studies of acute opioid dependence in animals and the human demonstrated that a broad range of opioid agonists, including levorphanol, heroin, methadone (and the structurally related compound levo-alpha-acetylmethadol [LAAM]), fentanyl, hydromorphone, and etorphine, in addition to morphine, produce similar rapid adaptive responses. Partial agonists such as buprenorphine have not been extensively evaluated, but available data suggest that they are considerably less efficacious than full agonists in producing an acute dependence response. While most opioid agonists that induce acute dependence are potent μ -opioid receptor agonists, most also have activity at other opioid receptors. Stimulation of only μ -opioid receptors with highly selective peptide agonists can induce acute dependence with similar intensity to that produced by morphine. Consistent with studies of chronic opioid dependence, while there is evidence that selective acute activation of κ - or δ -opioid receptors also produces a measurable degree of acute opioid dependence as measured by antagonist-precipitated withdrawal, the intensity of withdrawal is significantly reduced relative to that seen with μ -receptor activation. Because morphine is the agonist of choice in the overwhelming majority of acute opioid dependence work to date, further discussion focuses primarily on this classic opioid agonist.

Spontaneous versus Precipitated Withdrawal

The availability of competitive antagonists such as naloxone and naltrexone that can rapidly elicit withdrawal from opioid agonists offers distinct advantages for empirical study of opioid dependence. First, precipitated withdrawal provides a defined time window in which withdrawal signs can be observed, as compared to spontaneous withdrawal signs which may be observed infrequently and at unpredictable times in the hours or days following cessation of opioid agonist treatment. Second, leftward shifts in opioid antagonist dose–effect functions (i.e., increased potency of the antagonist to precipitate withdrawal) occur in proportion to the dose

Table 1 Summary of primary features of acute dependence on opioids, alcohol, benzodiazepines, and barbiturates

	Opioids	Alcohol	Benzodiazepines and barbiturates	
Agonists confirmed to produce acute dependence (most common in bold at top)	Morphine* Heroin Methadone LAAM Fentanyl Hydromorphone Levorphanol Etorphine	Ethanol	Benzodiazepines Chlordiazepoxide Diazepam Zolpidem Abecarnil Alprazolam Clonazepam Lorazepam Midazolam Triazolam	Barbiturates Pentobarbital
Partial agonists less efficacious in eliciting acute dependence?	Yes (e.g., buprenorphine)	N/A	Yes (e.g., bretazenil)	
Observed in human subjects?	Yes	Yes	Not systematically studied to date	
Animal species studied (most common in bold at top)	Mice Rats Dogs Primates Gerbils Hamsters Guinea pigs	Mice Rats	Mice Rats Primates	
Spontaneous withdrawal?	Yes, not commonly studied	Yes	Yes	
Precipitated withdrawal?	Yes	No	Yes	
Antagonists used (most common in bold at top)	Naloxone Naltrexone N-Allyl-normorphine 6-Alpha/beta-naloxol 6-Alpha/beta-naltrexol Methylnaloxonium*	N/A	Flumazenil Ro 15-1788 CGS-8216	
Somatic/autonomic withdrawal signs in animals common to acute and chronic dependence	Escape jumping Paw shakes/wet dog shakes Teeth chattering Swallowing/chewing Eye blinks/ptosis Urination Tachycardia Tachypnea	Reduced seizure thresholds Tremors	Reduced seizure thresholds Muscle hypertonus Abnormal gait Abnormal/arched posture	

Behavioral/emotional signs common to acute and chronic dependence	Suppression of operant responding Conditioned place aversion Anxiety-like behavior Elevated reward thresholds Elevated startle response	Anxiety-like behavior Elevated reward thresholds	Suppression of operant responding (others not systematically examined to date)
Potentiation of withdrawal Severity with repeated agonist:			
Daily intervals	Yes	Yes	Not systematically studied to date
Weekly intervals	Yes	Yes	Not systematically studied to date
Common neural or cellular Substrates of acute and chronic Dependence identified?			
	Yes	Yes	Yes

*bold-face type indicates most commonly studied drug or species.

and duration of agonist treatment. As a consequence, pharmacologists can employ quantitative antagonist dose-response analysis to estimate the magnitude of the underlying state of opioid dependence. Importantly, although opioid-dependent individuals typically experience spontaneous but not precipitated withdrawal, the range of withdrawal symptoms observed in the human or animals during spontaneous versus precipitated opioid withdrawal are quite similar, lending validity to the precipitated approach as an optimal empirical tool for quantitation of opioid dependence. While there is evidence of spontaneous withdrawal in some acute opioid-dependence studies, not surprisingly, these signs are subtle and difficult to detect after just a single treatment, and as a consequence the vast majority of studies employ the antagonist-precipitated approach.

Convergent Symptomatology of Acute and Chronic Dependence

Human

Clinical reports in the early 1970s of a naloxone-precipitated abstinence syndrome upon rescue from opioid anesthesia in otherwise opioid-naïve individuals suggested that acute opioid dependence was a clinically relevant phenomenon. These case reports were followed by systematic empirical investigations of the acute opioid-dependence phenomenon in human subjects. An early study of acute opioid dependence in subjects who had no prior history of recreational opioid use or abuse reported that the competitive antagonist naloxone given 24 h after an intramuscular administration of morphine (10–15 mg per 70 kg body weight) elicited opioid withdrawal-like symptoms such as yawning, nausea, irritability, feeling cold, and feeling sick (see Jones, 1980 in Further Reading). Subsequently Stitzer, Bigelow, and colleagues engaged in a series of parametric analyses of the conditions that support the development of acute opioid dependence. The majority of subjects employed in these studies were individuals with a history of prior opioid abuse and dependence, although the subjects were not currently physically dependent, nor seeking treatment for opioid addiction at the time of study. However, Stitzer and colleagues have also reported a similar spectrum of withdrawal signs following acute opioid exposure in individuals with no prior history of abuse or dependence (Azorlosa et al., 1994 in Further Reading), indicating that a prior history of chronic dependence is not a prerequisite to observe withdrawal following an acute morphine treatment.

In the majority of studies to date, the treatment regimen involved single intramuscular pretreatment with morphine (4–30 mg per 70 kg) followed 2–24 h later by intramuscular naloxone (3–30 mg per 70 kg). Although higher doses of morphine (18–30 mg per 70 kg) supported

a more robust, longer-lasting sensitivity to naloxone-precipitated withdrawal, doses of morphine as low as 8–10 mg per 70 kg – at the upper end of the typical clinical analgesic dose range for the intramuscular route of administration – elicited a significant state of acute dependence.

The most common signs of withdrawal elicited in these studies included:

1. a core set of physiological signs such as decrease in skin temperature and increase in heart rate;
2. observer-rated symptoms including yawning and restlessness;
3. subject-reported symptoms of hot/cold feelings, upset stomach, and irritability;
4. subjective ratings of ‘bad drug effect,’ ‘withdrawal sickness,’ and, in some studies, ‘dysphoria;’ and
5. in some recent studies, hyperalgesia (increased sensitivity to noxious, mildly painful stimuli) when morphine and naloxone were administered by the intravenous, instead of the intramuscular, route.

Other diagnostic criteria of opioid withdrawal such as muscle aches, lacrimation, rhinorrhea, diarrhea, and fever were less commonly or rarely observed after single doses of morphine. Thus, the withdrawal syndrome characteristically seen following acute pretreatment with morphine (8–30 mg per 70 kg) represents a subset of symptoms reported in chronically dependent addicts, and the intensity of symptoms following acute treatment is typically rated as less intense both by observers and the subjects themselves.

Nonetheless, a reliable cluster of physiological, somatic, and subjective withdrawal symptoms appears common to antagonist-precipitated withdrawal from acute or chronic opioid dependence. Since a prior history of opioid dependence is not necessary to elicit withdrawal following single opioid treatment, the convergence of symptoms of withdrawal from acute or chronic opioid dependence suggests that acute dependence may provide a reliable model of the initial stage in the development of chronic dependence.

Animal models

Although, as noted above, many early studies of acute opioid dependence in animals employed high doses of opioid agonist and antagonist to precipitate withdrawal jumping behavior in mice, one early report by Jacob and Michaud in dogs indicated that physiological signs of withdrawal such as hypersalivation, hyperthermia, increased heart rate and respiratory rate, and pupillary dilation could be precipitated by naloxone given 1–5 h after an intravenous injection of a very low dose of morphine (0.1 mg kg^{-1}).

More systematic examination of a broad spectrum of opioid withdrawal signs in a variety of species including mice, rats, hamsters, gerbils, and primates revealed that

certain profound symptoms such as escape jumps, diarrhea, body-weight loss, lacrimation, and rhinorrhea are observed only after high acute doses of morphine ($30\text{--}100\text{ mg kg}^{-1}$, intraperitoneal or subcutaneous); when such signs were observed after lower doses of the agonist ($5\text{--}20\text{ mg kg}^{-1}$), it was typically only after several repeated opioid treatments at daily intervals. In addition, typically these severe signs were precipitated only by higher doses of opioid antagonists such as naloxone or naltrexone.

However, other physiological and somatic signs such as elevations in plasma corticosterone, paw shakes, wet-dog shakes, teeth chattering, swallowing/chewing, eyeblinks, and nociceptive hyperalgesia were reliably observed after $5\text{--}10\text{ mg kg}^{-1}$ doses of morphine – an analgesic dose range in rodents – and could be precipitated by much lower doses of the antagonists. It is noteworthy that these signs are also precipitated by much lower doses of antagonist in rats chronically dependent on opioids than are more profound signs such as withdrawal jumping, diarrhea, and body-weight loss.

Perhaps the greatest utility of the animal models to date has been the identification of reliable markers of the aversive and negative emotional consequences of withdrawal from acute opioid dependence. Antagonist-precipitated withdrawal from either acute or chronic pretreatment with an opioid agonist such as morphine results in suppression of operant responding for food reward and conditioned place aversion. These two indices of opioid withdrawal have been used most extensively to date in characterizing withdrawal from acute opioid dependence. Dysphoria-like signs as measured by elevations in intracranial self-stimulation reward thresholds and anxiety-like signs, as measured by decreased exploration of the open arms of an elevated plus maze, also have been demonstrated during precipitated withdrawal from a single pretreatment with morphine as well as from chronic morphine exposure. While elevations in startle-reflex magnitude are reliably elicited by opioid antagonists several hours following acute pretreatment with morphine, studies of startle magnitude during withdrawal from chronic dependence have yielded mixed results, with different studies reporting elevated, decreased, or no change in startle-response amplitude.

Notably, escalation in withdrawal severity as measured by behavioral indices such as suppression of operant responding for food reward may be observed when morphine is administered repeatedly at intervals of 7 or even 21 days, whereas somatic signs such as those described above increased only when morphine was administered at daily, but not longer, intervals between successive treatments (Schulteis *et al.*, 1999 in Further Reading). Moreover, lower doses of opioid antagonist are required to precipitate the behavioral signs (e.g., $0.1\text{--}0.3\text{ mg kg}^{-1}$ naloxone) than are required to elicit most somatic signs of withdrawal (e.g., $1\text{--}3\text{ mg kg}^{-1}$ naloxone) from acute opioid dependence. These data suggest that aversive and negative

emotional signs of opioid withdrawal may be particularly sensitive to rapid escalation in severity early in the development of opioid dependence.

Factors Influencing Onset and Early Progression of Opioid Dependence

As is the case for withdrawal precipitated from chronic opioid dependence, severity of withdrawal from acute opioid dependence is critically dependent on the amount of agonist administered and the dose of antagonist used to precipitate the withdrawal response. Thus, in both human and animal studies, greater numbers of withdrawal signs are noted, and individual signs increase in intensity, as the pretreatment dose of morphine is increased. Similarly, higher doses of antagonist will precipitate greater intensity of individual signs of withdrawal, and recruit a broader spectrum of signs, following either acute or chronic opioid exposure. It has been known for some time that the potency of antagonists such as naloxone and naltrexone to precipitate opioid withdrawal increases in proportion to the duration of chronic agonist treatment. In the acute-dependence model, the same phenomenon is observed, with the potency of naloxone increasing progressively with repeated administration of single doses of morphine at daily, 7-day, or in some cases even 21-day intervals between successive morphine doses.

Stimulation of opioid receptors by the agonist is critical for the development of both acute and chronic opioid dependence, since coadministration of an antagonist concurrent with the acute or chronic agonist exposure will inhibit the subsequent ability of the antagonist to precipitate withdrawal. Thus, an interval separating the onset of agonist exposure and the administration of antagonist is a vital feature of models of acute opioid dependence. Both in human and animal studies, intervals of 2–12 h between morphine and naloxone/naltrexone administration typically produce maximal intensity of withdrawal for most signs. While certain signs may remain sensitive to precipitation at 18 and 24 h post morphine administration, the antagonists usually lose their effectiveness to precipitate any withdrawal signs by 36–48 h post morphine administration.

Conditioning Processes in Acute Opioid Dependence

While increases in naloxone potency to precipitate withdrawal with repeated intermittent (daily or weekly) pretreatments with morphine do not require that the antagonist be administered after each morphine dose, a number of studies have demonstrated that repeated experience with the antagonist following each morphine pretreatment can further potentiate the severity of withdrawal. Associative conditioning mechanisms support the development of conditioned withdrawal in response to

interoceptive drug cues or explicit external environmental cues that are paired, on several occasions, with precipitated withdrawal from acute intermittent pretreatments with morphine. For example, if naloxone-precipitation of withdrawal from four acute treatments with 5.6 mg kg⁻¹ of morphine at weekly intervals is associated with a discrete tone/light stimulus, subsequent presentation of the tone/light stimulus alone will elicit a significant suppression of operant responding for food reward similar to that produced by naloxone itself (Amitai *et al.*, 2006 in Further Reading). In addition, studies of conditioned place aversion demonstrate clearly that rats and mice can remember and subsequently avoid a distinct context in which they previously experienced antagonist-precipitated withdrawal from acute morphine pretreatment – even when the animals have received only one morphine and antagonist treatment, and hence have only a single opportunity to make the association between withdrawal and the distinct context (Parker and Joshi, 1998 in Further Reading). This suggests that conditioning factors – long believed to be an important factor in the maintenance of an addicted state, and in the promotion of relapse in abstinent individuals who are re-exposed to drug- and withdrawal-related environmental cues – may play an important role very early on in the development of dependence, and the transition from controlled drug use to loss-of-control and compulsive use patterns that characterize addiction.

Neuroanatomical and Neurochemical Substrates of Acute Opioid Dependence

The neuroanatomical substrates contributing to somatic and aversive behavioral indices of withdrawal from chronic morphine dependence have been studied with site-directed injections of a modified form of naloxone – methylnaloxonium – that is relatively lipophobic and hence does not spread rapidly from the site of intracerebral application. Aversive indices of withdrawal (e.g., conditioned place aversion, suppression of operant responding for food reward) both from acute and chronic opioid dependence are precipitated most potently by the application of methylnaloxonium to the nucleus accumbens, central nucleus of the amygdala, and bed nucleus of the stria terminalis (Criner *et al.*, 2007 in Further Reading). Studies involving lesions of some of these same brain regions can also abolish behavioral signs of antagonist-precipitated withdrawal from either acute (conditioned place aversion, elevated startle reflex) or chronic morphine exposure (conditioned place aversion). These studies demonstrate clearly that the expression of withdrawal from acute and chronic morphine involves common neural circuits.

With regard to neurochemical or molecular mechanisms mediating chronic opioid dependence, there is a substantial literature implicating adaptations in intracellular signaling systems such as the adenylate cyclase/cyclic

adenosine monophosphate (cAMP) cascade coupled to opioid receptors via G proteins. Protein kinases of various types alter the phosphorylation state of many proteins involved in the transduction of the opioid-receptor-mediated signaling, including the opioid receptor itself, receptor-coupled G proteins, other kinases, and additional intracellular proteins such as cAMP response-element-binding protein (CREB) which are involved in modifying gene expression. Although relatively few studies have directly examined the cellular adaptations that mediate acute opioid dependence, there are studies which indicate that inhibitors of protein kinases can attenuate the expression of withdrawal from both acute and chronic opioid dependence, as well as the increase in adenylate cyclase/cyclic-AMP signaling that is seen during precipitated opioid withdrawal, indicating at least some overlap in the cellular adaptations between the acute and chronic states of opioid dependence.

There may also be some important differences in mechanisms underlying acute and chronic opioid dependence that remain to be elucidated. For example, as documented above, antagonist-induced precipitation of withdrawal following acute administration of opioid agonists such as morphine is time dependent, with the potency of the antagonist to precipitate withdrawal declines by 24–36 h post morphine administration. However, when repeated treatments with morphine occur 1–3 weeks apart, the severity of withdrawal precipitated by the antagonists is greater with each successive morphine exposure. Clearly, there is some underlying residual trace that allows this progressive increase in withdrawal severity even though acute responsiveness to the antagonist has waned in the interim between successive morphine doses, much like a memory trace that can be maintained long term without the need to actively recall or rehearse the information constantly. Acute-dependence models should prove highly useful tools in distinguishing the transient changes in cellular signaling that temporarily increase antagonist sensitivity following a single dose of morphine from the residual adaptations that mediate the progression of dependence with repeated morphine exposure at infrequent intervals.

Acute Alcohol Dependence

Convergent Symptomatology of Acute and Chronic Dependence

The human

Studies of alcohol dependence and withdrawal cannot take advantage of a competitive antagonist to precipitate withdrawal, and therefore time-course analysis of the emergence and resolution of spontaneous withdrawal signs is the best research method available. Accordingly, withdrawal from chronic alcohol dependence in the

human is characterized by an abstinence syndrome in which symptoms resulting from CNS hyperexcitability emerge in a time-dependent fashion after drinking cessation. In addition to autonomic and somatic symptoms such as hyperventilation, tachycardia, hyperthermia, tremors, and convulsions, the withdrawal syndrome also includes emotional or affective symptoms such as anxiety, restlessness, hyperirritability, and depressed mood/dysphoria.

As is the case with opioid dependence and withdrawal, somatic and affective manifestations of alcohol withdrawal become more prevalent and severe with chronic use, but evidence from the human and animal models indicates that a subset of withdrawal signs may be observed after a single exposure to alcohol. For example, studies of human drinkers recovering from acute bouts of alcohol intoxication have identified withdrawal-like somatic signs such as tremors, and emotional signs such as anxiety/nervousness and depression/dysphoria. This milder form of withdrawal following acute binges of alcohol consumption may be one component of what is often termed alcohol hangover, but it must be recognized that the hangover syndrome reflects more than just an acute withdrawal reaction. Hangover from acute alcohol intoxication may include symptoms resulting not only from withdrawal from acute intoxication, but also from sickness related to build-up of acetaldehyde (the initial byproduct of ethanol metabolism), from dehydration resulting from the diuretic effects of alcohol, and/or from congeners present in certain types of dark liquors such as brandy, wine, whiskey, and tequila that seem to increase hangover but not withdrawal severity relative to clear liquors (vodka, gin, etc.).

Animal models

Acute alcohol dependence has received greater attention in animal models than in human drinkers, but the available data is still quite limited relative to the acute-opioid-dependence literature. A study as early as 1958 by McQuarrie and Fingl identified reductions in seizure thresholds as a common index of somatic withdrawal from both acute and chronic alcohol administration. In the most frequent application of this model, used extensively by Crabbe and colleagues to identify genetic markers of susceptibility to acute alcohol dependence in mice (see also below ‘Acute dependence on benzodiazepines and barbiturates’), a relatively high dose of alcohol (4 g kg^{-1}) which produces blood alcohol levels beyond what even the typical heavy drinker might achieve will induce increased susceptibility to handling-induced convulsions in mice from 4 to 12 h post alcohol administration.

More recent work has identified emotional withdrawal signs from acute bouts of alcohol intoxication, including:

1. increased anxiety-like behavior as measured in the elevated plus maze or in generalization of the acute withdrawal period to the discriminative stimulus effects of the anxiogenic drug pentylenetetrazole; and
2. dysphoria-like behavior, as measured by elevated intracranial self-stimulation reward thresholds.

Although early studies focused on high ($3\text{--}4\text{ g kg}^{-1}$) doses of alcohol to induce these acute withdrawal behaviors, more recently these emotional signs of withdrawal have been demonstrated to occur from 6 to 12 h after doses of alcohol as low as $1.5\text{--}2\text{ g kg}^{-1}$, which produce blood alcohol levels that would not be uncommon after a heavy bout of drinking. In addition, as was the case with acute opioid dependence, repeated bouts of alcohol intoxication at daily or weekly intervals results in a potentiation of the peak magnitude and an extended duration of anxiety-like and dysphoria-like behaviors, suggesting that withdrawal from acute bouts of ethanol intoxication may represent a valid index of the initial adaptive response in the development of chronic alcohol dependence.

Neurochemical Substrates in Acute Alcohol Dependence

As is true for opioid dependence, the literature on neural mechanisms mediating an established state of dependence resulting from chronic alcohol exposure is quite large, whereas the literature dealing with acute dependence is quite limited. To date, some of the best evidence available on potential neurochemical substrates of acute alcohol dependence is provided by genetic analyses in mouse models, where it appears that genes encoding certain subunits of the type-A receptor for the inhibitory neurotransmitter γ -aminobutyric acid (GABA) may account for the sensitivity to handling-induced seizures during withdrawal from an acute dose of alcohol. Notably, alterations in GABA-A receptor signaling are also prominent in dependence on and withdrawal from chronic alcohol.

Acute Dependence on Benzodiazepines and Barbiturates

To date, the literature on acute dependence to benzodiazepines and barbiturates, two classes of CNS-depressant drugs with significant clinical use, is rather limited. A study published in 1986 revealed acute dependence on the classic benzodiazepine chlordiazepoxide (Librium) at relatively high doses ($75\text{--}450\text{ mg kg}^{-1}$) administered to rats. Spontaneous withdrawal was observed between 100 and 124 h after administration of a single injection of 450 mg kg^{-1} chlordiazepoxide, as measured by a number of somatic signs characteristic of withdrawal from chronic benzodiazepine treatment (e.g., tail erection, arched back,

muscle hypertonus). The delayed emergence of withdrawal is accounted for by the high dose of chlordiazepoxide (450 mg kg^{-1} is the acute maximally tolerable dose in rats, well above the required dose to produce anxiolytic and sedative effects), along with the relatively long half-life of its metabolites – a number of which possess significant benzodiazepine activity. Withdrawal could also be precipitated from 28 to 52 h post chlordiazepoxide administration with the benzodiazepine binding-site antagonist RO 15-1788. As is the case with acute opioid dependence, the magnitude of precipitated withdrawal was found to be proportional to the dose of agonist, dose of antagonist, and the interval between the two.

Crabbe and colleagues, as part of their genetic analyses of acute alcohol dependence, reported acute benzodiazepine dependence as measured by antagonist-precipitated increases in handling-induced convulsions in a variety of inbred strains of mice, including selectively bred withdrawal-seizure-prone (WSP) and high alcohol withdrawal (HAW) mice. A broad range of benzodiazepine agonists have been demonstrated to support acute dependence in this model, with half-lives ranging from short (e.g., zolpidem/Ambien, triazolam/Halcion, midazolam/Versed, 1.5–6 h half-lives) to medium (e.g., alprazolam/Xanax, lorazepam/Ativan, 6–20 h) to long (e.g., diazepam/Valium, 20–80 h). The doses of benzodiazepine agonist required to induce acute dependence in this model were within the normal range that produce anxiolytic-like and sedative effects. Some of the shorter-acting benzodiazepines also supported significant spontaneous withdrawal within 6 h of treatment. Finally, acute dependence on barbiturates such as pentobarbital has been confirmed using handling-induced convulsions in mice as an index of spontaneous withdrawal. Available data from genetic analyses of withdrawal-seizure susceptibility following acute pretreatment with benzodiazepines and barbiturates implicate similar neurochemical targets to those identified in studies of acute alcohol dependence (e.g., specific alpha and gamma subunits of the GABA-A receptor).

With regard to behavioral indices of withdrawal from acute benzodiazepine dependence, suppression of operant responding for food reward could be precipitated by the competitive antagonist flumazenil at 18 h after administration of a high dose of chlordiazepoxide (100 mg kg^{-1}) in rats, or 24 hr after the administration of behaviorally active doses of chlordiazepoxide (10 mg kg^{-1}) or diazepam ($3\text{--}5.6 \text{ mg kg}^{-1}$) in squirrel monkeys. The only study, to date, to evaluate intracranial self-stimulation reward thresholds during precipitated withdrawal from acute pretreatment with chlordiazepoxide ($30\text{--}100 \text{ mg kg}^{-1}$) and diazepam ($3\text{--}10 \text{ mg kg}^{-1}$) found no dysphoria-like elevations in reward thresholds (Easterling *et al.*, 2000 in Further Reading), in contrast to what is seen during withdrawal from acute morphine or alcohol pretreatment (see

above). At present, studies with other behavioral indices of aversive emotional consequences of withdrawal (e.g., conditioned place aversion, anxiety-like behavior) are lacking for acute benzodiazepine dependence.

Acute Dependence on Psychostimulants, Phencyclidine, and Cannabis?

Somatic and physiological withdrawal symptoms vary widely across classes of abused drugs, especially when comparing stimulants such as cocaine, amphetamines, or nicotine to sedative drugs such as opioids, alcohol, benzodiazepines, and barbiturates. However, it is well-established that emotional components of withdrawal from chronic dependence (i.e., increased anxiety and depression/dysphoria) are common across most classes of abused drugs studied to date, including stimulants. To date, acute withdrawal from stimulants is a relatively unexplored phenomenon. There are a number of human and animal studies focused on acute tolerance to the physiological and subjective effects of nicotine that have yielded mixed findings, but none include an explicit examination of acute dependence and withdrawal from acute nicotine. Laboratory animal studies of acute dependence with indirect sympathomimetic stimulants are limited to observations by Markou and colleagues that withdrawal from a single injection of d-amphetamine (4 mg kg^{-1}), or three escalating dose injections of 1, 2, and 3 mg kg^{-1} at 6-h intervals within a single day, results in the elevation of intracranial self-stimulation reward thresholds from 8 to 24 h post amphetamine administration, suggesting that acute dependence on stimulants can be measured as transient reward deficits. Acute dependence on phencyclidine (PCP/Angel Dust, $5\text{--}10 \text{ mg kg}^{-1}$) and delta-9-tetrahydrocannabinol (1 mg kg^{-1}), the primary active ingredient in cannabis, similarly has been reported in one study each, as time-dependent (<24 h) withdrawal-induced elevations of brain reward thresholds. Further work is clearly needed to more thoroughly characterize acute dependence on addictive substances other than opioid narcotics and CNS depressants, but the work to date with brain stimulation reward is a promising start.

Motivational Relevance of Acute Dependence and Withdrawal

Not all substance use, whether initiated primarily for pleasure seeking (e.g., euphoria-inducing effects, increased sociability) or for self-medication of stress, mood, or anxiety disorders, leads to compulsive use patterns that characterize addiction, and a significant

research challenge is to understand factors that contribute to the transition from casual, controlled drug use to loss of control/compulsive use in vulnerable individuals. Differential susceptibility to rapid onset of neuroadaptive responses to acute drug administration could serve as one important factor in this process. For example, Stitzer and colleagues who have done the majority of work on acute opioid dependence in the human make note of large individual differences in sensitivity to antagonist-precipitated withdrawal from single doses of morphine across subjects.

Aversive withdrawal reactions following an acute bout of intoxication could serve as a punishing stimulus, deterring further substance use. Within this framework, individuals with reduced susceptibility to acute dependence will show minimal aversive withdrawal symptoms following initial bouts of substance use, and consequently might be at higher risk to develop patterns of excessive use than individuals showing a more profound withdrawal reaction from acute dependence. However, it is also possible that some individuals who are more susceptible to rapid induction of acute dependence, and therefore do experience mild aversive withdrawal symptoms in response to initial substance use, nonetheless choose to engage in repeated drug use due to factors such as peer pressure. In these individuals, progressive increases in severity of withdrawal signs with successive episodes may contribute to the escalation of subsequent substance intake as the individual recognizes that intake effectively alleviates the aversive state experienced during abstinence. For those with preexisting anxiety/mood symptoms who initiated substance use to self-medicate those symptoms, this latter possibility may be particularly prominent, as the user is well aware that self-medication will alleviate the negative emotional withdrawal symptoms. In this fashion, acute withdrawal symptoms from individual bouts of substance use may play an increasing role over time as a motivational stimulus for further consumption through a process of negative reinforcement. Further research employing the models described herein should prove illuminating with respect to the motivational valence of increased susceptibility to acute dependence (i.e., punishing or negatively reinforcing further use), as well as more precisely delineating the neurobiological mechanisms underlying said increased susceptibility.

See also: Animal Models of Behavior; Alcohol Addiction; Alcoholism; Animal Models of Learning and Memory; Brain Stimulation and Addiction; Comorbidity – Depression; Drug Addiction; Drug Cues: Significance of

Conditioning Factors in Drug Abuse and Addiction; Drug Withdrawal – Motivational View; Molecular Neurobiology of Addiction; Neurobiology of Opioid Addiction; Psychiatric and Substance Use Disorder Comorbidity; Psychostimulants; Transition to Addiction; Vulnerability Factors in Addiction Disorders.

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Alcoholism

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Glossary

Alcohol dependence – A problematic term in that it refers to two related, but independent, concepts. As a clinical diagnosis, it refers to the complex syndrome which could otherwise better be called alcohol addiction. At the same time, it is used to refer to physical dependence, that is, the presence of tolerance and withdrawal.

Allostasis (in the context of neuroadaptations to alcohol) – A new, pathological set point for any psychological or physiological measure, which has been achieved by the activation of an opponent process that counteracts the effects of alcohol. For instance, acute intake of alcohol reduces anxiety. Chronic alcohol use will activate opponent processes, so that normal anxiety levels are experienced with alcohol onboard. When alcohol intake is terminated, the influence of the opponent process will be uncovered, as abnormally high anxiety.

Ataxia – Loss of balance and coordination, which, when not caused by cerebellar or other disease, typically reflects suppression of cerebellar function by alcohol or another central nervous system (CNS) suppressant.

Delirium tremens – Shaking madness – the most severe form of alcohol withdrawal, during which there is clouding of consciousness, hallucinations, and somatic symptoms including tremor and hyperthermia. Untreated, this syndrome once had an approximately 20% mortality.

Fermentation (in the context of alcohol production) – The anaerobic conversion of sugar to carbon dioxide and alcohol by yeast.

Heritability (of alcoholism or related phenotypes) – The proportion of risk of the respective condition attributable to genetic factors.

Sedation – Suppression of psychomotor function and general excitability by alcohol, barbiturates, or similar agents.

Seizures (in the context of alcohol withdrawal) – Uncontrolled electrical activity in the brain, which may produce a physical convulsion, minor physical signs, thought disturbances, or a combination of symptoms. Alcohol withdrawal seizures are most commonly motor seizures, typically.

Tolerance to alcohol – The need to consume increasingly higher amounts of alcohol in order to

achieve the same level of intoxication. Together with withdrawal, an integral part of physical dependence. As such, tolerance is often a part of alcohol addiction, and provides support for this diagnosis; but is in itself neither necessary nor sufficient to establish it.

Withdrawal – Signs and symptoms that may emerge upon discontinuation of alcohol intake following an extended duration of high-level intake. It includes both psychological (e.g., anxiety) and somatic (e.g., tachycardia) symptoms (see tolerance, an integral part of physical dependence).

Alcoholism (Alcohol Addiction)

Although definitions of alcoholism vary, most views recognize as a key component a pattern of excessive and repetitive drinking of alcoholic beverages at a level that causes harm to the individual, others, or both. The harmful consequences of excessive alcohol use are unusually diverse, and in addition to medical–physical – as well as psychiatric – problems, commonly include impaired social function and legal problems. Similar to other addictions, a hallmark of alcoholism is that the normal behavioral repertoire is restricted in favor of drug seeking and consumption, and that excessive and harmful use occurs despite realization of its harmful consequences. This points to an impairment of normal behavioral control, and a transition from episodic thoughtlessness, or impulsive to habitual or compulsive drunkenness.

Alcohol as a Drug

The active ingredient in alcoholic drinks is ethyl alcohol, or ethanol (C_2H_5OH), typically produced by fermentation (anaerobic metabolism) of carbohydrates by certain yeast species. Other alcohols are primarily of interest in toxicology (e.g., methanol, butanol) or in the food-processing industry (sugar alcohols, such as xylitol), and will not be discussed further here. Ideally, alcoholism should, therefore, be referred to as ‘ethylysm,’ and corresponding terms should be used whenever possible. The term ‘alcoholism’ is, however, so established in lay language that it will also be used here. Unless otherwise specified, the word ‘alcohol’ will refer to ethanol.

By 4000 BC, production and consumption of alcohol-containing beverages such as wine is solidly documented in hieroglyphic writing from Egypt, while recipes for making wine or beer are found on clay tablets from Mesopotamia that are dated about a millennium later. In ancient Greece, fermented honey – or mead – seems to have been the original alcoholic beverage, but wine-producing skills reached Greece by about 2000 BC. Fermented beverages provided a certain degree of protection against overgrowth of pathogens, such as cholera, in drinking supplies. Because of this, fluid consumption in areas where fresh water was not readily available could, to a large extent, consist of beer or wine. At sea, all fluid carried onboard could be in the form of beer. The alcohol content of these beverages was typically relatively low, probably not exceeding 4%. Of note, antiseptic properties of alcohol are only partial at these concentrations, so other ingredients, as well as crowding out of bacteria by yeast, must have contributed.

Fermented beverages can typically not reach an alcohol content beyond ~15%, because, at higher concentrations, the yeast are metabolically impaired or die. Higher alcohol concentrations, likely leading to a higher addictive potential, can only be reached through distillation. Although there is historical account of small-scale distillation dating to prehistorical times (from Babylonia and Egypt), successful distillation of quantities suitable for human consumption was only achieved by Arab chemists around AD 800–900. By the thirteenth century, this expertise had spread to Europe, and by the fourteenth century, the use of distilled spirits as medicinal elixirs was widespread, for instance, to provide protection or cure from the Black Death.

Psychoactive Properties

In contrast to other addictive drugs, alcohol does not have a unique molecular target, such as a specific neurotransmitter receptor or transporter in the central nervous system (CNS). It was once thought that the CNS actions of alcohol were produced through interactions with membrane lipids, but this view has largely been abandoned. Work with the *Drosophila* odorant-recognizing protein LUSH, instead, suggests that alcohol may interact directly with protein motifs that encode hydrophobic pockets. The main direct targets of alcohol are ligand-gated ion channels, most importantly glutamatergic and γ -aminobutyric acid (GABA-ergic) receptors. Alcohol acutely inhibits glutamatergic, and potentiates GABA-ergic transmission, in both cases through pre- as well as postsynaptic mechanisms. It thus shifts the excitation-inhibition balance throughout the CNS in favor of inhibition. Consistent with this profile, high alcohol doses are hypnotic, while lower doses are sedative and ataxic.

Sensitivity to sedative/ataxic alcohol actions displays considerable individual variation, which is highly heritable. This variation is related to genetic risk for alcoholism. Similar to other sedative-hypnotics, alcohol also suppresses anxiety.

Secondary alcohol targets include several G-protein-coupled receptors, including – perhaps most importantly – μ -opioid and dopaminergic receptors. Through these, alcohol activates classical brain reward circuitry, resulting in positive reinforcement as well as psychomotor stimulation. Genetic and other host factors also determine the degree of these alcohol effects. The sex, age, and genetic makeup of the individual will thus determine the net balance between sedative-hypnotic and psychomotor-stimulating, positively reinforcing alcohol actions.

Following a prolonged history of brain alcohol exposure to repeated cycles of alcohol intoxication and withdrawal, an allostatic shift will occur with regard to molecular, physical, and motivational alcohol actions. Specifically, in the presence of alcohol, opponent processes are progressively recruited that counteract acute alcohol actions. When alcohol is withdrawn, these opponent processes become predominant. For instance, processes opposing the sedative-hypnotic actions of alcohol, when uncovered, will result in the hyperexcitability of alcohol withdrawal, potentially leading to withdrawal seizures, delirium tremens (DT) or withdrawal anxiety. Recruitment of processes opposing the psychomotor-stimulating and positively reinforcing alcohol actions will lead to attenuated reward-system function – as shown, for example, by attenuated signaling within the classical circuitry of the mesolimbic dopaminergic system – and impaired reward function, as illustrated by elevated thresholds for intracranial self-stimulation in experimental animals. Additionally, antireward systems, with corticotropin-releasing factor (CRF) as a prominent example, will be activated in the course of these neuroadaptations, and will, in the absence of alcohol, contribute to shifting affective balance into a negative direction. In a vicious circle, this will set the scene for increased negative reinforcement by renewed alcohol intake.

Alcoholism as a Clinical Condition

If consumed in excess or under the wrong circumstances, alcohol can clearly be harmful to anyone, and warnings against lack of temperance can be traced back to ancient Greece. It has, however, also been recognized that some individuals develop alcohol-use habits which differ from the majority of users. In these individuals, alcohol causes harm related to the overall amount, pattern, and manner of alcohol use. The notion that this type of habitual drunkenness is a disease dates back to antiquity, and Seneca classified it as a form of madness. The modern

term ‘(chronic) alcoholism’ was introduced in a classic paper, in 1849, by Magnus Huss, professor of medicine at the Karolinska Institute, Stockholm, Sweden. Chronic alcoholism quickly became the medical term for a syndrome related to habitual, rather than occasional, drunkenness.

A medical view of alcoholism was originally based on purely pharmacological and physiological phenomena, among which tolerance and withdrawal were key. This refers to the need for progressively increasing doses to produce desired effects, and a withdrawal syndrome when drinking is discontinued. This view, although still widespread among clinicians, is clearly insufficient. Compulsive alcohol use that leads to adverse behavioral and medical consequences does not require that tolerance and withdrawal are present. Furthermore, relapse is a core phenomenon of chronic alcoholism, and classical relapse studies indicate that over a 12-month period, equal proportions of alcoholics relapse within the first month – when withdrawal symptoms might be present – and in the next 11 months, during which withdrawal symptoms are long gone. Clearly, processes other than tolerance and withdrawal must be involved in maintaining the disease process.

In order to capture clinically relevant core phenomena of alcoholism, a focus on behavioral pathology is needed. In this perspective, alcoholism is a condition in which alcohol acquires a pathological degree of motivational salience, restricting the behavioral repertoire of the individual so that alcohol seeking and consumption assume a predominant role at the expense of other behaviors, and the individual loses control over his or her alcohol use. Physical dependence, when present, may contribute to this state, and its development may be a marker of brain plasticity which contributes to the evolution of the behavioral disorder. However, physical dependence may or may not be present, that is, it is neither necessary nor sufficient for the development of clinical alcoholism. Whatever the underlying neural processes, the result is a behavioral and motivational pathology that results in compulsive and excessive alcohol consumption sufficient to – ultimately – cause medical, psychological, socioeconomic, and legal problems.

In this clinical perspective, alcoholism is very similar to other conditions whose disease nature is rarely questioned. It has a considerable component of genetic vulnerability, persists over time, is maintained in part by specific patterns of behavioral choices, and is a major cause of death and disability. A disease view is further justified by the observation that alcohol induces persistent changes in brain function and structure in ways that affect motivation and decision making.

Based on the phenomena discussed above, reasonable consensus has been reached on criteria by which clinical alcoholism can be diagnosed, as reflected by the World

Health Organization International Classification of Diseases (ICD)-10 criteria, and the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders, version IV (DSM-IV) criteria. While these tools work quite well in a clinical context, caution is needed when they are applied in non-clinical situations, such as epidemiological studies. People who fulfill these criteria but have never sought treatment appear to be a population that is distinct from those who fulfill such criteria but have also sought treatment. While few clinicians would question the disease nature of people in the latter category, it is questionable whether the former group can be regarded as having a clinical disease.

Etiology

The common belief that alcoholism can be inherited dates back at least several hundred years, as documented, for example, in preserved population registries maintained by the Swedish church. By now, adoption and twin studies have consistently estimated the heritability of alcoholism in the 50–60% range. Some continuing controversy in this area is, therefore, unsubstantiated by data.

Available evidence suggests that the heritability of alcoholism is polygenic, with common estimates of how many loci that contribute currently running close to 20 – each contributing a small effect size. It also appears that ‘alcoholism’ is likely to be a group of phenocopies, that is, conditions that may appear very similar in a cross-sectional, snapshot view, but in fact represent the endpoint of very different trajectories, to which in turn different groups of genes may contribute. For instance, classical adoption studies showed, nearly three decades ago, that early-onset alcoholism with impulsive personality traits has a higher degree of heritability than disease with later onset where high trait anxiety is predominant. Defining less complex intermediate phenotypes provides the hope of identifying more homogenous groups of alcoholics, and thereby facilitating the identification of specific genetic risk factors. As an example, many – but not all – people at genetic risk of developing alcoholism have been shown to respond with a lower degree of sedative–ataxic effects to alcohol. This constitutes an intermediate phenotype that may lead to the discovery of specific genetic risk factors. Another intermediate phenotype, comprised of specific electroencephalogram (EEG) response patterns (P300), has already proved useful for this purpose (see below). In other cases, people with known genetic variants, such as a functional variant of the μ -opioid receptor, show increased subjective responses to alcohol. Other interesting intermediate phenotypes are currently being developed using functional brain imaging.

The first specific genetic factors that were discovered to modulate alcoholism risk were those that confer a

protective influence. Genetic variation in the aldehyde dehydrogenase gene common among people of Asian descent confers decreased capacity for degradation of acetaldehyde – the main alcohol metabolite. Because they accumulate this toxic metabolite, homozygous carriers of this variant have an unpleasant flushing response even to small quantities of alcohol. This clearly reduces their risk for developing alcoholism. More recently, increased susceptibility for developing alcoholism has been linked to variation in genes encoding CNS proteins more directly involved in the pharmacodynamic actions of alcohol. The first successful case in this category was variation in the gene encoding the GABA-A receptor α -2 subunit – first identified using an EEG-based intermediate phenotype, and subsequently directly linked to alcoholism risk. This finding has been confirmed in several independent replications. Variation in the serotonin transporter gene as well as the gene encoding the type 1 receptor for CRF has also been linked to increased risk and, in both cases, seems to interact with adverse life events to produce excessive drinking.

What is inherited is clearly not alcoholism *per se*, but rather an increased disease susceptibility, that is, a risk to develop this condition given exposure to specific environmental risk factors. The most important among these is availability of alcohol. Easy availability clearly increases alcoholism risk, and countries or communities where alcohol is cheap, for example, due to low taxation, and where societal control over sales is low have high rates of alcoholism. Peer and cultural influence have also been described to modulate risk, although they are difficult to dissociate from genetic factors. Several psychiatric conditions increase alcoholism risk – the most important among these being attention-deficit disorder, panic disorder, social phobia, schizophrenia, bipolar illness, and, with the highest quantitative contribution, antisocial disorder. Finally, unemployment, irregular working hours, or working in an environment with high availability of alcohol (e.g., diplomats, journalists, bartenders) increases alcoholism risk.

Clinical Characteristics

Acute Presentation

Alcohol-addicted subjects come to medical attention through an unusually wide range of medical problems. Studies in trauma centers have demonstrated that a large and long-overlooked component of this condition is accidental injury. Accident risk is generally increased by intoxication, but in the absence of addiction, most people confine episodes of heavy drinking to reasonably safe settings. It is when the addictive process pushes heavy drinking beyond these confines, for example, onto the road or into the workplace, that the risk of accidental

injury increases dramatically. Overall, mortality is approximately three- to fivefold elevated in alcoholism. Alcoholics are almost 30 times more likely to die from fatal poisoning, about 15 times more from a fall accident, and about 5 times more liable to die in a car crash. Alcoholism is also a major cause of suicide, ultimately causing death in about 3% of alcoholics. Conversely, a classic study found fatty liver – a condition that only rarely has other causes than alcoholism – in about half of the suicide victims, but only 3% of the general population. Risks of other violent death are also high: the risk of death through homicide or drowning is roughly doubled.

A set of more specific acute conditions, collectively labeled the alcohol withdrawal syndrome, often brings the patient to care when episodes of heavy drinking come to an end. To a large extent, these reflect the rebound CNS hyperexcitability in withdrawal. The common hangover illustrates, in miniature, some of these phenomena – such as headache, increased anxiety, and general malaise. The clinically significant phenomena of withdrawal are, however, absent in hangover. Following prolonged periods of heavy drinking, patients develop tremors, accompanied by a loss of appetite, inability to retain food, sweating, restlessness, and sleep disturbances. Unless confounded by the use of other sedative-hypnotic drugs, this group of symptoms typically peaks within 24 h from discontinuation of drinking, and subsides thereafter. In the most severe cases of CNS hyperexcitability and/or in combination with other risk factors such as fever, seizures can also occur in early withdrawal, occasionally developing into status epilepticus, a potentially fatal condition. Most seizures occur within the first 48 h. Finally, the risk for DT peaks around the third day of withdrawal. DT is a transient, but serious, psychotic condition, with confusion and visual hallucinations, accompanied by whole-body tremor and fever. Unless treated, typically with tapered doses of benzodiazepines, it can last up to a week. Before treatment was available, DT was fatal in up to 20% of cases.

Prolonged heavy drinking leads to an almost complete impairment of thiamin uptake. Some individuals have a low activity of the enzyme transketolase, but as long as thiamin (vitamin B1) is adequately supplied through the diet, this is inconsequential. Loss of absorption, however, will unmask the deficit in these individuals, potentially leading to neuronal death and scarring in the brainstem, cerebellum, and mamillary bodies. This is clinically expressed as Wernicke-Korsakoff's disease, marked classically by a clouding of consciousness, abnormal eye movements, and axial amnesia (both anterograde and retrograde loss of recent memory) accompanied by confabulation, a tendency to make up for the defect without regard for fact. Although one would think that a condition with these rather dramatic symptoms would be readily

diagnosed and treated, most Wernicke–Korsakoff's cases are, in fact, found only on autopsy.

Some degree of liver inflammation is common in the acute phase of alcoholism treatment. It usually resolves without the need for specific treatment, and it is the chronic liver complications that have the greater clinical significance. Other gastrointestinal conditions that are commonly encountered during the acute phase are gastritis, duodenal ulcers, and pancreatitis.

Chronic Disease

Although most often unrecognized, alcoholism is one of the most common causes of psychiatric problems, including insomnia, anxiety, and depression. In most individuals, these problems resolve after about 1 month of abstinence. In a minority, however, it is found that alcohol use has triggered or been comorbid with independent psychiatric disorders, making a careful differential diagnosis paramount.

Alcoholism also causes a cognitive impairment, which will initially resolve within some 6 months of abstinence, but will persist in later stages, reflecting a loss of brain tissue that is at least, in part, irreversible. In older alcoholics, enlargement of both gyri and ventricles is commonly seen on structural imaging as a result of the atrophy of gray matter. This atrophy is likely to in part reflect direct alcohol effects, and in part damage caused by accidents and blows.

Among the almost endless list of medical conditions that chronic heavy alcohol use can lead to, contribute to, or mimic, a few more are specifically enough linked to alcoholism to merit mention here. The classic chronic disease resulting from alcoholism is cirrhosis of the liver, typically preceded by steatosis (a fatty enlargement of the organ). In its most severe form, alcoholic liver cirrhosis leads to liver failure, and is ultimately fatal unless the patient receives a transplant. Chronic alcohol use is a common and commonly overlooked cause of hypertension and related cardiovascular morbidity, but a condition more specifically associated with chronic alcoholism is alcoholic cardiomyopathy, a disease of the cardiac muscle that can lead to terminal heart failure. Thiamin deficiency can chronically lead to polyneuropathy, a degenerative disease of the peripheral nerves with loss of vibratory sensation and proprioception that – together with cerebellar damage – contributes to the characteristically broad gait of late-stage alcoholics.

Natural History

Alcoholism evolves over 5–15 years. This is reflected by the fact that alcohol-related problems are most common among men in the age range of 18–30, but the

development of chronic, clinical alcohol addiction is most common in people between 25 and 50 years of age. Thus, among the many young people whose heavy drinking has the potential to lead to alcoholism, the process is not carried to completion in most cases, and by age 30 many hazardous drinkers will have returned to controlled social alcohol consumption.

Disease with early onset evolves more rapidly than that with onset later in life. Because of this, considerable emphasis has been placed on the harm of alcohol use in adolescence. The hope has been that delaying alcohol use would reduce the risk for rapid development of alcoholism. It is certainly desirable to refrain from alcohol use while the brain is still developing and the individual has not achieved a mature capacity for assessing and handling the risks involved in alcohol use. It is, however, unclear whether age of alcohol-use onset modulates addiction risk once the genetic risk is controlled for.

Alcoholism is a chronic, relapsing disorder. Short-term discontinuation of heavy drinking can readily be achieved in most cases. It is, however, the ability to prevent relapse, that is, a return to heavy drinking, that will determine long-term impact on health and social functioning. Patients with alcoholism are a heterogeneous group, and so are their outcomes. On the one hand, of those people who in epidemiological studies, at some point, fulfill diagnostic criteria for alcoholism, a majority will no longer do so 5–10 years later, and their health and social functioning will not be adversely affected. On the other hand, among clinical cases, that is, treatment-seeking people with an alcoholism diagnosis, about 70% will relapse within 12 months. Key relapse-triggers in this group are sampling a dose of alcohol, being exposed to alcohol-associated cues, or experiencing stress. Although formally fulfilling the same diagnostic criteria, it is unlikely that these groups share underlying pathophysiology. A disease view of alcoholism is certainly justified in the latter, but not necessarily in the former, group.

A particularly contentious issue related to outcomes is whether a return to controlled social drinking is possible for alcoholics. Classical long-term follow-up studies concluded that this may be possible in individuals who had abused alcohol for less than a year, but that, if alcohol dependence has persisted for more than 5 years and reached a certain degree of severity, efforts to return to social drinking lead to relapse for a vast majority of subjects. Other studies challenged this notion, describing considerable proportions of people with alcoholism who successfully returned to controlled drinking. If the heterogeneity of alcoholism is considered, this controversy is likely to melt away. At one end of the spectrum, there are people in whom the critical neuroadaptations of addiction are absent or have resolved. If the risk factors that once contributed to heavy drinking have been eliminated, it is not unreasonable that these individuals cautiously

attempt a return to controlled drinking. On the other hand, if the disease has had sufficient duration and/or severity that the critical neuroadaptations persist, risk of relapse remains very high, justifying the saying “once an alcoholic, always an alcoholic.” Therefore, a major objective of future research is to identify the critical neuroadaptations and their clinically useful biomarkers.

Prevalence of Alcoholism

Epidemiological studies in the United States of America (USA) and Western Europe commonly estimate lifetime prevalence of alcoholism at 10–20% in men and 5–10% in women. Somewhat higher rates are reported from Eastern Europe, while rates reported from countries in the Middle East and in Southeast Asia are much lower. Rates reported from rural areas in Africa are low, but are very high in the rapidly growing slums around the big cities.

Thus, alcoholism is generally common where alcohol is available, and about twice as much so among men than women. Some trends over time can also be discerned. Beyond that, the specific numbers should not be over-emphasized. Attitudes, criteria, and reporting among different countries are rarely comparable. Even when state-of-the-art methodology is used, there is reason for caution when up to one in five men receive diagnosis of a disease that, when present clinically, has considerable mortality and morbidity.

The diagnostic term alcoholism is perhaps better reserved for a condition of sufficient clinical significance to typically bring people to treatment. This is particularly true if a medical view of alcoholism as a disease is to be widely accepted. In other cases, it may be more meaningful to consider hazardous drinking. This refers to the use of alcohol at a level or in a pattern that, although not necessarily reflecting addiction, carries a high risk of negative medical or social consequences. Excellent and simple screening instruments have been developed that identify hazardous alcohol use, with the Alcohol Use Disorder Identification Test (AUDIT) being the most widely used. In people with hazardous drinking but without clinical alcoholism, considerable benefits in terms of reduced healthcare utilization and sick leave have been documented from simply identifying the problem through screening with these questionnaires, for example, in primary care, and providing feedback on the adverse consequences of consumption. The ability of these subjects to change their behavior based merely on information received perhaps most directly illustrates a fundamental difference from a person with an addictive disease.

Treatment of Alcoholism

The concept of treatment is closely related to the choice of outcome measures. Traditionally, abstinence has been the standard measure of success. Given the chronic, relapsing nature of alcoholism, such a notion is naive at best, and harmful at worst. As in any medical condition, a successful treatment is one that reduces morbidity and improves functional outcomes. Reduction of heavy drinking achieves these goals. Similar to most chronic diseases, alcoholism may not necessarily be possible to cure, but can certainly be successfully managed.

Treatments with measurable beneficial effects exist for alcoholism. Unfortunately, a classical systematic review found that the likelihood of a specific treatment being provided to a patient was inversely correlated with the strength of the evidence supporting its efficacy. An equally disturbing inverse correlation was found between the cost of delivering a specific treatment and the strength of evidence in its support. Clearly, while research efforts hope to bring forward new treatments, considerable gains could be achieved by simply implementing what is already known.

‘Detoxification’ – an ill-chosen but widely adopted term that refers to medically supervised withdrawal – has long been a mainstay of alcoholism treatment. Detoxification may be necessary to safely prevent or manage complications and achieve short-term sobriety, but research shows that this intervention has little if any impact on long-term outcomes in alcoholism. The same is true for the widespread and costly 28-day residential treatments. Expensive long-term psychotherapies focusing on underlying psychological problems rather than alcohol use are also as common as they are ineffective.

Among psychological-behavioral treatments, short- and medium-term efficacy has been found for those that are nonconfrontational, specifically focus on the alcohol use, and apply methods that increase motivation for change; actively practice, for instance through role-play, thoughts and behaviors that minimize relapse risk by eliminating or avoiding relapse triggers; practice strategies to avoid progression from a slip to a full-blown relapse; and reinforce alternative behaviors. Among various methods that have successfully applied these elements are motivational interviewing, community reinforcement, and relapse prevention based on cognitive-behavioral therapy principles. While much has been made of the specifics of these therapies by their respective advocates, large-scale studies have failed to show clinically meaningful differences in outcomes between them, or evidence for beneficial matching effects.

Modern pharmacotherapy of alcoholism is in its early days. It was preceded by disulfiram, an inhibitor of aldehyde-dehydrogenase, which was introduced in the 1950s.

Disulfiram does not affect the motivational pathology of alcohol addiction. Instead, it leads to accumulation of acet-aldehyde upon intake of alcohol, resulting in severe flushing and tachycardia thought to deter alcohol use. Unsupervised disulfiram has, however, no documented efficacy, presumably because compliance is low. When used, it has the potential for serious complications. Outside special settings, for example, when short-term abstinence must be achieved to allow a psychiatric evaluation, or with supervised administration to professionals at risk for losing their credentials, disulfiram therefore should have little place in the modern treatment of alcoholism.

The era of modern alcoholism pharmacotherapy started in the early 1990s, with the discovery that the opioid antagonist naltrexone reduces relapse to heavy drinking. This has subsequently been confirmed in meta-analyses of the many controlled trials that followed, and naltrexone is approved for treatment of alcoholism in the USA, Europe, and many other countries. Naltrexone directly inhibits positively reinforcing properties of alcohol that are mediated by endogenous opioids in response to alcohol intake. Average effect sizes in clinical treatment are relatively low. Recent research has, however, shown interesting pharmacogenetic diversity that can help improve outcomes by personalizing treatment. Carriers of a functional 118G variant of the μ -opioid receptor, who constitute nearly 15% of Caucasian populations, appear to respond well to naltrexone, while patients homozygous for the major 118A allele do not seem to respond at all.

The functional glutamate antagonist acamprosate is the second currently approved modern alcoholism pharmacotherapy. Its mechanism of action is less clear. Although meta-analyses of European studies solidly support the efficacy of acamprosate, American studies have not been able to replicate it. Animal data may be helpful in understanding this apparent discrepancy. Basic studies strongly suggest that a hyperglutamatergic state is progressively recruited in alcoholism. In animal models, acamprosate is only effective in subjects with a prolonged history of pronounced dependence. This appears to be consistent with the human data, since the European studies in which acamprosate was found effective enrolled patients with more severe disease than the American studies that did not.

In addition to the currently approved medications, initial positive data are currently available for the 5HT₃ antagonist ondansetron, the anticonvulsant topiramate, and several other experimental compounds. A particularly interesting category among these comprises compounds that target the negative affective states that maintain alcoholism in its later stages, such as antagonists for CRF1 or neurokinin-1 (NK1) receptors. As new target mechanisms are added over the next decade or so, there is considerable hope that effective, personalized alcoholism

treatments that are tailored to the genetics and pathophysiology of the individual patient will become possible.

See also: Animal Tests for Anxiety; Antisocial Substance Dependence; Brain Imaging and Addiction; Cellular Plasticity in Cocaine and Alcohol Addiction; Cognition: Attention and Impulsivity; Drug Addiction; Drug Withdrawal – Motivational View; Ethanol and Nicotine Interactions; Korsakoff's Syndrome; Molecular Neurobiology of Addiction; Neural Systems of Motivation; Stress and Drug Craving; Stress and Emotionality; Stress and Reward; Transition to Addiction.

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Animal Models of Behavior: Alcohol Addiction

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Glossary

Alcohol reinforcement – In its reinforcing capacity, alcohol increases the frequency of preceding responses, and, accordingly, is called a reinforcer. Alcohol is a primary reinforcer and leads to self-administration.

Allostatic state – A state of chronic deviation of a regulatory system from its normal (homeostatic) operating level is defined as an allostatic state. In the context of drug addiction, this term has been introduced by George Koob and Michel Le Moal and represents a chronic deviation of reward set point by dysregulation of reward circuits and brain stress systems that provide a negative motivational state that drives addictive behavior.

Craving – There are opposing views in the field regarding the term ‘craving,’ whether it describes a physiological, subjective, or behavioral state, if it is necessary at all to explain addictive behavior or is an epiphenomenon which is not necessary for the production of continued drug use in addicts. The World Health Organization (WHO) agreed on the definition of craving as “the desire to experience the effect(s) of a previously experienced psychoactive substance.” Behavioral researchers conceptualize craving within the framework of incentive motivational theories of behavior and modify the definition of craving as “incentive motivation to self-administer a psychoactive substance.” Such an operational definition of craving has the advantage of making the phenomenon of craving accessible to experimental investigation and making it measurable.

Reinstatement – The reinstatement model is the first choice for the measurement of craving and relapse behavior. In this paradigm, animals are trained to self-administer a drug and are then subjected to extinction – that is, they are tested under conditions of nonreinforcement until operant responding appears to be extinguished. When the animals reach some criterion of unresponsiveness, various stimuli are presented. A stimulus is said to reinstate drug-seeking behavior if it causes renewed responding, that is, lever pressing, without any further response-contingent drug reward. At least three conditions can reinstate responding: drug priming – that is the injection of a small dose of the drug, stress, and conditioned stimuli.

Tolerance – Tolerance has developed when, after repeated administration, a given dose of a drug produces

a decreased effect. It is important to note that tolerance does not only develop with alcohol and other drugs of abuse but also after repeated administration with a wide variety with drugs that are not self-administered by animals or used compulsively by man.

Alcohol Drinking in Laboratory Animals

In 1940, Curt Richter observed that laboratory rats consume alcohol on a voluntary basis. In other words, if a water bottle and a bottle with an alcohol solution are presented to the home cage, rats will consume alcohol and some animals will even prefer alcohol over water. The large variability in alcohol preference among individual animals and strains has allowed researchers to selectively breed rats for differential alcohol preference, generating pairs of animal strains that are characterized by particularly low or high alcohol consumption levels. The best-studied pairs of lines were generated in Finland, the United States, and Sardinia. For example, the Finnish model – called Alko Alcohol (AA) and Alko Nonalcohol (ANA) rats – comprises two strains of albino rats based on their selection or rejection of a 10% alcohol solution and water. The AA rats were selectively bred starting in 1963 and voluntarily consume more than 5 gram alcohol per kilogram body weight per day ($\text{g kg}^{-1} \text{ day}^{-1}$), attaining high blood alcohol concentrations, whereas the ANA rats consume less than $0.5 \text{ g kg}^{-1} \text{ day}^{-1}$ of alcohol. Alcohol-preferring rat lines and drinking experiments in a laboratory setting were extremely helpful in obtaining a solid knowledge about the genetic factors modulating initial alcohol consumption and the neurobiological underpinnings of primary alcohol reinforcement processes. However, the question remains whether voluntary alcohol consumption in rodents in a laboratory setting is translatable to the human condition. This might well be the case as chronic high consumption of alcohol already occurred early on in primate evolution. It is suggested that the trait of alcohol consumption is actively maintained during evolution and should therefore be considered as part of the behavioral repertoire of mammals. Thus, data deriving from alcohol-drinking experiments in rodents in a laboratory setting may well translate into the human condition as these animal models show strong face validity. Moreover, because alcohol

reinforcement is mediated by brain structures that have been strongly conserved during evolution (i.e., subcortical structures), rodent-drinking models have also a good construct validity and, therefore, potential for further elucidating the neurobiological basis of alcohol consumption and alcohol reinforcement processes in humans.

A major limitation of these drinking models, however, is that alcohol preference alone does not necessarily indicate addictive behavior but often reflects controlled alcohol consumption. For example, animals from an alcohol-preferring strain have a high alcohol preference but do not meet important criteria of addictive behavior, such as loss of control over drinking. Thus, the animals' alcohol intake decreases dramatically when they are offered alternative diets augmented with sugar. Furthermore, the close correlation of food and alcohol consumption, and the occurrence of alcohol consumption at normal times in the circadian cycle demonstrate that alcohol intake is controlled as part of normal behavior. In contrast, the main symptom of alcohol addiction is the progressive loss of control over the amount and the contextual circumstances accompanying alcohol use. This loss of control results not only in higher alcohol consumption, but also in a compulsive search for, and inability to refrain from, its use even after long periods of abstinence (relapse), especially when the individual is exposed to stimuli previously associated with alcohol consumption. Compulsive alcohol drinking can pervade all life activities of the consumer. As a consequence, life becomes governed by alcohol and the addicted patient can subsequently lose social compatibility (e.g., loss of partner and friends, loss of job, and crime).

According to this definition of alcohol addiction, and in contrast to controlled alcohol drinking, addiction is a behavioral disorder that seems to appear exclusively in humans. This conclusion raises a very important question: If addiction does not appear in animals living in their naturalistic environments, can these animals be appropriate subjects to reproduce alcohol addiction in a laboratory setting? Although addiction can hardly be reproduced as a whole in an experimental situation, specific features of addictive behavior can be adequately modeled. In particular, different phases of long-term alcohol consumption can be consistently observed in laboratory animals.

From Controlled Drinking to Alcohol Addiction

Large epidemiological data sets on alcohol-drinking behavior led to an alcohol-specific symptom progression model for alcohol addiction, describing transition probabilities from one phase to another (nonuse, use, heavy use, abuse, and addiction) in relation to biological, psychological and social vulnerability, and risk factors.

Similarly to the human condition, alcohol drinking in rats over a long time period can be also separated into different phases. The acquisition of alcohol drinking is the first phase, followed by a second phase of controlled alcohol-drinking behavior. As described, most animal work in the past has focused on the acquisition of alcohol drinking or the maintenance of an established controlled alcohol-seeking behavior. A third phase, in which uncontrolled excessive alcohol-drinking behavior occurs, relates to addictive behavior in humans and has been intensively studied in recent years. In this phase, positive reinforcement processes become less important. There is a shift from liking to wanting alcohol as habit-forming properties and opponent motivational processes – mainly triggered by acute, protracted, and conditioned withdrawal – come increasingly into play. Subsequent allostatic dysregulation of the reinforcement system may then occur. One animal model which captures these different drinking phases is the long-term self-administration procedure with repeated deprivation phases.

An Animal Model to Study Different Phases of Alcohol Consumption

In the long-term alcohol self-administration procedure with repeated deprivation phases, Wistar rats receive, in addition to food and tap water, different concentrations of ethanol solutions *ad libitum* in four bottles per cage (5%, 10%, and 20%, reflecting alcoholic beverages consumed by humans such as beer, wine, and spirits). A four-bottle paradigm has the advantage of overcoming initial preference problems. Rats usually prefer lower-concentration alcohol solutions (<6%) over higher-concentration alcohol solutions. Following a period of taste adaptation, a shift toward preference for higher concentrations of alcohol solutions is observed. Furthermore, individual sensitivities and preferences to alcohol solutions are usually observed. The free-choice presentation of various concentrations of alcohol solutions bypasses the problem of individual preferences – in this model, a rat is allowed to drink what it likes most. Indeed, in a four-bottle paradigm, high alcohol intake and preference in common stock rats are observed during the acquisition of alcohol-drinking behavior in male as well as in female rats. After 2 months of continuous access to alcohol, the rats are deprived of alcohol for 3 days. Following this deprivation phase, all alcohol solutions are presented again. This procedure is repeated monthly for the following 10 months. The introduction of repeated deprivation (withdrawal) phases for several days/weeks is crucial in developing an addictive behavior, as the negative consequences of acute, protracted, and conditioned withdrawal triggers further drinking and induces relapse behavior.

The alcohol deprivation effect

Following a deprivation phase, re-presentation of the alcohol solutions leads to a pronounced transient rise in alcohol intake and preference. This is termed the alcohol deprivation effect (ADE). This relapse-like drinking phenomenon is observed across several species, including rats, mice, monkeys, and human social drinkers. The increase in alcohol drinking probably reflects an increase in alcohol-seeking, which, according to self-reports of some alcohol-dependent subjects, can also increase progressively during abstinence and decrease after relapse during a drinking bout. In summary, ADE in long-term voluntary alcohol-drinking rats is used as a measure of high motivation to drink alcohol and as a measure of relapse-like behavior.

In this long-term drinking model, changes in alcohol-drinking behavior occur over time. During the first days of alcohol exposure, male rats have a high daily consumption of around 6 g kg^{-1} and an alcohol preference of 60%. Overall, female rats consume greater amounts of alcohol than male rats. Such a sex difference is also seen in other species such as mice and monkeys. At first glance, this appears to be in stark contrast to observations in humans, since epidemiological and clinical studies demonstrate that women consume less alcohol than men. However, if alcohol intake in humans were to be calculated on a g kg^{-1} base instead of the number of drinks consumed, consumption in females would be much the same or even more than that in males. Thus, contrasting sex differences in humans and animals are mainly related to social barriers in different populations and to an artifact in calculating exact alcohol intake.

After this short initiation phase, large daily fluctuations in drinking behavior are observed though, over a period of months, there is a clear tendency for a decline in alcohol consumption, resulting in a stable average daily intake of between and 4 g kg^{-1} alcohol. In the first 8 weeks of the acquisition phase of alcohol drinking, there is a clear sequence in preferences for the different concentrated alcohol solutions: $5 >> 10 > 20\%$. However, there is a change in this sequence from week 9 onward to $5 < 10 \leq 20\%$. This change in preferences coincides with the introduction of the first alcohol deprivation period and this relation remains stable for up to 1 year. Alcohol-drinking behavior during this time can be regarded as controlled (phase of maintenance). However, following repeated ADEs, alcohol-drinking behavior can become uncontrolled and compulsive. Uncontrolled drinking behavior can be assessed by the adulteration of the alcohol solution with quinine, thus altering its taste. In this experiment, quinine is added to the alcohol solution, but not to the water. Quinine is a very bitter tasting substance that usually produces a strong taste aversion in rats. Despite the aversive taste, however, the long-term alcohol-drinking rats consume large amounts of the quinine-

containing alcohol solution following a deprivation phase. In fact, alcohol intake and preference and the time course of the ADE of quinine-exposed animals are similar to those of control animals that have had the same experimental history and which have received unadulterated alcohol. In long-term alcohol-drinking rats, alcohol intake following a deprivation period is thus relatively resistant to modification by taste adulteration, that is, drinking behavior becomes compulsive and uncontrolled. In addition, pronounced changes in the diurnal rhythm of drinking activity are observed following alcohol deprivation in chronic drinking rats. In particular, most of the animals still show high-drinking activity during the inactive phase, and some animals even show no differences in drinking activity during the dark and light phases of the daily cycle. Such a level of drinking activity is far beyond the normal controlled behavior seen in the appropriate control animals, and points to alterations in circadian rhythmicity.

In summary, alcohol consumption behavior following long-term consumption and subsequent deprivation is characterized by changes in the alcohol-intake patterns of animals. The animals consume not only more alcohol, but also large amounts of highly concentrated alcohol solutions at inappropriate times during their daily cycle in an uncontrolled and compulsive manner, that is, during the light phase when the animals are normally inactive and drinking activity is low. Finally, the fact that the clinically effective antirelapse drugs acamprosate and naltrexone reduce or even abolish the ADE lends predictive value to this animal model for the development of novel and improved drugs for the treatment of craving and relapse.

Dependence-Induced Alcohol Drinking – A Procedure that Leads to Allostatic Dysregulation of the Reward System

George Koob and Michel Le Moal describe the development of addiction as an allostatic dysregulation of the brain reward system. They suggest that chronic alcohol intake induces counteradaptive processes within the reward system that fail to return within the normal homeostatic range resulting into an allostatic state of anhedonia. Thus, the allostatic state represents a chronic deviation of reward set point. This state is not only caused by dysregulation of reward circuits, but also by the activation of brain and hormonal stress responses.

In order to induce an allostatic dysregulation of reward function in animals, repeated alcohol vapor exposure is used. In rodents, chronic intermittent alcohol vapor exposure (14 h ON/10 h OFF) for 8 weeks leads to long-lasting neural and behavioral plasticity. This type of manipulation produces persistently increased alcohol intake in genetically nonselected rats. Similar effects are also

observed in alcohol-preferring rat strains. For example, if alcohol-preferring rats are trained to respond for a 10% alcohol solution in an operant situation, then are exposed to chronic intermittent alcohol vapor for 8 weeks, and subsequently tested for operant alcohol responding at multiple time points during acute alcohol withdrawal and protracted abstinence (1–15 days), persistent augmented alcohol responding will be observed. In conclusion, exposure to repeated cycles of intoxication and withdrawal, which mimics the course of the clinical condition, is most effective for inducing increased alcohol drinking. Examination of drinking during protracted abstinence allows for complete dissociation of the somatic and motivational aspects of alcohol dependence, as well as separation of their respective effects on dependence-induced increases in alcohol drinking. Similar to the human condition, a minimum duration of dependence is required for lasting upregulation of alcohol preference. Importantly, elevated alcohol intake in post-dependent rats is sensitive to the clinically effective compound, acamprosate, while alcohol intake of nondependent rats is unaffected by the same treatment. Furthermore, the post-dependent state is characterized by a persistently upregulated behavioral sensitivity to stress. Together, these findings indicate that neuroadaptive processes induced by a prolonged exposure to cycles of intoxication and withdrawal parallel those in alcohol-dependent patients and will therefore be helpful for preclinical medication development.

An Animal Model to Study Alcohol-Seeking Behavior

The most common procedure to study alcohol-seeking behavior – which can be considered as the motivational component of alcohol craving – is the reinstatement model. The origin of the reinstatement model can be traced to some findings of Pavlov and Skinner, but its application in the context of drug abuse research did not appear until 1970. The first report using this procedure, as it is now actually understood, was published in the classical paper by de Wit and Stewart in 1981 and, over the last 10 years, the number of studies referring to the use of this paradigm has grown exponentially. From a procedural point of view, it is clear that any assessment of reinstatement needs to first instate the self-administration behavior to an adequate level, followed by an alcohol-free period (i.e., extinction) after which the resumption of the extinguished behavior in response to a specific trigger will be tested. These three phases can be implemented in a single (within-session design) or, more often, several experimental sessions. It is usually an operant procedure, whose schedule of reinforcement is generally a low fixed ratio (i.e., FR1) that regulates alcohol delivery and, very often, the appearance of an initially neutral stimulus (i.e.,

light). After this self-administration period, reinstatement procedures usually include an extinction phase (i.e., allowing the subject to perform the operant response, without programmed consequences) rather than an abstinence process. This procedure imposes a reduction of the face validity of the model, since rarely do humans undergo extinction, although no systematic studies have been conducted to assess its possible consequences on the construct or predictive validity of these methods. Finally, the re-emergence of this behavior in response to a specific trigger is conducted under an alcohol-free condition. This allows for studying the recovery of the extinguished behavior without the interference produced by the psychoactive effects of the drug, and the increase in the number of operant responses (compared to that observed during extinction) is understood as an enhancement of the subject's alcohol-seeking behavior. However, this factor also distinguishes preclinical reinstatement procedures of human relapse episodes. In fact, in these methods, the operant response is reinstated but the subjects, *sensu strictu*, do not relapse because they actually do not resume alcohol consumption.

In humans, alcohol priming, negative mood states, and stress or drug-associated cues are able to produce an increase of self-reported craving. Similarly, reinstatement of alcohol-seeking behavior in rodents can be induced by a small quantity of alcohol. This phenomenon is consistent with the widely reported description of the first-drink phenomenon by which ingestion of a small amount of alcohol may induce a strong subjective state of craving in abstinent alcohol-dependent subjects. This priming effect can even occur in alcohol-dependent subjects who have been abstinent for years. Stress caused by intermittent mild electric shocks to the animals' feet as well as alcohol-associated olfactory cues can also reinstate previously extinguished responding for alcohol. Data derived from studies using the reinstatement model suggest that the neuronal substrates mediating alcohol-, stress-, and cue-induced reinstatement are not identical. For example, naltrexone reduces cue-induced reinstatement of alcohol-seeking behavior whereas stress-induced reinstatement is not affected by this drug but can be blocked by CRH1 receptor antagonists. Furthermore, foot-shock stress and response-contingent presentation of an alcohol-associated light cue, acting as a conditioned stimulus also augments reinstated responding. Thus, additive effects of these stimuli on responding are observed, which demonstrate that more than one neurobiological pathway is involved in provoking alcohol-seeking behavior.

The reinstatement model of alcohol-seeking behavior is now a well-established model in rats, and has recently been replicated in mice. However, some methodological transfer problems from rats to mice have to be considered: operant tasks have to be achieved by the individual during the course of a reinstatement experiment and usually

rats acquire alcohol-directed behavior more easily than mice. The first goal which has to be achieved by an individual in the reinstatement procedure is selective responding on a reinforced lever. Usually an easy task for a rat; for mice, due to higher motor activity, it is more difficult to achieve this task. Another confounding variable in some mice strains is that lever pressing is reinforcing *per se*. The same problem might also relate to nose-poking. Thus, whether a reinforcer follows a lever press/nose poke or not, it might not influence further behavior of the mouse. Despite the problems of high-motor activity and reinforcing effects of lever pressing/nose poking, mice usually acquire selective responding under a simple FR1 for the drug although on the control lever – the nonreinforced lever – a higher number of responses is seen than in rats. The next task which has to be achieved is extinction of lever responding which means that responding is without any further consequence and the individual does not receive the drug anymore. Rats usually show a short burst of responding and then responding gradually declines over days. Usually following 4–6 days rats show only some spontaneous responses; however, this occurs on a low rate. Extinction in mice seems to be more difficult and one needs more extinction days compared to rats; again much higher rates of spontaneous responding are seen and even if a satisfying degree of extinction is achieved, spontaneous recovery of responding often occurs. In the third task, reinstatement of alcohol-seeking behavior has to be elicited. Using the reinstatement paradigm, only a few studies with mice have been reported so far. However, in the future, this paradigm will be frequently used to study conditional knock-out mice in order to pin down precisely the genes and brain sites involved in alcohol-seeking behavior.

In conclusion, the reinstatement model can be used to study the neurobiological and molecular basis of alcohol-seeking behavior since there appears to be a good correspondence between the events that induce this behavior in laboratory animals and those that provoke craving in humans. Furthermore, naltrexone is known to reduce craving in alcohol-dependent patients and can also reduce or even block cue-induced reinstatement of alcohol-seeking behavior. Nevertheless, the usefulness of the reinstatement model in mimicking craving in humans has some limitations. First, the phenomenon of craving is complex. Thus although the operational definition of craving as incentive motivation to drink alcohol has the advantage of making the phenomenon of craving measurable, such a definition neglects the fact that craving also has a subjective dimension that is difficult, if at all possible, to assess in laboratory animals. Second, it appears that extinction of alcohol-seeking behavior usually plays only a minor role in alcohol-dependent patients trying to achieve and maintain abstinence. With the exception

of patients undergoing focused extinction therapy, alcoholics generally try to avoid exposure to external alcohol cues during abstinence. In most cases, alcoholics stay abstinent for a while but may experience craving and subsequent relapse if they are re-exposed to external cues (e.g., the smell of alcohol), particularly if they are in a vulnerable internal state. Consequently, the animal reinstatement procedure may not accurately reflect the situation of abstinent alcoholics experiencing craving.

Conclusions

In summary, the last decade has witnessed advances in the field of alcohol research with the development of new animal models mimicking core features of an addictive behavior. The validity of animal models is typically assessed using three evaluation criteria, including face, construct, and predictive validity. Reliability is also a critical issue in complex animal models. At the present time, the reinstatement and alcohol-deprivation paradigms are the models for which these issues have been addressed most systematically. Another animal model in which excessive drinking occurs following a history of dependence is used by several laboratories to study the consequences of allostatic dysregulation of the reward system and, thereby, the neurochemical substrates of the addicted brain. Considerable work remains to be done to establish whether measures obtained in these and other models are valid and reliable. The refinement of these animal models and the characterization of specific reliable phenotypes within these models is a challenging process that requires a multidisciplinary research approach, involving collaboration between experimental and clinical psychologists, clinicians and, of course, the patients themselves. Nevertheless, these models can already be used to study the neurobiological foundation of alcohol craving, relapse, loss of control, and alcohol intake despite the negative consequences.

See also: Animal Models of Learning and Memory; Animal Tests for Anxiety; Cognition: Learning and Memory: Pavlovian; Drug Addiction; Feeding; Genes and Behavior: Animal Models; Knock-Outs: Learning and Memory; Motivation; Mouse Genetic Approaches to Psychiatric Disorders.

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Antisocial Substance Dependence

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Glossary

Adult antisocial behavior (AAB) – A behavioral syndrome in which a person meets the other diagnostic criteria of the *Diagnostic and Statistical Manual of Mental Disorders Fourth Edition* (DSM-IV) for antisocial personality disorder, but not the criterion requiring the presence of conduct disorder before age 15.

Anterior cingulate cortex (ACC) – A region of the medial surface of the cerebral cortex.

Antisocial personality disorder (ASPD) – DSM-IV defines this condition as “A pervasive pattern of disregard for, and violation of, the rights of others that begins in childhood or early adolescence and continues into adulthood.”

Antisocial substance dependence (ASD) – This term may name a categorical diagnosis, combining ASPD and substance dependence, or it may name the extreme end of an externalizing trait of behavioral disinhibition.

Attention deficit/hyperactivity disorder (ADHD) – A behavioral disorder characterized by excessive inattention and/or a combination of impulsivity and hyperactivity that begins in childhood.

Cholinergic receptors (CHRs) – A family of proteins that alters the activity of cells in response to acetylcholine or related chemicals.

Conduct disorder (CD) – According to DSM-IV, this condition of children and adolescents is “a repetitive and persistent pattern of behavior in which the basic rights of others or major age-appropriate societal norms or rules are violated.”

Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (Text Revision) (DSM-IV TR) – A widely used manual of psychiatric diagnoses published by the American Psychiatric Association, Washington, DC, in 2000.

Functional magnetic resonance imaging (fMRI) – A powerful technique for producing pictures that identify regions of the brain involved in processing cognitive or motor tasks.

Genome-wide association studies (GWASs) – Studies aiming to find unique DNA structures that contribute to a particular trait or disorder by examining

DNA structure across the whole genome, comparing DNA in large groups of persons who do, versus those who do not, have the trait or disorder.

Lateral prefrontal cortex (LPFC) – Usually divided into dorsolateral (upper lateral) prefrontal cortex and ventrolateral (lower lateral) prefrontal cortex, it lies on the right and left surfaces of the front of the brain.

Magnetic resonance imaging (MRI) – A powerful technique that can produce detailed pictures of brain structure. The technique allows the comparison of brain structure in persons with, or without, a particular trait or disorder.

Multisystemic treatment (MST) – One of several family and community treatments that seek to strengthen multiple domains of functioning in the lives of antisocial youths by involving the entire community (i.e., school, family, church, neighborhood, peers, and courts) to modify interactions with the youth to promote and sustain prosocial behavior.

Nucleus accumbens (NAcc) – These paired deep-brain structures are way stations in the brain reward system that responds to reinforcers. Through the mechanism of changing dopamine release, NAcc is in a key position to recognize changing contingencies and to guide behavioral change.

Orbitofrontal cortex (OFC) – It lies at the inferior front of the brain just over the eyes. Different sections of OFC, working closely with ACC, have roles in stopping (inhibiting) some risky responses; in monitoring, learning, and remembering the value of reinforcers and punishers; and in assessing the future consequences of behavior.

Posterior medial frontal cortices (pMFCs) – Two regions of cortex that face one another, each forming a part of the middle wall of a hemisphere. Included in the right and left pMFC are the right and left dorsal ACCs. The pMFCs help to detect response conflict.

Substance use disorders (SUDs) – In DSM-IV, SUDs include substance abuse and substance dependence, two related diagnoses that both involve continued use of substances despite life problems from those substances.

Definition, Comorbidity, and Clinical Course

The term ‘antisocial substance dependence (ASD)’ may have two meanings. First, it names a categorical diagnosis. Categorical disorders are discrete; each person falls into one of two categories – they do or do not have the disorder. ASD also may imply the severe end of an externalizing dimensional trait that is called behavioral (or neurobehavioral) disinhibition. Dimensional traits are continuous; each person lies somewhere on a continuum of severity ranging from trait absent to trait very severe.

ASD as a Categorical Diagnosis

In this sense, ASD is the co-occurrence within an individual of serious antisocial behavior and serious substance use disorder. This term is not used in the standard psychiatric nomenclature, the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV).

ASD's antisocial component

DSM-IV does include the antisocial diagnoses ‘conduct disorder’ (CD) for children and adolescents, and ‘antisocial personality disorder’ (ASPD) for adults. Most persons with ASD would receive one of these diagnoses. To receive the ASPD diagnosis one must have had CD before age 15. According to a recent large national survey, ASPD affects about 5.5% of adult male Americans, and about 1.9% of female Americans. Fortunately, not all youths with CD develop ASPD in adulthood, but of the 4.7% of adults who reported having met the diagnostic criteria for CD in childhood or adolescence, about three-fourths did develop ASPD.

Studies indicate that antisocial problems developing early in childhood are more likely to persist into adult ASPD. Among persons with ASD, the conduct problems usually antedate the substance problems, sometimes appearing as early as ages 3–6 years. Compared with others, these children often are excessively impulsive, exploratory, excitable, curious, and distractible, with reduced cautiousness, fearfulness, shyness, and inhibition. Such early antisocial characteristics often antedate, and lead adolescents into, extensive illicit drug use. Persons developing such early-onset substance use disorders (SUDs) tend to have early antisocial problems as well, while those developing substance problems in their third or fourth decades of life tend to have less severe antisocial problems.

ASD's substance component

In addition to an antisocial diagnosis, most persons with ASD would also be diagnosed with an SUD. DSM-IV includes two SUDs: substance dependence and substance abuse; either can be fatal, but substance dependence usually is the more serious condition. Therefore, we focus here on

the comorbid association of antisocial problems with substance dependence. A recent national survey estimated the ever-in-your-life prevalence of substance dependence among adult Americans to be 12.5% for alcohol dependence and 2.6% for other substances (excluding nicotine, for which dependence is much more common).

Often, in fact, persons with ASD receive several diagnoses. One person, for example, might have DSM-IV diagnoses of ASPD, nicotine dependence, cannabis dependence, and methamphetamine dependence. However, despite such multiple, co-occurring DSM diagnoses, the combined antisocial and substance problems *per se* warrant clinical and research attention because of their comorbid prevalence (discussed next), and because they may share a common etiology (discussed below).

The comorbid prevalence of antisocial and substance use disorders strongly argues for considering them together. For example, alcohol dependence is about 8 times more prevalent among persons with ASPD than among the general population. For other drugs, such as stimulants, opioids, or marijuana, dependence is 17–23 times more common among those with ASPD, compared with persons in the general population. Clearly, these disorders co-occur far more frequently than would be expected by chance alone. This strong comorbidity of antisocial and substance problems holds true in both sexes, and in Asian as well as Western countries. It also holds among younger persons. A large survey of adolescents in the Great Smoky Mountain region found that (in comparison with other youths) boys were about 6 times, and girls about 30 times, more likely to have SUD if they had CD.

Additional comorbid disorders

In addition to their antisocial and substance use disorders, persons with ASD often have other comorbid disorders. Thirty to fifty percent of adolescents with substance dependence and CD also have attention-deficit/hyperactivity disorder (ADHD), and the reverse also is true. Compared with youths in the general population, those with CD are about 24 times more likely to have ADHD. Furthermore, many youths with ASD, having experienced significant abuse, neglect, or other trauma, suffer from posttraumatic stress disorder. Finally, major depression is common in adolescents with ASD.

ASD as a Dimensional Trait

Many clinicians and researchers productively consider ASD to be the severe end of an absent-to-very-severe dimensional continuum of combined impulsive, risk-taking antisocial, and drug-using behaviors. For example, one research group examined the number of adolescent CD symptoms reported by a large sample of

American adults; many had no CD symptoms, others had a few, and some had many. The range of those adolescent symptom counts associated very significantly with the number of different substances that, by adulthood, had caused problems for the subjects; more symptoms of adolescent CD predicted more drug involvement.

Many researchers have studied adult antisocial behavior (AAB), a syndrome consisting of the adult symptoms of ASPD, but with no history of childhood CD. A large national survey found that over 12% of adult Americans meet those criteria, and SUD is about as common in them as in ASPD. Since they do not have ASPD, they could not qualify for a categorical diagnosis of ASD, as described above. However, combined antisocial and substance problems would place many of them at the severe end of a dimensional trait of ASD.

Risk-taking, behavioral disinhibition, and ASD

Risk-taking is a behavior that unpredictably may result in either, or both, reinforcing or punishing outcomes. Risk-taking in competitive sports, high-altitude mountaineering, or stock day trading is not pathological and may be adaptive. However, when the possible punishing outcomes are very severe or endanger others, continued risk-taking is pathological, and tends to be associated with substance dependence.

Extensive substance use is itself a risk-taking behavior that often produces severe, punishing outcomes. Abused drugs do produce rewards by directly activating a brain reward system, but with uncertain street doses, those rewards are unpredictable. Drugs also unpredictably may result in severe punishments, such as overdoses, arrests, school expulsions, psychosis, seizures, cerebrovascular pathology, compulsive drug seeking, or withdrawal.

Antisocial behaviors can also result in either reinforcing or punishing outcomes, or both, for the perpetrator, often harming others as well. Risk-taking actually is a central characteristic of CD and ASPD, with many of the defining symptoms of those disorders being risk-taking behaviors: stealing, fire-setting, break-ins, truancy, reckless disregard for safety, etc. Patients describe such behaviors as exciting, but doing them requires considerable disregard for potential adverse consequences. For example, early-onset sexual intercourse, with possibly major adverse consequences, is much more common among adolescent girls with CD than among others. Antisocial persons' risk-taking disposition also makes them more likely to try available substances, and less likely to abstain when adverse effects arise, thus enhancing their vulnerability to SUD.

So risk-taking is one important component that ties together antisocial and substance problems. However, this three-cornered relationship is complex, because substances themselves may induce greater risk-taking. Even

when abstinent, adults and adolescents with antisocial and substance problems do take more risks than others in standardized laboratory tests, but intoxication with either alcohol or marijuana further increases risk-taking in such tests.

Summary

Persons who had antisocial, aggressive, and hyperactive behaviors in early childhood are quite likely to develop adolescent CD and adult antisocial problems, usually with serious SUD. Those, whose problems begin before age 10, especially if they later developed ASD, have an especially unfavorable prognosis. Indeed, many adolescents with CD will have persisting antisocial problems for decades. Mortality rates among such adolescents considerably exceed those of their age-mates. ASD, the combination of serious antisocial problems and substance dependence, comprises a profound, devastating, often persisting, life-threatening condition.

Etiology

Contributions of Genes

The strong comorbidity of SUDs and antisocial disorders in ASD apparently is no random accident. Several research groups have used CD symptom counts, symptom counts of other childhood disorders, and various psychological measures (including novelty seeking) to define a latent psychological trait that may underlie this comorbidity. They variously have called this an externalizing trait, behavioral disinhibition, or neurobehavioral disinhibition. Research shows that high neurobehavioral-disinhibition scores at ages 12–14 strongly predict SUD in early adulthood.

Persons with ASD, having many antisocial and substance problems, comprise the severe end of a behavioral disinhibition continuum, and genes contribute to this continuum. For example, early adoption studies showed that adoptees whose biological parents had antisocial and substance problems were at increased risk to develop similar problems. Moreover, alcohol problems in the biological families predicted increased nonalcohol drug use in the adoptees. These probably are genetic effects, since biological parents determine an adopted child's genetic make-up, but have little impact on the environment in which the child is raised.

Two research groups found that behavioral disinhibition was very strongly heritable (i.e., heavily influenced by genes); genes accounted for about 80% of the variance among individuals on this underlying trait. By contrast, studies that separately consider antisocial disorders or SUDs find that genes contribute only about half of the

variance for the individual disorders. Another group studied SUD, CD, and AAB in young adults using a gambling task and found that insensitivity to future consequences was related to the severity of the combined scores for the disorders, a disinhibitory dimension, but not to the severity of any one disorder. Similarly, a twin study found evidence for a single externalizing genetic influence (which could be multiple genes working together) on SUD, CD, and AAB.

After studying antisocial and substance disorders, Hicks and his colleagues concluded that, “parents pass on to the next generation . . . a general vulnerability to a spectrum of [these] disorders, with each disorder representing a different expression of this general vulnerability.” Dick and her associates agree, stating “that shared genetic factors influence a spectrum of externalizing disorders, including alcohol dependence, illicit drug dependence, conduct disorder, antisocial behavior, and disinhibitory personality traits.” However, although behavioral disinhibition appears to be highly heritable, researchers do suspect that several genes are involved, each contributing only a small effect.

Several genes have been suggested to play a role in antisocial and substance problems. Acetylcholine-related CHRM2 may contribute to an externalizing trait, and perhaps specifically to alcohol dependence. Other acetylcholine-related genes, CHRNa6 and CHRNb3, may be involved in nicotine dependence. In addition, GABRA2 and ADH4 are suggested as important in alcohol dependence. Newer genome-wide association studies (GWASs) are the most promising contemporary approaches for identifying genes influencing complex traits (such as ASD), traits influenced by the environment and by many genes, each with a small individual effect. GWASs require that several thousand individuals, some with the disorder and some community controls, provide both genetic samples and information on their behaviors (e.g., their antisocial and substance using behaviors). Several such studies currently are in progress, and it is widely hoped that complex statistical procedures will reveal relationships between specific genetic variants and the broadly externalizing behaviors of ASD, an approach already successful with more isolated disorders related to nicotine and alcohol use.

However, practical applications of ASD genetics remain for the future. Complex traits are aptly named, and identifying numerous genes, each with a small effect on the disorder, will not lead immediately to genetic or pharmaceutical interventions. Rather, GWASs findings will gradually shed light on the biological basis of these disorders, including chemical mechanisms and neural pathways. Only then will converging evidence suggest new interventions for the genetic factors contributing to these disorders.

Contributions of Environment

Environment also contributes to ASD. Among children adopted away at birth, in addition to genetic influences from biological parents, environmental factors present in adoptive families, most notably divorce and psychiatric disturbance, apparently contribute to increased drug abuse. In addition, adopted children raised in an antisocial environment are at greater risk for antisocial behaviors and SUD. Similarly, children with highly antisocial fathers tend to fare better when raised by a single mother, rather than by both parents. Furthermore, childhood maltreatment (abuse and neglect) apparently exacerbates adolescent ASD.

Gene–Environment Correlation

In addition to independent risks for ASD from genetic and environmental factors, a gene–environment correlation for ASD produces a double-whammy of risk factors. Some children are at high biological risk for ASD because they inherited deleterious gene variants from parents with ASD. Then, during the toddler years, the parents, often antisocial and substance dependent themselves, fail to effectively reward prosocial behavior or to punish non-compliant, aggressive behavior. The family models antisocial and substance-using behaviors, providing an environment that further enhances risks for the genetically at-risk child.

Neuroscience

The shared genetic influences on antisocial and SUDs, discussed above, presumably are mediated in the brain, which clearly is central to ASD’s behavioral abnormalities. Other articles in this encyclopedia extensively address neural mechanisms in drug seeking, drug reward, and drug withdrawal. However, our review suggests that a genetically influenced externalizing trait called behavioral (or neurobehavioral) disinhibition underlies the excessive risk-taking that leads persons with ASD to recklessly engage in sometimes-rewarding, but often punishing, antisocial and drug-using behaviors. Thus, we focus here on neural mechanisms that may contribute to a failure of restraint in persons with ASD.

One consistent brain finding in ASD concerns abnormal electroencephalographic (EEG) responses to infrequent oddball stimuli. The normal EEG response to such stimuli mainly occurs over the frontal cortex as a P300 wave. P300 is suppressed in adults with antisocial and substance problems, and even in their children not yet exposed to substances. Among nearly 1000 community adolescents, externalizing scores, constructed from symptom counts for CD, ASPD, and SUD, better predicted P300 suppression

than did symptom counts for any of the disorders considered alone. This indicates a strong relationship between the P300 brain response and the externalizing trait that underlies both the antisocial and substance problems of ASD.

Frontal lobe injuries produce a frontal disinhibition syndrome, with impairments of judgment, insight, and foresight. The behavior of persons with ASD often resembles that syndrome. Indeed, in a few case reports, youths with CD-like syndromes actually did suffer early frontal brain injuries. Brain imaging techniques, such as positron emission tomography, and especially magnetic resonance imaging (MRI) and functional magnetic resonance imaging (fMRI), now provide extraordinary four-dimensional images of the structure and neural activity of human brain. These techniques allow researchers to ask whether there are structural differences, or differences in neural activity, between the brains of living human beings who do, or do not, have ASD.

Growing evidence suggests that without brain injury even substance dependent or antisocial adults have subtle structural abnormalities in frontal lobes and their connections, perhaps contributing to flawed decision making, increased risk-taking, and ASD. Even youngsters with CD reportedly have reduced structural volumes of insula, amygdala, temporal lobes, hippocampus, and ventromedial cortex. Brain structure may affect brain function. For example, researchers recently measured the neurobehavioral disinhibition of young adolescents. Then, in an fMRI machine, the youths did a task that required occasionally inhibiting a movement of the eyes. Those youths with higher neurobehavioral disinhibition scores had significantly less frontal-lobe activation as they tried to inhibit the movement; their frontal lobes functioned differently.

Neural Equipment for Risk Decisions

Each person's world changes constantly, and a behavior that safely gained things of value yesterday may not do so today. Therefore, the brain must continually monitor ones' success at gaining reinforcers and avoiding punishers. A network of brain regions constantly monitors, anticipates, and weighs response conflicts and risky potential gains and losses (Figure 1). Persons with ASD excessively do risky antisocial and drug-using behaviors, pursuing reinforcers despite the danger of very harmful punishers. We now review evidence that substance-involved adults considering risky decisions show hypoactivity in the brain's risk-decision network. Our review above suggests that genes and life experience in the environment may influence this hypoactivity. In addition, the drugs consumed by persons with ASD may persistently, adversely affect brain function.

The posterior medial frontal cortices (pMFCs) comprise parts of the risk-decision network. These two

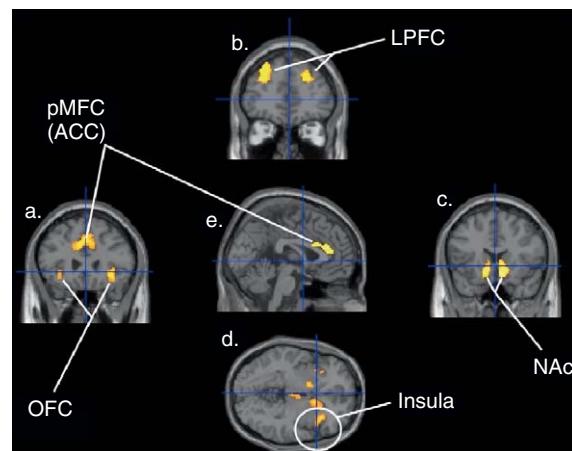


Figure 1 Functional magnetic resonance images showing group-average activation sites among adolescent boys as they worked on a cognitive risk-taking task. (a–c) coronal slices (as though viewed looking forward through the back of the head). (d) Axial slice (as though viewed looking down through the top of the head, forehead at right). (e) Sagittal slice through brain midline (as though viewed from right side of head). Abbreviations: ACC, anterior cingulate cortex; LPFC, lateral prefrontal cortex (shown is the dorsal, or upper, portion of LPFC); NAc, nucleus accumbens; OFC, orbitofrontal cortex (regions shown are in Brodmann Areas 47, considered part of OFC by most authors); pMFC, posterior medial frontal cortex, including ACC (which is activated here). See text for description of sites' functions.

regions of cortex face each other, each forming part of the middle wall of a hemisphere. Included in the right and left pMFCs are the right and left dorsal anterior cingulate cortices (ACCs). The pMFCs are critical for detecting response conflict (e.g., choosing whether to do, or not do, behaviors that could produce either rewards or punishments). The pMFCs also monitor behavioral outcomes (reinforcement and punishment), and based on that monitoring, the pMFCs signal other brain regions to adjust behavior to maximize reinforcement. People with ASD often persist with antisocial or drug-using behaviors that had been reinforced, but which later mostly earned punishment; they fail to adjust behavior normally. Among now-abstinent adults with substance dependence, the pMFCs are hypoactive during risk-taking tasks. Children and adolescents with antisocial problems and psychopathic traits show neural hypoactivity in the nearby ventromedial prefrontal cortex while doing a task requiring rapid strategy changes to earn rewards. These critical outcome-monitoring and behavior-adjusting regions apparently fail to operate normally among persons with antisocial and substance problems.

Orbitofrontal cortex (OFC), another part of the risk-decision network, lies at the inferior front of the brain just over the eyes. Different sections of OFC, working closely with ACC, have roles in stopping (inhibiting) some risky responses; in monitoring, learning, and remembering the

value of reinforcers and punishers; and in assessing the future consequences of behavior. OFC definitely malfunctions in substance-dependent persons, among whom several studies report structural gray-matter volume reductions in OFC, while functional studies find neural hypoactivity there.

Insula, an infolded, buried region of prefrontal cortex, is another part of the risk-decision network. In risky decision making it apparently evaluates recent, and estimates future, losses or punishments from risky behaviors. Neural activity of the insula correlates with the ambiguity, uncertainty, and complexity of risky choices, and the insula also is thought to adjust its assessment of risk when contingencies change. Boys with CD reportedly lack gray-matter volume in the insula, and the extent of that reduction correlates with their aggressive behavior and lack of empathy. Some researchers even suggest that the intensity of neural activity of the insula is an endophenotype (an internal biological marker) for a risk-taking personality, which is central to ASD.

NAcc, paired deep-brain structures, also participate in the risk-decision network. Way stations in the brain reward system that responds to reinforcers, the NAcc help drive repetition of whatever behaviors have gained reinforcement. All abused drugs activate that system, and that behavior-driving effect of the reward system is thought to underlie the compulsive, driven nature of substance dependence; drugs commandeer the reward system that normally drives people to repeat behaviors that gain valuable reinforcers, such as food, water, social contact, or sexual opportunity. The drugs' activation of the reward system similarly drives repetition of drug-using behaviors. When a new reinforcer is presented, NAcc activates. However, after repeated experience with the reinforcer, NAcc begins to activate in anticipation of the reinforcer, presumably responding to stimuli associated with the reinforcer (e.g., a sound announcing the imminent delivery of food). Moreover, NAcc responds more strongly to unexpected reinforcers, and deactivates when a reinforcer is expected but not delivered. Thus, through its principal mechanism of changing dopamine release, NAcc is in a key position to recognize changing contingencies and to guide behavioral change. However, among detoxified alcohol-dependent persons dopamine release in NAcc is profoundly attenuated, suggesting that for these people NAcc's ability to guide adaptive behavior change is impaired.

Lateral prefrontal cortex (LPFC, usually divided into dorsolateral prefrontal cortex and ventrolateral prefrontal cortex) lies on the right and left sides of the front of the brain. Subdivisions of the LPFC are involved in critical executive functions: inhibiting some behaviors; formulating conscious plans and intentions; regulating choices

among different actions, based on current environmental circumstances; directing attention to the most salient current stimuli; monitoring the contents of working memory to use memories in choosing behaviors; and using information stored in other brain regions to make active judgments. As currently drug-free, substance-dependent persons work on a risk-taking task, the right dorsolateral PFC, so crucial to planned behavior, is hypoactive. Curiously, however, the left dorsolateral PFC reportedly is overactive.

An Integrated Network

The above structures and others, probably including thalamus, caudate nucleus, putamen, cerebellum, and some cortex of the superior temporal gyrus, function as an integrated network to make choices about doing, or not doing, risky behaviors. In some regions of this network, persons with ASD have less gray matter than do normal controls. Moreover, among such persons several of these regions show functional hypoactivity on fMRI during decision making. These findings support the view that reduced neural activity in this network may underlie the excessive antisocial and drug-use risk-taking of persons with ASD.

Prevention and Treatment

Early Prevention-Intervention Strategies

ASD typically begins in childhood or adolescence, so early interventions have been widely studied. Such interventions fall into three major groups. First, parent management training is aimed at modifying the interactions between parent and child that may support antisocial and drug-using behaviors. Second, social-cognitive and problem-solving skills training aim to improve problem-solving skills, perceptions, self-statements, and self-attributions. In particular, these cognitive interventions help adolescents consider precursors, correlates, and consequences of their antisocial and drug-using behaviors. Of Dutch youths treated with these procedures for CD-like disorders as pre-teens, fewer (compared to a treatment-as-usual group) were found at a 5-year follow-up to be using drugs, and delinquency rates were similar to those of community controls. Third, for children at high risk, school-based interventions show considerable promise. A recent study followed children who, in first- or second-grade, had received an intervention rewarding teams of children for avoiding disruptive behaviors. When followed in early adulthood, students who had received the intervention (compared to those without it), especially boys who were very aggressive when beginning school, had significantly lower rates of ASPD and violent crime.

Psychosocial Treatments

Once CD and SUD develop in adolescence, family and community treatments, such as multisystemic therapy (MST), seek to strengthen multiple domains of functioning in the lives of the youth. These therapies aim to enlist the entire community (i.e., school, family, church, court, neighborhood, and peers) to modify interactions with the youth to promote and sustain prosocial behavior. Such interventions appear to be effective, although such short-term treatments may not adequately address the long-term needs of these youths and their families. For example, among juvenile offenders with SUD, a 4-year follow-up after MST (compared with treatment-as-usual) found in the MST group reduced aggressive crimes, no difference in property crimes, and a worse outcome on biological tests for substances.

For drug-dependent adults, many of whom have ASD, many studies document the benefit of several psychosocial treatments. However, an extensive review of that literature is beyond the scope of this article.

Medications

Many medications have been shown to improve outcome among nicotine, alcohol, and opioid-dependent persons. Strong evidence indicates that concurrent psychosocial treatments improve responses to such medications. Unfortunately, no medications have been shown consistently to improve antisocial disorders. Furthermore, although multiple medications may benefit other psychiatric disorders commonly comorbid with ASD, such as depression, anxiety disorders, or attention-deficit/hyperactivity disorder, such treatments do not appear to alter the course of the ASD itself.

Treatment or Punishment

The antisocial behavior of persons with ASD often involves them in the criminal justice system. Many serve prison sentences without effective treatment and without the on-site family and social supports that help to treat these disorders. However, some collaborations of the criminal justice and treatment systems show great promise. For example, specialized drug courts can deploy their coercive authority, giving offenders a choice: successfully participate in effective treatment or go immediately to jail. Although more research is needed, that imposed change in the reward–punishment equation appears to motivate more pro-therapeutic and prosocial decision making.

Summary

Antisocial and substance problems co-occur much more often than would be expected by chance. Genes strongly

influence the development of these disorders, with the same or closely linked genes contributing to both the antisocial and substance problems. Those genetic abnormalities presumably contribute to the brain-structure abnormalities found in children with CD, even before the children would have had much drug exposure. In addition, many of these youths are raised by antisocial and substance-involved parents in a vulnerability-enhancing environment. Abuse and neglect may further exacerbate their problems. Then, during adolescence, most youths with CD develop serious substance problems, apparently related to the risk-taking behavioral disinhibition that underlies both the antisocial and substance problems. The abused drugs may further adversely affect brain function. By adulthood, persons with ASD have significant neural hypofunction in several regions of a network that regulates risk-taking behavior, and that presumably contributes to the persistent high-risk antisocial and substance-using behaviors that characterize ASD.

The substance use and antisocial behavior of ASD appear to be different manifestations of a primary neurogenetic disorder that produces a trait of behavioral disinhibition, which in turn may be exacerbated by life experiences and by ingested substances. Environment apparently can make it worse (e.g., by gang involvement) or better (e.g., by treatment). Treatment clearly is effective in the short run. However, ASD treatments usually last, at most, a few months, and the underlying brain disorder still can be detected even during treatment. After treatment ends, the persisting brain disorder and its associated behavioral disinhibition presumably contribute to the observed high rates of relapse. Our review suggests that effective ASD treatment should not be episodic, with each brief episode aiming at a cure. Rather, ASD treatment, like effective diabetes treatment, should aim at lifelong management of this chronic disorder.

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See also: Alcoholism; Brain Imaging and Addiction; Brain Imaging; Brain Stimulation and Addiction; Molecular

Psychology of Personality; Vulnerability Factors in Addiction Disorders.

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Relevant Websites

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Brain Imaging and Addiction

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Glossary

Addiction – A chronic, relapsing brain disease that is characterized by compulsive drug seeking and use, despite harmful consequences. It is a brain disease that results from the long-lasting changes in brain neurochemistry and function that occur with repeated drug exposures in vulnerable individuals (because of genetics, development, or adverse environments). These brain changes are long lasting and persist for months after drug discontinuation, which accounts for the chronic nature of addiction. Abuse and addiction to alcohol, nicotine, and illegal substances cost Americans upward of half a trillion dollars a year, considering their combined medical, economic, criminal, and social impact. Every year, abuse of illicit drugs and alcohol contributes to the death of more than 100 000 Americans, while tobacco is linked to an estimated 440 000 deaths per year.

Dopamine (DA) – All drugs of abuse directly or indirectly target the brain's reward system by flooding the circuit with DA, which is a neurotransmitter present in regions of the brain that regulate movement, emotion, cognition, motivation, reward and prediction of reward, and learning (conditioning, habits). The overstimulation of this system, which rewards our natural behaviors, is associated with the euphoric effects sought by people who abuse drugs increasing the likelihood that they will repeat the behavior. It also associates the environment where the drug was taken with the drug itself (conditioning) so that when the individual is in the same environment DA will increase in the limbic brain in expectation of receiving the reward.

Mesocorticolimbic – A circuit of extended brain network that spans the midbrain (predominantly the ventral tegmental area) and the limbic system (the nucleus accumbens (NAcc), amygdala, hippocampus, hypothalamus, and the medial prefrontal cortex). The functional integration within this vast brain system, which relies heavily on dopaminergic transmission, contributes to or mediates complex behavioral outputs, such as response to emotional and rewarding stimuli, motivation, learning, memory, and higher cognitive function.

Positron emission tomography (PET) – The imaging technique that takes advantage of short-lived positron emitters used to label various compounds that are used

to measure the concentration of receptors, transporters, and enzymes in the human brain as well as to measure brain glucose metabolism and cerebral blood flow and to measure the distribution and movement of a drug.

Striatum – Dopaminergic cells, which are predominantly located in the ventral tegmental area (VTA) and the substantia nigra (SN) in mesencephalon send projections to various cortical (most dense in prefrontal cortex) and subcortical regions. The main projection is to the striatum, which receives innervation from SN (predominantly dorsal striatum) and the VTA (predominantly to the ventral striatum where the NAcc is located). All of the drugs of abuse increase DA in the NAcc (their mechanism by which they do it being distinct for the various classes of drugs) and this effect is believed to underlie their reinforcing effects.

Imaging the Relationship between Acute Dopamine Increases in the Human Brain and Drug Reinforcement

One of the major roles of dopamine (DA) is to optimize memory, learning, motivation, and attentional processes along the mesocorticolimbic axis. Addictions involve profound disruptions in these cognitive domains, and it is not surprising that some form of DA dysregulation can be found in substance use disorders. Indeed, the vast majority of addictive drugs has the ability to rapidly increase extracellular DA levels in nucleus accumbens (NAcc), a key structure of the limbic system. These DA surges resemble but greatly surpass the physiological increases that are triggered by the phasic DA cell firing that conveys information about saliency, reward, and reward expectation.

Human brain imaging studies have been mostly consistent with the notion that drug-induced increases in DA in the dorsal and ventral striatum (the location of the NAcc) are closely linked to the subjective experience of reward or euphoria. However, as drug use continues, the repeated firing of DA cells begins to upset the balanced neurochemistry required to support plastic changes in associative learning circuits, facilitating the consolidation of maladaptive memory traces that are connected to the drug. These can then trigger the firing of DA cells upon

exposure to any number of contextual stimuli that happen to be associated with the drug (in expectation of the reward). In addition, due to DA's role in motivation, its increased association with the drug-cues, or with the drug itself, is also likely to modulate the drive to secure the reward.

This better understanding of DA's multiple roles in the reinforcement process has resulted in a more coherent model of drug addiction, according to which drugs are reinforcing not only because they are pleasurable but also because, by increasing DA, they are processed as salient stimuli that will inherently motivate the procurement of more drug (whether the drug is consciously perceived as pleasurable or not). This model continues to evolve, largely thanks to the increasing use of sophisticated brain imaging techniques that allow us to (1) measure neurochemical and metabolic processes in the living human brain; (2) investigate the nature of the changes in DA induced by drugs of abuse and their behavioral impact; and (3) study the long-term plastic changes in brain DA activity and its functional consequences in drug-addicted subjects.

The use of positron emission tomography (PET) with DA, D2 receptor radioligands (e.g., [¹¹C]raclopride, [¹⁸F]N-methylspiroperidol, and the more recent [¹¹C]-(+)-4-propyl-9-hydroxynaphthoxazine) have allowed the study of the involvement of DA in drug reward and addiction in the human brain. This includes studies that investigate the relationships between the ability of various drugs to modulate DA and their reinforcing (i.e., euphorogenic, high-inducing, and drug-liking) effects, both in nondependent and in drug-dependent individuals. Using [¹¹C]raclopride, these studies have shown that stimulant drugs (such as methylphenidate, amphetamine, and cocaine), nicotine and alcohol, increased DA in striatum in the human brain, whereas opiates did not. Failure to see an effect with opiates could reflect the limited sensitivity of the PET method since opiates elicit relatively small DA responses in rats, but it could also reflect a minor role of DA in the subjective experience of heroin in opioid addicts. This is in contrast to other drugs for which the DA increases have been associated with their reinforcing effects. For example, both the intravenous (i.v.) administration of methylphenidate (0.5 mg kg^{-1}), which like cocaine, increases DA by blocking DA transporters (DAT), as well as that of amphetamine (0.3 mg kg^{-1}), which like methamphetamine, increases DA by releasing it from the terminal through DAT, can increase extracellular DA concentration in the striatum and that such increases are associated with self-reports of 'high' and 'euphoria.' In contrast, orally administered methylphenidate ($0.75\text{--}1 \text{ mg kg}^{-1}$), which can also increase DA, is not typically perceived as reinforcing. The difference between the i.v. and oral administration of methylphenidate with respect

to their reinforcing effects most likely reflects the fact that the i.v. route leads to DA changes that are much faster than those observed after oral administration. Thus, the failure of oral methylphenidate – or amphetamine – to induce a 'high' is likely due to slower pharmacokinetics (slower brain uptake). This is because the speed with which drugs of abuse enter the brain is a key parameter that affects their reinforcing effects; the faster their brain uptake, the more intense are their reinforcing effects. Similarly, DA increases in ventral striatum induced by smoking, which has very fast rate of brain uptake, are also associated with its reinforcing effects. More precise details on DA's actions in the human brain and their relationship to rewards will continue to evolve as new radioligands with improved selectivity and sensitivity are developed. For example, recent experiments with [¹¹C]-(+)-4-propyl-9-hydroxynaphthoxazine (PHNO), a novel D2/3 agonist radioligand in humans appears to be more sensitive to the DA-releasing effect of amphetamine than the D2/3 antagonist [¹¹C]raclopride (Figure 1). The collected evidence, albeit not conclusive, appears to point to an important contribution of D3 receptor binding to the differential patterns of displacement observed with this novel ligand. The DA D3 receptor is of particular interest because it has been implicated in the establishment of addictive behaviors and is a target for medication development in addiction.

The close correlation between the fast uptake of a drug into the brain, the rapid changes in extracellular DA in the striatum, and its reinforcing properties suggests the involvement of phasic DA firing. Phasic release at frequencies of $>30 \text{ Hz}$ cause abrupt fluctuations in DA levels that contribute to highlighting the saliency of a stimulus. In contrast, tonic DA cell firing, with frequencies of $\sim 5 \text{ Hz}$, serves to maintain the baseline steady-state DA levels that set the threshold of the DA system's responsiveness. This has led to the notion that drugs of abuse manage to induce changes in DA concentration that mimic, but greatly exceed those produced by physiologic phasic DA cell firing. However, the outcome of such DA increases – even when supraphysiologic – will be contingent upon other factors, such as expectation of a particular outcome and context of administration. This dependency of a drug's effects on its context of administration is likely to reflect prefrontocortical regulation of DA cell activity and DA release in the NAcc.

In contrast, oral administration of stimulant drugs, which is the therapeutic route, is more likely to induce slow DA changes, more akin to those provoked by tonic DA cell firing. However, since stimulant drugs block DATs, which are the main mechanism for DA removal, they have the potential to increase the reinforcing value of other reinforcers (natural or drug rewards), even when administered orally by amplifying weak DA signals.

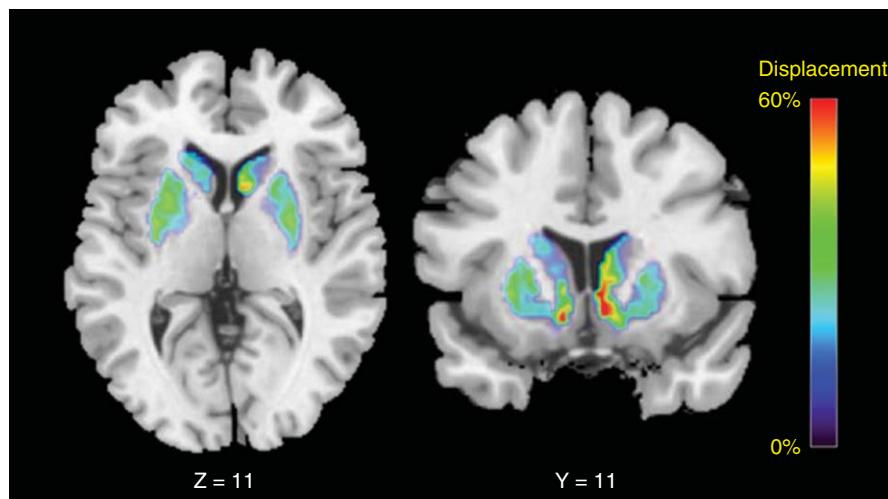


Figure 1 Colored areas show percent decrease in $[^{11}\text{C}]$ - $(+)$ -PHNO binding potentials (BPs) in *d*-amphetamine scans as compared to placebo scans. Image represents mean image of nine individual subtraction images calculated as $(\text{BPmap placebo-BPmap } d\text{-amphetamine/BPmap placebo}) \times 100$. Reproduced with permission from Willeit M, Ginovart N, Graff A, Rusjan P, et al. (2008) First human evidence of *d*-amphetamine induced displacement of a D2/3 agonist radioligand: A $[^{11}\text{C}]$ - $(+)$ -PHNO Positron emission tomography study. *Neuropsychopharmacology* 33(2): 279–289.

Similarly, nicotine, which facilitates DA cell firing, can also enhance the reinforcing value of stimuli with which it is paired. Thus, the combination of nicotine with natural rewards becomes inextricably linked to its reinforcing effects. Since nicotine is smoked throughout the day, blending with an individual's everyday activities, it makes smoking a particularly entrenched behavior, since a virtually unlimited number of everyday activities can become conditioned to nicotine and thus trigger drug craving in the smoker. This is in sharp contrast to other drugs, which are typically abused in very specific and therefore, more limited settings (i.e., bar, dealer's house, and parties).

The usefulness of the PET technique to explore the importance of pharmacokinetics on the reinforcing effects of drugs of abuse stems in part from the ability to label the drugs of abuse with the positron-emitting isotope ^{11}C , which can be substituted for a regular C without affecting the pharmacological properties of the drug. This allows not only for the measurement of its *in vivo* kinetics in the human brain, but also for a detailed mapping of the drug's potential target sites in the brain and periphery. Using this strategy it was possible to measure the pharmacokinetics in brain of cocaine, methamphetamine, toluene, nicotine, and salvinorin. In addition, it is worth mentioning the use of PET to study the availability of drugs in the fetus of nonhuman primates, as illustrated by a PET study done in pregnant nonhuman primates which showed that $[^{11}\text{C}]$ cocaine readily crosses the placenta and accumulates in the fetal liver and to a lesser extent in the brain.

Long-Term Effects of Drugs of Abuse on DA in the Human Brain: Involvement in Addiction

It is important to underscore the fact that even though drug-induced surges in synaptic DA occur in both addicted and nonaddicted individuals, only a minority of exposed subjects – the actual fraction being a function of the type of drug used – ever develop a compulsive drive to continue taking the drug. This is the reason that DA increases alone are insufficient to explain the start of an addiction trajectory. Chronic drug administration is a prerequisite for the development of a drug addiction, which suggests that addictions hinge – in vulnerable individuals – on the ‘repeated’ perturbation of the DA system. Over time, these perturbations are thought to induce neuroadaptations in reward/saliency, motivation/drive, inhibitory control/executive function, memory/conditioning, and mood/stress circuits, all of which are known to be modulated by dopaminergic pathways.

In fact, there is growing evidence that supports this notion. Chronic exposure to stimulants, nicotine, or opiates can induce persistent adaptive changes in the structure of dendrites and dendritic spines on neurons in key brain circuits with roles in motivation, reward, judgment, and inhibitory control of behavior. This observation becomes particularly significant when we consider that the induction of long-term potentiation (LTP) is often associated with measurable increases in the size of dendritic spines and their associated structures.

Drug-induced DA perturbations are likely to have both direct and indirect effects on the maladaptive rewiring of neural circuits. DA (as well as other neurotransmitter systems) is a versatile modulator of synaptic plasticity in its own right. In addition, chronic adaptations in DA receptor signaling may trigger, for example, compensatory glutamate receptor responses with the potential to affect synaptic plasticity. It is interesting to note in this context that, while DA receptors can be found throughout the neuron, there is growing evidence of their increased concentration in dendritic spines, which also feature the highest density of glutamatergic synapses. Thus, the various combinations of postsynaptic DA receptor types are strategically located to influence the synaptic properties of spines through the accurate decodification of tonic and phasic trains of DA signals, while also modulating glutamatergic neurotransmission.

When combined, these observations draw a direct path connecting the effects of drugs of abuse with the adaptive alterations, not only in reward centers but also in many other circuits, through the strengthening, formation, and elimination of synapses.

Effects on Reward and Motivation Circuits

The availability of several radiotracers has allowed researchers to monitor both transient as well as persistent

neurochemical changes in the DA network of the human brain (**Table 1**). It has been shown, using [¹⁸F]N-methylspiroperidol, [¹¹C]raclopride, or [¹¹C]-(+)-4-propyl-9-hydroxynaphthoxazine that subjects addicted to a wide range of drugs (e.g., cocaine, heroin, alcohol, methamphetamine, and nicotine) exhibit significant reductions in D2 DA receptor availability in the striatum (including ventral striatum) that persist for months after protracted detoxification.

It has also been observed that the striatal increases in DA levels induced by i.v. methylphenidate or i.v. amphetamine (and assessed with [¹¹C]raclopride) in cocaine abusers and alcoholics are at least half of what is seen in control subjects. Since DA increases induced by methylphenidate are dependent on DA release – a function of DA cell firing – it is reasonable to hypothesize that the difference likely reflects decreased dopaminergic cell activity in these drug-abusing populations.

While evaluating the results of PET studies based on the competition of [¹¹C]raclopride by endogenous DA, it is critical to remember that the results merely reflect the fraction of D2 DA receptors that is vacant and thus capable of binding the tracer. As a consequence, any reduction in D2 DA receptor availability measured with this technique could reflect either ‘decreases’ in levels of D2 DA receptors and/or ‘increases’ in DA release (competing for binding with [¹¹C]raclopride for the D2 DA

Table 1 Summary of PET findings comparing various targets involved in DA neurotransmission between substance abusers and control subjects for which statistically significant differences between the groups were identified

Target investigated	Drug used	Finding
D2 DA receptors	Cocaine	↓ Acute withdrawal ↓ Detoxified
	Alcohol	↓ 1–68-week abstinence ↓ Detoxified
	Methamphetamine	↓ Detoxified
	Heroin	↓ Active user
	Nicotine (via cigarette smoking)	↓ Active user
	Cannabis	0 Detoxified 0 Early remission
DA transporters	Cocaine	↑ 4 weeks abstinence 0 Detoxified
	Alcohol	↓ Acute withdrawal 0 Detoxified
	Methamphetamine	↓ Detoxified
	Cigarettes	↓ Active user
Vesicular monoamine transporters-2 Metabolism (MAO A and B) Synthesis (dopa decarboxylase)	Methamphetamine	↓ Detoxified
	Cigarettes	↓ Active user
	Cigarettes	↓ Detoxified
	Cocaine	↓ Active user
	Alcohol	↓ Detoxified
DA release	Alcohol	0 Detoxified
	Cocaine	↑ Active user
	Alcohol	↓ Detoxified ↓ Detoxified

Reproduced with permission from Volkow ND, Fowler JS, Wang GJ, Baler R, and Telang F (2008) Imaging dopamine's role in drug abuse and addiction. *Neuropharmacology* 56: 3–8.

receptors) in striatum (including NAcc). However, the fact that cocaine abusers as well as alcoholics show blunted reductions in specific binding (indicative of decrease DA release) when administered i.v. MP indicates that these individuals had both a reduction in the levels of D2 DA receptors as well as a decrease in DA release in striatum. Each deficiency would contribute to the overall decreased sensitivity in addicted subjects to natural reinforcers.

On the other hand, drugs are much more potent at stimulating DA-regulated reward circuits and at triggering persistent circuit changes than natural reinforcers. Therefore, drugs would still have an advantage in individuals attempting to activate their depressed reward circuits. This decreased sensitivity would also result in a reduced interest for environmental stimuli, possibly predisposing subjects for seeking drug stimulation as a means of temporarily activating an underresponsive reward network. As time progresses, the chronic nature of this behavior may sustain the transition from taking drugs in order to feel 'high' to taking them just to feel 'normal.'

Executive Function and Inhibitory Control

The long-term imbalances in dopaminergic function can eventually trigger profound metabolic and functional consequences. The use of the PET radiotracer $[^{18}\text{F}]$ fluoro-deoxyglucose (FDG) that measures regional

brain glucose metabolism, allowed researchers to document decreased activity in orbitofrontal cortex (OFC), cingulate gyrus (CG), and dorsolateral prefrontal cortex (DLPFC) in addicted subjects (e.g., alcoholics, cocaine abusers, marijuana abusers, and methamphetamine abusers). Moreover, there are significant correlations between reduced metabolic activity in OFC, CG, and DLPFC and decreased D2 DA receptor availability in the striatum of cocaine- and methamphetamine-addicted subjects (**Figure 2**) and of alcoholics. Since the OFC, CG, and DLPFC play critical roles in inhibitory control and emotional regulation, it has been postulated that their abnormal modulation by DA, characteristic of addiction, could underlie the subjects' loss of control over drug intake and their poor emotional self-regulation. Indeed, in alcoholics, reductions in D2 DA receptor availability in ventral striatum appear to be associated with alcohol-craving severity and with greater cue-induced activation of the medial prefrontal cortex and anterior CG, as assessed with fMRI. In addition, because damage to the OFC results in perseverative behaviors – and, in humans, impairments in OFC and CG are associated with obsessive-compulsive behaviors – it has also been postulated that DA impairment of these regions could underlie the compulsive drug intake that characterizes addiction.

However, the implication of inhibitory control areas should be considered with caution, because weakened

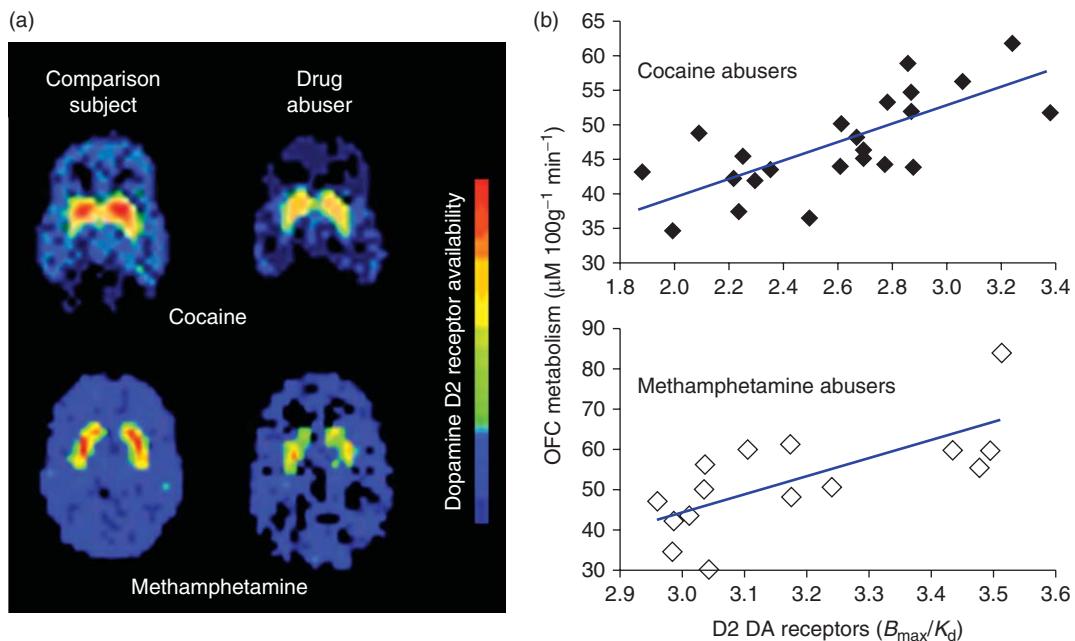


Figure 2 (a) Comparison of dopamine D2 receptor images at the level of the striatum of cocaine and methamphetamine abusers and non-drug-abusing control subjects. (b) Correlation of DA receptor availability (B_{\max}/K_d) in the striatum with measures of metabolic activity in the orbitofrontal cortex (OFC) in cocaine (closed diamonds) and methamphetamine (open diamonds) abusers. Reproduced with permission from Volkow ND, Chang L, Wang GJ, et al. (2001) Low level of brain dopamine D2 receptors in methamphetamine abusers: Association with metabolism in the orbitofrontal cortex. *American Journal of Psychiatry* 158(12): 2015–2021; and Reproduced with permission from Volkow ND, Fowler JS, Wang GJ, et al. (1993) Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. *Synapse* 14(2): 169–177.

prefrontal regions could also increase the drive to engage in risky behaviors in general, which could ‘secondarily’ put individuals at risk for drug abuse. Alternatively, low D2 DA receptors during fetal developmental periods may also influence prefrontal activity in adulthood.

Effects on Conditioning Circuits

It is well known that the hippocampus (declarative memory), amygdala (conditioning), and dorsal striatum (habits) play critical roles in learning and memory. All these areas have been shown to undergo significant adaptations in preclinical models of drug abuse, leading to a growing recognition of the relevance and likely involvement of memory and learning mechanisms in drug addiction. It is highly relevant then, that DA (interdependently with 5-hydroxytryptamine (5-HT)) can modulate the activity of, and affect adaptive changes in the circuits that support learning/memory, conditioning, and habit formation. Moreover, the effects of drugs of abuse on memory systems suggest a likely mechanism – conditioned-incentive learning – through which neutral stimuli can acquire reinforcing properties and motivational salience.

A central question in relapse research is: Why do drug-addicted subjects experience such an intense desire for the drug when exposed to places, people, or things associated with drug taking behaviors? A better understanding of the mechanisms involved could have profound clinical implications, for exposure to conditioned cues (stimuli that had become strongly linked to the drug experience) is a known, powerful trigger of relapse. Due to its involvement in the prediction of reward, DA has been proposed to underlie the conditioned responses that trigger craving. Animal studies support this hypothesis: when neutral stimuli are paired with a drug, animals will – with repeated associations – acquire the ability to increase DA in NAcc and dorsal striatum when exposed to the now conditioned cue. Predictably, these neurochemical responses have been found to be associated with drug-seeking behaviors.

Human PET studies using [¹¹C]raclopride have recently confirmed this hypothesis by showing that in cocaine abusers, drug cues (video images of subjects taking cocaine) significantly increased DA in dorsal striatum, and that these increases were also associated with cocaine craving in a cue-dependent fashion. If we consider the fact that the dorsal striatum is implicated in habit learning, this association is likely to reflect the strengthening of habits as addiction develops. This suggests that the DA-triggered conditioned responses that produce, first habits and then compulsive drug consumption, may reflect a fundamental neurobiological perturbation in addiction. In addition, it is likely that these conditioned responses involve adaptations in corticostriatal glutamatergic pathways that regulate DA release.

Do DA increases induce cravings by themselves or do they represent a secondary response to the cue? A recent study investigated this important question directly in cocaine-addicted individuals, by imaging the effects of oral administration of methylphenidate (which increases DA) in the presence or absence of a drug cue. The results of the study revealed a clear dissociation between oral methylphenidate-induced DA increases and cue-associated cravings, suggesting that cue-induced DA increases are not the primary effectors but rather reflect downstream stimulation of DA cells (corticostriatal glutamatergic pathways that regulate DA release). This observation sheds light on the relevance of the ‘rate’ of DA firing in addiction circuits, for the failure of methylphenidate-induced DA increases to induce craving in this paradigm could be explained by the slow nature of the DA increases. On the other hand, fast DA changes as triggered by phasic DA cell firing – as a secondary response to the activation of descending pathways – may underlie the successful induction of cravings during exposure to a cue. It is worth highlighting one study that found a negative correlation between the DA increases induced by i.v. amphetamine in cocaine abusers and their choice of cocaine over money when tested on a separate paradigm. That is, the subjects that showed the lower DA increases, when given amphetamine, were the ones more likely to select cocaine over a monetary reinforcer. Because in their studies they also reported reduced DA increases in cocaine abusers when compared with controls, this could indicate that cocaine abusers with the most severe decreases in brain dopaminergic activity are the ones more likely to choose cocaine over other reinforcers (such as money).

DA and Vulnerability to Drug Abuse

Understanding why some individuals are more vulnerable to becoming addicted to drugs than others remains one of the most challenging questions for drug-abuse researchers. Only a largely unpredictable minority of drug abusers progress to drug addiction (approximately 10% of those exposed), a fact that hints at a complex interaction between genetic and environmental risk and protective factors. Twin data, for example, suggest that about half of the overall vulnerability toward addiction is heritable. However, the neurobiological mechanisms underlying the role of genes in vulnerability or protection are not understood. Imaging studies are starting to shed light on these mechanisms as illustrated by the following examples.

Recent studies found that allelic variations in the D2 DA gene contribute to significantly higher scores in novelty seeking among methamphetamine-addicted patients and among children who were reared in a punitive environment. There is also preliminary evidence

suggesting that specific DA receptor gene variants (particularly in the D2 and D4 subtypes) modulate smoking progression (initiation and continuation) in adolescence. Finally, there is also good evidence that the availability of D2 DA receptors in the striatum can modulate the subjective responses of healthy nondrug-abusing controls to the stimulant drug methylphenidate. In that experiment, subjects describing the experience as pleasant displayed significantly lower levels of receptors than those describing methylphenidate as unpleasant (**Figure 3**). This suggests that the relationship between DA levels and reinforcing responses follows an inverted U-shaped curve: too little being suboptimal for reinforcement while too much may become aversive.

Could this result mean that high D2 DA receptor levels are protective against drug self-administration? Interestingly, there is a substantial amount of evidence that supports this hypothesis. On the preclinical side, higher levels of D2 DA receptors in NAcc significantly reduce alcohol or cocaine intake in animals previously trained to self-administer alcohol or cocaine, respectively; and, switching cynomolgus macaques from individual to group-housing conditions, exposes a robust (inverse) correlation between individual changes in striatal DA D2 receptor levels and the tendency to self-administer cocaine. Evidence in favor of this relationship has also emerged from human studies. First, there is evidence of depressed

DA activity in specific brain regions of adults with attention-deficit hyperactivity disorder (ADHD) compared to controls. Deficiencies were observed at the level of both D2 DA receptors and DA release in the caudate and in the ventral striatum. Importantly, and consistent with this model, the depressed DA phenotype was associated with higher scores on self-reports of liking when methylphenidate was administered intravenously. It is not surprising then, that individuals with ADHD have a high risk for substance-abuse disorders. Second, it has been observed that subjects who, despite having a dense family history for alcoholism, were not alcoholics had significantly higher D2 DA receptors in striatum than individuals without such family histories. Interestingly, the higher the D2 DA receptors in these subjects, the higher their metabolic activity in OFC and CG. Thus, it can be postulated that high levels of D2 DA receptors may protect against alcoholism by modulating frontal circuits involved in salience attribution and inhibitory control. In this respect, it is worth noting that in the rodent model low levels of D2 DA receptors are associated with impulsive behaviors, which in turn predict compulsive administration of cocaine. Inasmuch as the prefrontal cortex is involved in modulating impulsivity, this may be another mechanism by which low D2 DA levels may increase an individual's vulnerability to drug abuse and addictions and/or by which high D2 DA receptor level may protect against drug abuse.

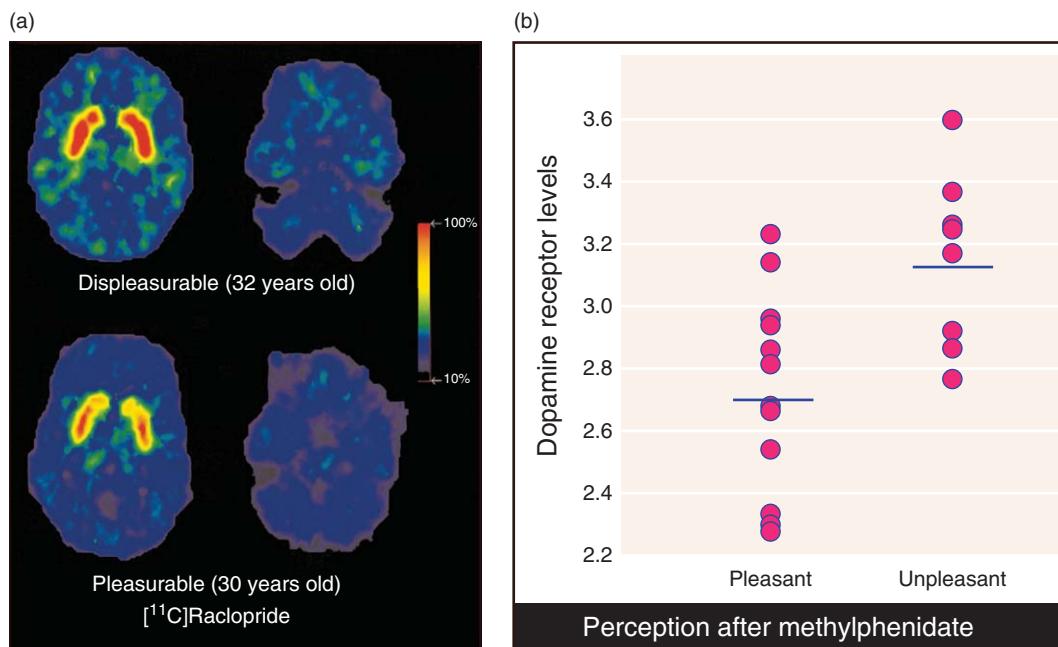


Figure 3 Striatal D2DA is predictive of methylphenidate liking in humans (a) Distribution volume images of $[^{11}\text{C}]$ raclopride at the levels of the striatum (left) and cerebellum (right) in a healthy male subject who reported the effects of methylphenidate as pleasant and in a healthy male subject who reported them as unpleasant. (b) D2DA receptor levels (B_{max}/K_d) in 21 healthy male subjects who reported the effects of methylphenidate as pleasant or unpleasant. Reproduced with permission from Volkow ND, Wang GJ, Fowler JS, et al. (1999) Prediction of reinforcing responses to psychostimulants in humans by brain dopamine D2 receptor levels. *American Journal of Psychiatry* 156(9): 1440–1443.

There are many other variables that are likely or known to significantly modulate the risk of abuse, addiction, and/or relapse. For example, sexual dimorphisms have been observed repeatedly in addictive disorders and recently proposed to be strongly mediated by epigenetic mechanisms. Preclinical evidence suggests that such differences may be due in part to striatal DA system differences and/or differences in the activity of prefrontal regions. Currently, the data do not permit a clear-cut conclusion as to whether men or women display greater DA responses when exposed to drugs. It is also likely that the patterns will be sensitive to experimental conditions, such as context, age, and stage of menstrual cycle.

Another relevant component in drug addiction is the connection that exists between the stress response and addiction vulnerabilities. For, in addition to drug-related cues, stress is a major contributing factor to the increased risk of relapse in an addictive disorder. Indeed, there are substantial overlaps between the circuits in charge of processing stress signals and drug cues and those responsible for processing reward information. Since chronic stress is often accompanied by some degree of sleep disturbances or full-fledged sleep deprivation (SD), it is also pertinent to mention in this context, the recent finding that a single night of SD was associated with a significant reduction in specific binding of [¹¹C]raclopride in the striatum, which was interpreted as a reflection of DA increases. Thus, DA increases with SD may be one of the mechanisms linking sleep deprivation and relapse to drug taking.

When combined, these and other observations provide critical insight into the contribution of the striatal DA system to addiction vulnerability, to the observed sexually dimorphic patterns of substance abuse, and the emergence of frequent psychiatric comorbid conditions.

Treatment Implications

Imaging studies have corroborated the role of DA in the reinforcing effects of drugs of abuse in humans and have dramatically extended the traditional views of DA involvement in drug addiction. These findings suggest multipronged strategies for the treatment of drug addiction designed to (1) decrease the reward value of the drug of choice and increase the reward value of nondrug reinforcers; (2) weaken conditioned drug behaviors, and the motivational drive to take the drug; (3) strengthen frontal inhibitory and executive control; and (4) improve mood and decrease reactivity to stressors. Additional circuits that play a role in various aspects of addiction and that provide potential targets for therapeutic interventions are interoceptive circuits (perception of needs and desires), which are mediated in part by the insula and the brain stress circuits associated with the extended amygdala.

See also: Animal Models of Behavior: Alcohol Addiction; Alcoholism; Cognition: Attention and Impulsivity; Basal Ganglia; Brain Imaging and Addiction; Brain Imaging; Brain Stimulation and Addiction; Cellular Plasticity in Cocaine and Alcohol Addiction; Cognitive Control in the Service of Self-Regulation; Comorbidity – Depression; Drug Addiction; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Drug Sensitization and Drug Abuse; Drug Withdrawal – Motivational View; Emotion–Cognition Interactions; Genes and Behavior: Animal Models; Habituation; Hallucinogens; Incentive Motivation and Incentive Salience; Molecular Neurobiology of Addiction; Motivation; Neurophysiology of Drug Reward; Neural Systems of Motivation; Neurobiology of Opioid Addiction; Neurotransmitters and Neuromodulators Regulating Sleep and Wakefulness; Nicotine; Novelty; Pain and Addiction; Psychostimulants; Psychiatric and Substance Use Disorder Comorbidity; Rewarding Brain Stimulation; Stress and Drug Craving; Stress and Reward; Δ9-THC; Transition to Addiction; Vulnerability Factors in Addiction Disorders.

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Brain Stimulation and Addiction

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Glossary

Anhedonia – Inability to gain pleasure from normally pleasurable experiences – a clinical feature of depression.

Brain-stimulation reward (BSR) – The outcome in operant intracranial self-stimulation (ICSS) procedures that serves as the reinforcing stimulus to maintain the ICSS behavior.

Dysphoria – A state of feeling unwell or unhappy, opposite to euphoria; a clinical feature of depression.

Dysphoria-like – A treatment-induced (e.g., withdrawal-induced) hedonic state resembling the human hedonic experience of dysphoria.

Euphoria – A feeling of well-being or elation, opposite to dysphoria.

Euphoria-like – A treatment-induced (e.g., drug-induced) hedonic state resembling the human hedonic experience of euphoria/pleasure/reward.

Intracranial self-stimulation (ICSS) – An operant behavioral response such as a nose-poke, lever-press, or turn of a wheel, the successful completion of which results in electrical stimulation of reward pathways.

Brain-stimulation reward is measured by an animal's willingness to perform a particular response (e.g., pressing a lever, turning a wheel manipulandum, nose-poking) in order to receive a response-contingent electrical stimulation of a particular region of the brain. The behavioral response is often referred to as intracranial self-stimulation (ICSS), with the outcome of said behavioral response being brain-stimulation reward (BSR). A broad range of abused substances including cocaine, amphetamines, nicotine, opioid narcotics, cannabinoids, alcohol, benzodiazepines, barbiturates, phencyclidine (PCP/Angel Dust), and even inhaled solvents such as toluene and benzene, can facilitate ICSS behavior and lower thresholds of electrical stimulation required to maintain responding (see **Table 1**). Conversely, in dependent subjects an elevation of thresholds is observed during withdrawal from many of these same substances, and this reward-deficit state is commonly used as a model of the depression-like symptoms experienced by human addicts undergoing withdrawal.

Most often studied in rodents, BSR can be elicited from many parts of the brain, from cortical (e.g., medial

prefrontal cortex) all the way down to brainstem structures (e.g., cerebellum, nucleus of the solitary tract). However, in studying the effects of abused substances on BSR, the majority of studies have focused on electrical stimulation of either the medial forebrain bundle (MFB) as it courses through the lateral hypothalamus, or the ventral tegmental area (VTA) which serves as the mid-brain origin of mesolimbic dopamine neurons critical to reward.

Why Use ICSS to Study Addiction?

Addiction is a chronic, relapsing disorder characterized by loss of control over the ability to limit one's intake of a drug and a high probability of relapse during periods of successful abstinence. Initiation of recreational (nonprescription) drug use is typically motivated by a desire to experience pleasurable effects (e.g., euphoria), or in some cases for self-medication of existing states of pain, anxiety, or depression. With continued drug use, the body and in particular the brain adapt to drug-induced disruptions in normal function, and dependence on the drug may develop – which is expressed as a withdrawal syndrome when drug use is discontinued. Most significant among the varied symptoms of withdrawal across classes of abused drugs are negative emotional states, including profound depression-like symptoms such as dysphoria and anhedonia. The greatest utility of animal models of BSR lies in their ability to readily measure both elements of the hedonic continuum experienced by addicts: the euphoria elicited by acute drug intoxication, and the dysphoria/anhedonia experienced during bouts of abstinence. Because animals, unlike the human, cannot directly tell us that they are experiencing euphoria or dysphoria, we will refer to the effects of acute drug intoxication and drug withdrawal on BSR thresholds as euphoria-like and dysphoria-like, respectively.

ICSS paradigms are but one of several commonly used procedures to assess the rewarding effects of acute intoxication and aversive consequences of withdrawal in dependent subjects, and to study the underlying neurobiological substrates. Other useful paradigms include drug self-administration, drug discrimination, and place-conditioning procedures. The advantages and limitations of ICSS for studying acute drug reward, dependence, and withdrawal, and for assessment of abuse liability are considered briefly below relative to these other techniques.

Table 1 Euphoria-like effects of acute intoxication and dysphoria-like effects of withdrawal from various abused substances as measured by shifts in brain-stimulation reward thresholds

		<i>Dysphoria-like effect of withdrawal?</i>		
	<i>Euphoria-like effect of acute intoxication?</i>	<i>Acute dependence</i>	<i>Chronic dependence</i>	<i>Spontaneous, precipitated, or both?</i>
Stimulants:				
D-Amphetamine	Yes	Yes	Yes	Spontaneous
Methamphetamine	Yes	N.D.	N.D.	N.D.
Cocaine	Yes	N.D.	Yes	Spontaneous
Nicotine	Yes	N.D.	Yes	Spontaneous, Precipitated
Caffeine	No/Yes ^a	N.D.	N.D.	N.D.
Opioid Narcotics:				
Morphine	Yes	Yes	Yes	Spontaneous, Precipitated
6-acetyl-morphine	Yes	N.D.	N.D.	N.D.
Heroin	Yes	N.D.	N.D.	N.D.
Fentanyl	Yes	N.D.	Yes	Spontaneous, Precipitated
Nalbuphine	Yes	N.D.	N.D.	N.D.
Buprenorphine (Partial Agonist)	Yes	N.D.	No ^c	Spontaneous
General CNS Depressants:				
Alcohol	Yes ^b	Yes	N.D.	Spontaneous
Diazepam (Valium)	Yes	No ^c	N.D.	Precipitated
Chlordiazepoxide (Librium)	Yes	No ^c	N.D.	Precipitated
Pentobarbital	No ^c	N.D.	N.D.	N.D.
Inhaled Solvents	Yes	N.D.	N.D.	N.D.
Psychedelics:				
PCP	Yes	Yes	Yes	Spontaneous
Delta-9-Tetrahydrocannabinol (THC)	Yes/No ^d	Yes	N.D.	Spontaneous
MDMA/Ecstasy	Yes	N.D.	N.D.	N.D.
LSD	No	N.D.	N.D.	N.D.

^anot shown to have significant euphoria-like effects on its own, but has been shown to produce synergistic euphoria-like interactions with other compounds.

^bafter oral self-administration, or on rising portion of blood alcohol curve with passive administration.

^cassessed under very limited conditions thus far, not systematically evaluated.

^dConflicting results, see text for details.

N.D. = not determined to date.

ICSS Compared to Other Operant Techniques: Self-Administration and Drug Discrimination

In self-administration, an animal completes an operant response such as a lever press to administer a drug, either intravenously or in some cases orally. Therefore the animal's behavior directly leads to consumption of the drug itself – which by inference suggests that the drug produces rewarding effects. ICSS, in contrast to self-administration, provides not a direct measure of the drug's potential to act as a reinforcer, but instead measures its ability to enhance the reinforcing efficacy of rewarding electrical stimulation. Thus, face validity for modeling human drug-taking behavior is greatest for self-administration, but the ICSS procedure nonetheless possesses excellent predictive validity for the assessment of a drug's potential abuse liability and its euphoria-like effects, with virtually all substances that are self-administered by the human demonstrating facilitatory effects on BSR (see **Table 1**). Indeed, in some cases (e.g., nicotine, alcohol, phencyclidine/PCP) it has been possible to establish reliable drug-induced reductions in BSR thresholds in rodents without special manipulations required to establish self-administration (e.g., adulteration of alcohol solutions with sweeteners to initiate self-administration followed by gradual fading out of the sweetener; food-restriction or addition of explicit drug-predictive cues to initiate nicotine self-administration), or for abused substances such as inhaled solvents, where self-administration techniques are technically difficult.

Drug discrimination involves training an animal to recognize the interoceptive stimulus properties of a particular drug, which the animal demonstrates by pressing one lever for food reward when intoxicated with the drug, and another lever when injected with placebo or with a different drug. This has utility in assessment of abuse liability of novel compounds when they are substituted for the training drug with known abuse liability. If a novel compound shares discriminative stimulus effects of a known abused substance, this overlap implies potential abuse liability for the novel compound as well. However, drug discrimination, unlike ICSS, provides no direct measure of a drug's euphoria-like effects, as the discriminative state includes interoceptive components beyond just the euphoria-like properties of the drug, also including physiological, somatic, and other behavioral states. For example, the subjective state produced by lysergic acid diethylamide (LSD) is readily discriminated by animals, but they will not self-administer LSD, nor does it facilitate ICSS behavior as other abused drugs do.

In addition, relative to self-administration and drug discrimination, ICSS has the distinct advantage of requiring no exposure to an abused drug during the acquisition of stable operant responding. Therefore, the euphoria-like effects of a drug, and experimental manipulations to

elucidate the underlying neural substrates thereof, can be assessed on the very first exposure to the drug in ICSS procedures, eliminating possible complicating influences such as the development of tolerance, sensitization, and/or dependence during training.

With regard to dependence and withdrawal, the motivational significance of avoiding or terminating withdrawal certainly is most directly demonstrated in self-administration paradigms where animals actually increase their drug intake in order to terminate the withdrawal state. Withdrawal can also be studied in drug-discrimination paradigms, as the interoceptive state of withdrawal from some drugs is readily discriminable, and the withdrawal interoceptive state of many drugs can be generalized to the discriminative stimulus effects of anxiogenic agents such as pentylenetetrazol, providing an indirect assessment of potential anxiogenic effects of withdrawal. However, withdrawal from most abused substances involves a constellation of somatic, physiological, and affective/emotional signs that vary according to the class of drug. Neither increased self-administration during withdrawal or recognition of the interoceptive stimulus characteristics of withdrawal can identify the specific withdrawal symptom(s) that support the operant behavior. The dysphoria-like effects of withdrawal may indeed be one relevant component that supports increased self-administration during withdrawal, or the ability to discriminate the withdrawal interoceptive state, but only ICSS provides a selective assessment of a reduced functioning or deficit state of the reward system during drug withdrawal – as measured by an increase in BSR thresholds. Moreover, in examining the neural substrates of withdrawal from acute dependence on a drug (i.e., withdrawal from a single acute administration in otherwise drug-naïve animals), ICSS again enjoys an advantage over self-administration and drug discrimination in requiring no prior exposure to the drug during training and establishment of stable baseline performance.

ICSS Compared to Place Conditioning

In conditioned place-preference procedures the potential reinforcing, presumed euphoria-like, effects of drugs are assessed through a demonstrated preference for an environment that was previously paired with the subjective experience of drug intoxication, relative to another environment paired with the absence of drug (i.e., placebo treatment). In contrast, the aversive consequences of drug withdrawal can be assessed through avoidance of a withdrawal-paired environment relative to a neutral environment where no withdrawal has been experienced – so-called conditioned place aversion.

Relative to operant paradigms such as self-administration, drug discrimination, and ICSS, place conditioning

can assess the potential euphoria-like effects of drug intoxication, or aversive consequences of withdrawal, while the animal is drug-free during the test session where preference or aversion for the conditioned environment is expressed. Place conditioning also requires substantially less training than operant paradigms. However, the place-preference paradigm is limited relative to the operant paradigms in that it cannot be used in within-subjects designs of treatment effects on drug-induced reward (once preference has been assessed, it cannot be re-assessed without some extinction of the conditioned association between drug and the paired environment). While repeated testing is feasible in self-administration and drug-discrimination studies – for example, when testing different experimental manipulations (e.g., different doses of a test agent, different types of antagonists to block a drug effect, etc.) – in both procedures re-establishing baseline performance between days of experimental treatment requires further exposure to the abused substance. In contrast, baseline performance in ICSS studies can be re-established between test sessions without further drug exposure.

Conditioned place preference also is hampered by the fact that it provides only a conditioned, not direct, measure of possible euphoria-like or aversive effects. Therefore, treatments that alter learning and memory processes can modulate the strength of the memory for the conditioned environment without necessarily directly altering the underlying hedonic effects of drug intoxication or withdrawal. Conditioning procedures can be successfully integrated into operant techniques, including the ICSS procedure, such that both direct (unconditioned) and conditioned alterations in hedonic processing (both euphoria-like and dysphoria-like) can be studied.

Conditioned place aversion is one of the most widely used measures of the aversive stimulus effects of precipitated withdrawal from drugs such as opioid narcotics, nicotine, and cannabis, where competitive antagonists are readily available and can facilitate precise temporal contiguity of withdrawal and confinement to the conditioning environment. However, establishing the required temporal contiguity between the withdrawal state and the conditioning environment can be problematic for drugs such as alcohol, cocaine, and amphetamines where the lack of available competitive antagonists necessitates the examination of spontaneous withdrawal. Spontaneous withdrawal takes place over an extended time course, and association of a novel context with just a portion of the withdrawal period may not engender a strong conditioned association. Finally, while the dysphoria-like effects of withdrawal indeed may contribute to a conditioned place aversion, other aspects of withdrawal malaise can also readily elicit avoidance of the conditioned environment. Therefore, although ICSS experiments are more time-intensive than those utilizing place conditioning,

this is offset substantially by the utility of ICSS for within-subjects designs. In addition, ICSS provides a selective measure of dysphoria-like effects – not a non-specific index of withdrawal malaise – can be used to study both spontaneous and precipitated withdrawal, and can be used to assess both direct and conditioned alterations in hedonic processing.

Commonly Employed ICSS Techniques for Studying Abused Substances

Many early studies of BSR employed ICSS techniques based on simple fixed-ratio schedules of reinforcement, often a continuous reinforcement schedule in which each successfully completed response, most often a lever-press, resulted in delivery of a brief (typically 100–500-ms) delivery of biphasic square- or sine-wave stimulation. Under such schedules of reinforcement, the absolute response rate for stimulation at a fixed current intensity, stimulation frequency (e.g., 60 Hz), and duration of stimulation has been used as an index of the reward-facilitating effects of abused substances with some degree of success. However, most abused substances can exert nonspecific effects such as attentional deficits, behavioral stimulation, sedation, or behaviors that compete with high rates of operant responding, and these effects usually increase as the dose of the abused substance is increased, thereby confounding dose-dependent reward-facilitation with nonspecific alterations in operant performance. For example, early studies of morphine effects on BSR often failed to find significant facilitatory effects on the first few administrations using rate-dependent measures, likely due to the rate-suppressing motoric effects of morphine that predominate until tolerance develops. However, procedures that can separate reward threshold from absolute response rate (see below) detect changes in BSR threshold on first morphine exposure.

Some researchers have proposed alternative response requirements to lever-pressing (not a natural component of a rodent's behavioral repertoire) that are more readily elicited in rodents (e.g., licking a surface, nose-poking into a small aperture, locomotion from one location to another, turning of a wheel manipulandum), but even these simpler responses are susceptible to performance-altering effects at higher drug doses. Therefore, irrespective of the physical-response requirement, researchers have applied a number of alternative approaches to mere assessment of absolute response rate at fixed stimulation parameters, including autotitration schedules, progressive ratio schedules, partial reinforcement schedules, differential reinforcement of low rates of responding, discrete trial current-intensity-threshold procedures, and curve-shift procedures. Rather than reviewing all of their relative advantages and limitations

in exhaustive detail here, several of the most frequently employed techniques are described.

Curve-Shift-Analysis Procedures

By selectively manipulating one stimulation parameter while holding the others constant, rates of responding are observed to increase in direct proportion to current intensity, stimulus frequency, or train duration up to a certain point, at which an asymptote in the rate function is reached. Several measures derived from these rate functions can be used to separate effects on reward from effects on performance. Taking rate–frequency functions as a prominent example utilized in studying drug- and withdrawal-induced alterations in BSR thresholds, the critical measures include the slope of the rate–frequency curve, the asymptotic maximal response level, the M50 (frequency at which 50% of maximal responding is achieved), and Theta-0 (theoretical frequency at which responding is first initiated). Factors that affect only reinforcement magnitude (e.g., lowering or increasing stimulation frequency), but not the effort required to complete the response, will generally produce parallel shifts in the rate curves without altering the slope of the function or the asymptotic maximal response rate. Under conditions where parallel shifts are observed, both M50 and Theta-0 serve as reliable indices of relative magnitude of BSR, and both measures are used extensively to estimate BSR thresholds. In contrast, manipulations that affect the ability of the animal to complete the response (e.g., increased force required to depress a lever) will lower the slope of the function and decrease the asymptotic maximal response rate. When a drug produces such a nonparallel shift in the rate–frequency curve, Theta-0 is more resilient than M50 to nonspecific performance effects because M50 is dependent on slope; therefore, Theta-0 provides the best estimate of BSR threshold when a drug affects slope and maximal response rate.

Two-Lever Autotitration Procedure

In this procedure, each response on one lever in an operant chamber results in delivery of a suprathreshold level of electrical stimulation that reliably maintains responding, but after completion of a defined number of responses on that same lever, the current intensity or the frequency of stimulation is automatically stepped down. Thus, as animals continue to respond, the reward magnitude is progressively lowered. A second lever in the chamber serves as a reset lever, and each response on that lever will reset the current intensity or stimulation frequency to the starting level, and the titration point (current intensity or stimulation frequency at which the reset lever is pressed) serves as a measure of BSR threshold. Although response rates generally remain quite high

in this procedure, it is possible to manipulate the response rate independent of titration point – which can serve as a reasonably resilient index of BSR threshold even when drugs affect response rate on the reward lever.

Psychophysical Discrete Trial Procedure

Kornetsky and colleagues have championed a psychophysical, discrete trial method to estimate the threshold for BSR. The major advantage of the procedure is that it requires a single discrete response by the subject in each trial to receive the electrical stimulation, and the total response requirement of a typical 30–45-min session can be 60 responses or fewer. In this procedure, train duration and frequency of stimulation are held constant, and each trial begins with a noncontingent electrical stimulation at a given current intensity, after which the animal has a defined interval, typically 7.5 s, to complete a response requirement of one-fourth rotation of a wheel manipulandum in order to receive a response-contingent stimulation of identical current intensity. If the response does not occur within the specified interval, the second stimulation is omitted on that trial. Each operant response completed during a variable inter-trial interval (e.g., 5–20 s, average of 10–15 s) resets the inter-trial interval and delays the onset of the next trial. In this fashion, animals are trained to elicit only the minimal response required to earn the response-contingent stimulation in each given trial where they detect the initial noncontingent stimulation. Beginning at a current intensity that is suprathreshold for a given subject, intensity is lowered and raised in two series of ascending and descending trials, with changes in stimulus intensity dependent on whether the animal successfully responded on more than 50% of the trials at the prior intensity (3–5 trials are typically conducted at each current intensity step). The midpoint between the lowest current step that supported 50% or greater successful responding and the highest current intensity that failed to support that level of responding is determined for each ascending and descending series, and the average of the midpoint for all four series is taken as the threshold current intensity. Response latency serves as an independent measure of possible nonspecific performance effects of a drug in this procedure.

The discrete trial component of the procedure ensures that responding is truly contingent upon the intensity of the priming stimulation provided to start each trial, and the extremely low response requirements ensure that all but the most severe of nonspecific performance-altering effects will have a minimal influence on threshold. For example, suppression of operant responding during antagonist-precipitated withdrawal from morphine serves as a sensitive behavioral index of opioid withdrawal, but an animal will successfully complete a discrete trial current-intensity ICSS session at doses of the antagonist that

would dramatically reduce operant response rates under high-demand schedules of reinforcement.

Measuring the Effects of Acute Drug Intoxication and Neuroadaptive Responses in Reward Circuitry Using ICSS

As shown in **Table 1**, the majority of substances that are abused by humans and self-administered by animals also will facilitate BSR, with the most notable exception being pure psychedelic/hallucinogenic compounds such as LSD, which appear not to be rewarding to animals in either self-administration or ICSS paradigms. The vast majority of ICSS studies examining euphoria-like effects of acute drug intoxication and dysphoria-like effects of withdrawal have employed rats, but models with mice have begun to emerge for cocaine, amphetamine, morphine, and nicotine. These should prove useful through leveraging of the vast array of mutant mouse lines for examination of molecular/genetic substrates, but to date the studies with rats and mice have yielded qualitatively similar results, and consequently the discussion below does not distinguish among them.

Euphoria-Like Effects of Acute Drug Intoxication

The euphoria-like effects of most abused substances as measured in ICSS procedures appear to be mediated by reward circuitry in the extended amygdala, with the mesolimbic dopamine system sitting at its core. BSR thresholds are reduced by the activation of this system at the level of its midbrain site of origin (VTA) and/or its forebrain terminal regions (e.g., nucleus accumbens, medial prefrontal cortex) via actions on nicotinic receptors (nicotine), opioid receptors (opioid narcotics), dopaminergic axon terminals (e.g., cocaine, D-amphetamine), or glutamatergic N-methyl-D-aspartate (NMDA) receptors (phencyclidine/PCP/Angel Dust, MK-801). Combinations of these drugs can facilitate BSR in a synergistic fashion, with administration of ineffective or minimally effective doses of each compound producing a potent, combined euphoria-like effect (e.g., cocaine–ethanol, cocaine–caffeine, morphine–amphetamine, morphine–MK-801, and caffeine–MK-801), thereby providing further evidence that most drugs of abuse produce their euphoria-like effects through action on common neural circuitry.

Stimulants

D-amphetamine dose-dependently elicits euphoria-like effects in progressive ratio, autotitration, rate–frequency and rate–current intensity curve-shift, and psychophysical discrete-trial threshold-determination techniques,

with both MFB and VTA as sites of stimulation. Significant effects are observed in a dose range of 0.5–5 mg kg⁻¹, with maximal threshold reductions of about 40% below baseline reached at 3–4 mg kg⁻¹. D-amphetamine administered directly into the nucleus accumbens – a primary forebrain terminal projection site of mesolimbic dopamine fibers from the VTA – mimics the euphoria-like effects of systemically administered D-amphetamine, and dopaminergic antagonists will oppose the euphoria-like effects of D-amphetamine.

Although methamphetamine has not been as extensively evaluated as D-amphetamine for effects on BSR thresholds, one study demonstrated 50% reductions from baseline threshold in a rate–frequency curve-shift procedure, with MFB as the stimulation site. The optimal dose of 0.65 mg kg⁻¹ was lower than optimal doses of D-amphetamine – consistent with methamphetamine’s greater potency in other measures of drug reward such as self-administration and conditioned place preference.

Cocaine also dose-dependently lowers BSR thresholds measured in rate–frequency, rate–duration, or rate–current intensity curve-shift analyses, as well as psychophysical discrete-trial current-intensity-threshold procedures, irrespective of whether the stimulation site is the VTA or MFB. In addition, while it has been difficult to demonstrate euphoria-like effects of D-amphetamine or morphine on electrical stimulation of the medial prefrontal cortex, the effects of cocaine appear reliable and reproducible with this stimulation site. Peak effects observed after administration of 16–30 mg kg⁻¹ doses of cocaine result in threshold reductions of up to 60% below baseline, making the euphoria-like effects of cocaine in ICSS procedures the largest documented to date among all abused substances tested. Dopaminergic antagonists reverse the euphoria-like effects of cocaine.

Nicotine dose-dependently reduces BSR thresholds for stimulation of the VTA or MFB in the dose range of 0.1–0.8 mg kg⁻¹, with peak effects in rate–frequency curve-shift or psychophysical discrete-trial procedures of about 30–40% reductions from the baseline threshold. Slightly lower doses are effective in facilitating BSR thresholds when the VTA is the site of stimulation than when MFB is the stimulation site. The euphoria-like effects of nicotine appear to rely upon the activation of nicotinic receptors in the VTA, which in turn facilitates mesolimbic dopamine activity, and dopaminergic antagonists will reverse threshold-lowering effects of nicotine.

Caffeine produces stimulant effects by acting as an antagonist of the inhibitory neurotransmitter adenosine at A1- and A2a-receptors in the brain. Caffeine-induced reductions in BSR thresholds are weak at best when administered on its own (maximal effect at 3–5.6 mg kg⁻¹ usually <10% reductions from baseline), and higher doses (10 mg kg⁻¹ and above) produce dose-dependent reductions in response rate and elevations in threshold.

However, the combined administration of ineffective doses of caffeine and MK-801, a noncompetitive NMDA antagonist that mimics PCP effects (see below), produce significant reductions in BSR thresholds. This finding is consistent with observations that selective antagonists of the A2a-receptor do not alter reward thresholds on their own, but can potently reverse the dysphoria-like effects of withdrawal from chronic cocaine. Therefore, caffeine and other adenosine antagonists, while weakly euphoria-like on their own, may potentiate the positive hedonic efficacy of acute drug intoxication and reduce the negative hedonic consequences of drug withdrawal.

Opioid narcotics

Numerous ICSS studies have documented the euphoria-like effects of opioid agonists such as morphine, heroin, and 6-acetylmorphine, among others (see **Table 1**). Activation of either μ - or δ -opioid receptors in the shell region of the nucleus accumbens, or in the VTA, results in reliable reductions in BSR threshold. However, κ -opioid receptor activation results in dysphoria-like effects, indicating that significant κ -receptor activation by nonspecific opioid agonists that can interact with multiple receptor types may oppose the euphoria-like effects of μ - and δ -receptor activation.

Morphine in the dose range of 2–10 mg kg⁻¹ elicits dose-dependent reductions in threshold for electrical stimulation of the VTA or MFB using autotitration, rate–frequency curve-shift, or psychophysical discrete-trial procedures. Peak effects are observed at doses of 4–8 mg kg⁻¹ (30–40% reduction from baseline threshold). Both heroin (diacetylmorphine) and 6-acetylmorphine are roughly 40 times as potent as morphine itself in reducing BSR thresholds. The partial opioid agonist buprenorphine, approved for use in the treatment of opioid addiction, has inconsistent effects on its own at doses of 20–80 μ g kg⁻¹ in a psychophysical discrete-trial procedure (significant threshold reduction in one study, no effect in another). However, it has been shown that buprenorphine can potently reverse the dysphoria-like effects of spontaneous withdrawal from the full opioid agonist fentanyl, and marked buprenorphine-induced reductions in BSR threshold of 25–30% below baseline persist after the dysphoria-like effects of fentanyl withdrawal have dissipated. This suggests that opioid withdrawal may unmask euphoria-like potential of partial opioid agonists. This latter observation has significant implications for buprenorphine treatment of addiction, as administration of the compound during periods where peak withdrawal dysphoria is present may result in increased risk of abuse of buprenorphine itself.

General central nervous system (CNS) depressants

Using their classic discrete-trial current-intensity method, Kornetsky and colleagues demonstrated that BSR thresholds for electrical stimulation of the MFB were significantly reduced by alcohol that was self-administered by the animals immediately prior to the ICSS session to a blood alcohol concentration of 0.03–0.05 g%. Lewis and June found that passively administered alcohol also could lower BSR thresholds during the rising phase in the blood alcohol concentration curve (15–20% reductions from baseline threshold in the first 20 min postinjection), but not after concentrations had peaked, suggesting a potential rapid acute tolerance to the euphoria-like effects of alcohol.

Among other classes of general CNS depressants, benzodiazepines and barbiturates have been found to facilitate rates of responding for electrical stimulation of the MFB at lower doses, and suppress rates at higher doses as would be expected of a sedative drug. To date only benzodiazepines such as chlordiazepoxide (Librium) and diazepam (Valium) have been assessed in ICSS threshold procedures, and both drugs reduce BSR thresholds in autotitration or rate–current intensity and rate–frequency curve-shift paradigms. Maximal reductions of about 15–20% below baseline are observed at 2.5–5 mg kg⁻¹ doses of diazepam and 5–15 mg kg⁻¹ doses of chlordiazepoxide. Barbiturates and newer-generation anxiolytic agents such as midazolam (Versed) and alprazolam (Xanax) have yet to be systematically evaluated for effects on BSR threshold.

While the maximum reductions in threshold produced by diazepam, chlordiazepoxide, and alcohol are relatively modest, a recent study provided intriguing evidence that another group of CNS-depressant agents may produce more profound euphoria-like effects. Autotitration ICSS thresholds determined in sealed vapor-inhalation chambers during active delivery of abused inhalant solvents such as toluene, cyclohexane, acetone, and benzene revealed concentration-dependent reductions in BSR thresholds, with up to 50% reductions in threshold produced by benzene (maximal effect of 20–30% with all other agents investigated).

Psychedelics

Drugs such as LSD and dimethyl-tryptamine (DMT), which exert profound psychedelic effects through action on serotonergic systems but do not stimulate mesolimbic reward circuitry, appear to lack euphoria-like effects in ICSS threshold-determination paradigms. This finding is consistent with the fact that these compounds also are not self-administered by animals. However, reductions in BSR thresholds are produced by several mixed-class drugs that have psychedelic properties but additionally stimulate either directly or indirectly mesolimbic reward

circuitry, suggesting that psychedelic properties *per se* do not preclude an agent from exerting euphoria-like effects in ICSS paradigms. For example, methylenedioxymethamphetamine (MDMA/Ecstasy) lowers BSR thresholds in rate–frequency curve-shift and discrete-trial current-intensity ICSS paradigms, with peak effects observed at $2\text{--}4 \text{ mg kg}^{-1}$. The maximum threshold reduction produced by MDMA is comparable to that seen after administration of 1 mg kg^{-1} of D-amphetamine or 15 mg kg^{-1} of cocaine (30–40% reduction from baseline), suggesting similar euphoria-like efficacy.

Phencyclidine (PCP/Angel Dust) and the related compound ketamine were developed initially as dissociative anesthetic agents, but ultimately became limited in their clinical use in the human because of their psychedelic effects at moderate doses and psychomimetic effects at higher doses. Systemic administration of $2.5\text{--}10 \text{ mg kg}^{-1}$ doses of PCP lowers BSR thresholds for electrical stimulation of the MFB by about 20–25% as measured by parallel leftward shifts in rate–frequency curves. Microinjection of $5\text{--}20 \mu\text{g}$ doses directly into the shell region of the nucleus accumbens produces similar effects, implicating glutamatergic inputs to this component of mesolimbic reward circuitry as a substrate for the reward-facilitating effects of PCP. To date, ketamine has been examined only in a rate-dependent ICSS paradigm, where it was found to increase responding for stimulation of the MFB after systemic administration of 3.0 mg kg^{-1} , followed by response suppression at higher sedative doses ($30\text{--}100 \text{ mg kg}^{-1}$). Although PCP acts both as a noncompetitive antagonist of NMDA receptors and a blocker of monoamine (dopamine, serotonin, norepinephrine) reuptake, the reward-facilitating actions of PCP after systemic or intra-accumbens administration are mimicked by MK-801 – which like PCP is a noncompetitive antagonist at NMDA receptors, but unlike PCP is devoid of activity at monoamine reuptake transporters. This indicates that PCP effects at NMDA receptors in the nucleus accumbens are sufficient to produce reward facilitation.

Gardner and colleagues have consistently reported that δ -9-tetrahydrocannabinol (THC), the principal active ingredient in cannabis, reduces reward thresholds by 15–25% at doses of $1\text{--}1.5 \text{ mg kg}^{-1}$, as measured by the Theta-0 index in rate–frequency curve-shift paradigms, or in autotitration procedures. However, this group demonstrated that the effects are variable across strains of rats, with some strains showing no euphoria-like effects. Moreover, other groups have failed to detect THC-induced reductions in reward threshold using M50 as the threshold index in rate–frequency curve-shift analyses, or in psychophysical discrete-trial procedures. To date, systemic administration of synthetic cannabinoid receptor agonists most often have been shown to exert no effect, or in some cases modestly increase, BSR thresholds in curve-shift paradigms. Thus, while THC is self-

administered directly into the VTA and shell of the nucleus accumbens in a cannabinoid-receptor-dependent fashion, the available data from the BSR literature indicates that cannabinoid-receptor stimulation by systemically administered agonists is not consistently euphoria-like, suggesting that additional actions outside this circuit may diminish euphoria-like efficacy.

Neuroadaptive Responses in Reward Circuitry I: Chronic Dependence and Withdrawal

Initiation of drug use is typically motivated by pleasure seeking (e.g., euphoria-inducing effects, increased sociability) or for self-medication of preexisting pain, stress, mood, or anxiety disorders. However, as the body and particularly the nervous system adapts to the disruptions in homeostatic balance produced by drug intoxication, states of acute and/or chronic dependence will emerge, and avoidance or termination of the aversive consequences of withdrawal come to play an increasing role in the transition to and maintenance of addiction. Of particular motivational relevance are negative emotional signs of withdrawal such as anxiety and depression/dysphoria, which for the addict stand in stark contrast to the positive emotional state experienced during drug intoxication. Animal models of these negative emotional consequences of withdrawal from acute and chronic dependence are critical to the study of their underlying neurobiological mechanisms, and one of the most widely employed models is the dysphoria-like effects of withdrawal measured in ICSS paradigms.

Stimulants

Depression-like symptoms during withdrawal from abused substances were first reported in rodents in the mid-1970s by Barrett and colleagues following chronic treatment with D-amphetamine. Since then the reliability and robustness of the effect has been established in a number of studies under varying regimens of drug treatment, including repeated daily injections for as little as 4 days, and continuous infusion via osmotic minipumps for 7–14 days. Multiple threshold-detection techniques, including rate–intensity curve-shift, rate–frequency curve-shift, and psychophysical discrete-trial methods, have proven to be sensitive indices of the dysphoria-like effects of spontaneous D-amphetamine withdrawal. Peak threshold elevations of 175% and duration of effect as long as 18 days have been reported following more chronic exposure regimens, with lesser duration and peak magnitude observed under shorter-term D-amphetamine-exposure schedules.

Both curve-shift and psychophysical discrete-trial threshold-detection methods have been used to document elevations in BSR thresholds during withdrawal from chronic exposure to cocaine via repeated injections

(40–120 mg kg⁻¹ day⁻¹), or in animals allowed to continuously self-administer cocaine for 12–48-h periods. Maximum threshold elevations of 200% of baseline are seen from 1- to 6-h postcocaine administration, with a maximum duration of 72 h documented to date.

Both spontaneous and antagonist-precipitated withdrawal from chronic nicotine infusion via osmotic minipumps (7 days or longer, 3.2 mg kg⁻¹ day⁻¹ or higher) results in elevations in BSR thresholds of about 140% above baseline at peak intensity, as measured by the psychophysical discrete-trial procedure. The most frequently used administration regimen, 3.2 mg kg⁻¹ day⁻¹, results in plasma nicotine levels comparable to those observed in 30 cigarettes/day smokers. Some studies have reported the duration of spontaneous nicotine withdrawal as long as 104 h after cessation of infusion. Administration of nicotinic receptor antagonists directly into the VTA, but not into forebrain structures implicated in drug reward such as the nucleus accumbens, central nucleus of the amygdala, or bed nucleus of the stria terminalis, will precipitate withdrawal-induced dysphoria-like effects after chronic nicotine infusion.

Opioid narcotics

Naloxone-precipitated withdrawal from chronic administration of morphine via osmotic minipumps or slow-release pellets is well-established to elicit significant dysphoria-like effects, with peak magnitude of 170% above baseline threshold in psychophysical discrete-trial paradigms, and still significant but slightly lower peak elevations (140%) in an autotitration threshold-detection task. It has been suggested that high rates of self-stimulation in autotitration and curve-shift paradigms may in some cases act as a hedonic buffer against dysphoria-like effects, allowing for discrete-trial procedures with much less frequent electrical stimulation to detect larger threshold shifts during withdrawal.

Spontaneous withdrawal from morphine reduces the rate of responding in ICSS autotitration paradigms for 24–48 h after cessation of morphine delivery without significant alteration in threshold; spontaneous withdrawal from the partial agonist buprenorphine does not significantly alter thresholds in a discrete-trial paradigm. Recently, however, spontaneous withdrawal from the full μ -opioid-receptor agonist fentanyl was shown to result in dramatic elevations in BSR threshold in psychophysical discrete-trial procedures. This effect is dependent on dose of fentanyl administered per day, with 0.3, 0.6, and 1.2 mg kg⁻¹ day⁻¹ resulting in threshold elevations of 140%, 170%, and 180% above baseline threshold, respectively. The duration of threshold elevation is also dose-dependent (0.3 mg kg⁻¹ day⁻¹: 12 h; 0.6 mg kg⁻¹ day⁻¹: 36 h; 1.2 mg kg⁻¹ day⁻¹: 48 h). Precipitated withdrawal from chronic fentanyl exposure (1.2 mg kg⁻¹ day⁻¹) results in peak threshold elevations of

200% above baseline, comparable to the peak effects observed during cocaine withdrawal. It is notable that peak intensity of precipitated withdrawal during infusion of 0.6 mg kg⁻¹ day⁻¹ fentanyl is reached within 20 h of onset of infusion, and remains stable thereafter, whereas precipitation of somatic withdrawal signs is modest at 20 h, and rises gradually to a maximal level after 120 h of continuous infusion. This difference suggests that neuroadaptation in brain-reward circuitry may be more rapid than adaptation in other systems mediating somatic manifestations of opioid withdrawal.

General CNS depressants

To date, among all the CNS depressants shown to exert facilitatory effects on BSR during acute bouts of intoxication (alcohol, benzodiazepines, inhaled solvents), only withdrawal from chronic alcohol has been systematically examined for its dysphoria-like effects. Passive exposure to alcohol via vapor inhalation for 2 weeks at levels that resulted in average blood alcohol concentrations of 0.23 g% at the time of withdrawal resulted in time-dependent BSR-threshold elevations as measured by psychophysical discrete-trial current-intensity thresholds. Peak magnitude threshold elevation of 140% above baseline was observed from 6 to 12 h after cessation of alcohol exposure, and thresholds remained significantly elevated for 48 h. Similar results using this same ICSS threshold-detection procedure were recently obtained in animals that were actively consuming alcohol through forced consumption of a liquid diet containing 10% ethyl alcohol.

Psychedelics

Dependence on pure psychedelic compounds such as LSD is extremely rare, because even just a few doses produce almost complete tolerance to their psychedelic effects. However, spontaneous withdrawal from continuous infusion of 15–20 mg kg⁻¹ day⁻¹ via osmotic minipump of the mixed-class psychedelic compound PCP results in lasting increases in BSR threshold for up to 30 days after pump removal, as measured in a discrete-trial current-intensity-threshold paradigm. Thus, while peak magnitude of threshold elevations (125–140% of baseline threshold) during PCP withdrawal was substantially less than that observed during spontaneous withdrawal from cocaine, amphetamine, or the opioid narcotic fentanyl, the duration of the reward-deficit state post-PCP was considerably longer. The dysphoria-like effects of withdrawal following chronic exposure to other mixed-class psychedelic compounds such as MDMA and THC have yet to be evaluated in ICSS paradigms (but see below for evidence of BSR threshold elevations during withdrawal from acute dependence on THC).

Neuroadaptive Responses in Reward Circuitry II: Acute Dependence and Withdrawal

Dysphoria-like effects of withdrawal from single treatments with abused substances as measured in ICSS paradigms to date have been reported for morphine, alcohol, d-amphetamine, THC, and PCP. Greatest attention thus far has been given to withdrawal from acute intoxication with morphine. When an acute bolus dose of morphine ($3\text{--}10 \text{ mg kg}^{-1}$) is followed 4–8 h later by an antagonist such as naloxone or naltrexone, antagonist-precipitated elevations in BSR thresholds (peak effect of 115–125% of baseline threshold) are evident in several ICSS paradigms, including progressive ratio breakpoint, an autotitration threshold procedure, and the psychophysical discrete-trial current-intensity-threshold procedure. Although spontaneous withdrawal has yet to be demonstrated following a single acute morphine treatment at doses up to 10 mg kg^{-1} , repeated daily treatment with 5.6 mg kg^{-1} morphine results in a progressive increase in dysphoria-like effects of precipitated withdrawal (threshold elevations of 140–150% above baseline threshold), and under these conditions spontaneous withdrawal (115% of baseline threshold) has been observed 20 h after as few as two daily treatments.

Spontaneous withdrawal from a single 2.0-g kg^{-1} dose of alcohol also transiently elevate BSR thresholds in a discrete-trial procedure from 6–9 h post-ethanol administration, and repeated treatment at weekly intervals results in a further increase in both peak magnitude (up to 140% of baseline), and duration (up to 48 h) of threshold elevations. Spontaneous withdrawal from 4.0 mg kg^{-1} d-amphetamine, $5\text{--}10 \text{ mg kg}^{-1}$ PCP, and 1 mg kg^{-1} THC also produce transient elevations in BSR thresholds (about 120% of baseline) from 8 to 24 h postinjection in either the psychophysical discrete-trial current-intensity (amphetamine or PCP) or rate-frequency curve-shift (THC) threshold procedures.

A single study to date reported a lack of precipitated withdrawal by the benzodiazepine antagonist flumazenil when administered 4–24 h after diazepam ($3\text{--}10 \text{ mg kg}^{-1}$) or chlordiazepoxide ($30\text{--}100 \text{ mg kg}^{-1}$), but this effect has only been examined with progressive ratio breakpoint analysis to date. It has been suggested by Easterling and colleagues, the study authors, that high rates of responding for BSR may act as a hedonic buffer of sorts against milder withdrawal-induced reward deficits. Consistent with this suggestion, the magnitude of change in BSR threshold observed with precipitated morphine withdrawal is greater in the discrete-trial procedure, which has extremely low response requirements, than in the autotitration and progressive ratio procedures where high rates of ICSS behavior are maintained even during precipitated withdrawal. Therefore, further evaluation of possible dysphoria-like effects of withdrawal from acute

benzodiazepine administration should be conducted using a procedure with low response requirements before drawing any final conclusions. Among other substances with known euphoria-like effects in ICSS procedures, withdrawal from acute cocaine, nicotine, and inhaled solvents have yet to be examined.

Neuroadaptive Responses in Reward Circuitry III: Tolerance and Sensitization

Tolerance to euphoria-like effects

Among studies seeking evidence of tolerance (i.e., decreased responsiveness to the euphoria-like effects of acute drug intoxication upon repeated administration), only a few have provided positive evidence, whereas others have indicated no tolerance with similar treatment regimens. For example, one study reported tolerance to the threshold-lowering effects of 5 mg kg^{-1} morphine in an autotitration ICSS procedure after 5 days of treatment with this dose. However, another study using similar treatment regimens of morphine (repeated daily or twice-daily injections with $4\text{--}6 \text{ mg kg}^{-1}$) failed to produce any evidence of tolerance to the euphoria-like effects even after chronic (up to 30 days+) treatment, although rapid tolerance did develop to the rate-suppressing effects of morphine. Repeated intermittent injections with a number of other drugs (nicotine, caffeine, THC, etc.) have produced evidence of differential tolerance to rate-suppressing, but not euphoria-like, effects.

One study reporting tolerance to the euphoria-like effects of cocaine administered $120 \text{ mg kg}^{-1} \text{ day}^{-1}$ for 3 days (four divided doses of 30 mg kg^{-1}). However, a number of other studies employing $40\text{--}75 \text{ mg kg}^{-1}$ daily doses of cocaine failed to produce evidence of tolerance, suggesting perhaps that extremely high doses of passively administered cocaine are necessary before tolerance is induced. Koob, Markou and colleagues have similarly demonstrated apparent dose-dependent tolerance with self-administered cocaine. In their study, rats allowed to self-administer 10–20 injections of cocaine ($0.25 \text{ mg per injection}$) for several weeks showed reliable reductions in discrete-trial current-intensity BSR thresholds 15 min after the conclusion of most self-administration sessions throughout the testing period, whereas rats allowed to self-administer 80 injections per session showed no facilitation of BSR thresholds after several weeks of testing, suggestive of dose-dependent tolerance.

In addition to repeated intermittent injection regimens or self-administration procedures, a number of studies examined chronic, continuous infusion of nicotine, d-amphetamine, PCP, or the opioid narcotic fentanyl using osmotic minipumps. It must be noted that the primary objective of these studies was to study dysphoria-like effects of withdrawal after cessation of chronic delivery, not tolerance to the euphoria-like effects, and therefore

treatment regimens rarely were optimized for observing tolerance. That said, tolerance to the rate-suppressing effects were evident on the first day or two of continuous infusion, even though tolerance to threshold-lowering effects were not observed at any time during the course of chronic infusion.

Sensitization of euphoria-like effects

Sensitization of drug-induced behavioral effects with repeated intermittent drug administration has been characterized most thoroughly as increased responsiveness to the locomotor stimulating effects of agents such as cocaine, D-amphetamine, morphine, PCP, etc. Positive evidence of sensitization to the effects of abused substances on responding for BSR has been observed primarily by one group of investigators employing measures requiring high rates of responding, while several other groups have failed to demonstrate sensitization of BSR threshold-lowering effects of drugs. For example, Kokkinidis and colleagues have observed sensitization of the rate-increasing effects of 1–2 mg kg⁻¹ D-amphetamine administered daily for 10–20 days in ICSS studies using the VTA, substantia nigra, or nucleus accumbens as the stimulation site. Evidence of sensitization included progressive potentiation of the rate of responding elicited by the daily-treatment dose, and a significant increase in responding for low (0.3–0.5 mg kg⁻¹) challenge doses of D-amphetamine that do not alter ICSS responding in drug-naïve animals. Notably, this research group has demonstrated that the emergence of sensitization to a threshold challenge dose of D-amphetamine (0.5 mg kg⁻¹) was more pronounced when daily ICSS testing occurred immediately after each daily injection of D-amphetamine (2 mg kg⁻¹) than when ICSS testing occurred prior to daily treatment. This temporal contiguity effect suggests a critical role for contextual conditioning factors in development of the sensitized ICSS response – a factor long known to play a facilitatory role in the development of locomotor sensitization to drugs such as cocaine, amphetamines, and morphine. In contrast, the vast majority of studies employing threshold procedures to more clearly separate euphoria-like effects from rate-altering effects have yielded negative evidence of sensitization of threshold-lowering effects of nicotine, cocaine, D-amphetamine, PCP, or morphine. Taken together, the results to date provide ample evidence of sensitization to the rate-altering, but not the threshold-altering or euphoria-like, effects of abused substances in ICSS paradigms.

Concluding Thoughts

As described in the preceding sections, reductions in BSR thresholds during acute drug intoxication and withdrawal-related increases in BSR thresholds are among the

most reliable and reproducible of findings across multiple drug classes. However, evidence of tolerance and/or sensitization to the euphoria-like effects of abused drugs in ICSS have proven difficult to demonstrate on a consistent basis. Studies with both tolerance- and sensitization-inducing regimens of drug treatment have provided numerous positive results in rate-dependent measures of ICSS behavior, but rarely yield positive evidence when BSR thresholds are assessed separate from pure rate of responding. The few positive findings of tolerance to the threshold-lowering effects of morphine and cocaine suggest that there may be parameters (e.g., very high doses of cocaine) that may support these adaptive responses to euphoria-like effects measured in ICSS procedures. Perhaps thorough parametric analysis of tolerance and sensitization using shifts in drug dose-effect functions rather than probing the effects of single test doses will provide a clearer picture of the permissive conditions.

The broad range of conditions that fail to support the development of tolerance or sensitization to the euphoria-like effects of abused substances stands in stark contrast to the ability to demonstrate dysphoria-like effects of withdrawal, another index of neuroadaptation in reward circuitry, across a vast array of parameters and abused substances. In this regard, it is notable that some studies on tolerance and sensitization of BSR thresholds measured ICSS behavior at times when withdrawal from the preceding drug injection may have been present, and this factor should be systemically examined in future studies for its possible confounding influence. Moreover, some work by the Wise and Carlezon research groups suggests that repeated electrical stimulation of brain-reward circuitry during ICSS training and testing may by itself induce neuroadaptation that confounds ready demonstration of tolerance or sensitization to drug-induced alterations in BSR thresholds. If this is true, then ICSS may not be an optimal paradigm with which to assess development of tolerance and sensitization phenomena. Nonetheless, its utility in measuring the direct, unconditioned, euphoria-like effects of acute drug intoxication and dysphoria-like effects of withdrawal from both acute and chronic drug exposure cannot be questioned.

See also: Acute Dependence; Animal Models of Behavior; Alcohol Addiction; Alcoholism; Cellular Plasticity in Cocaine and Alcohol Addiction; Comorbidity – Depression; Depression; Drug Addiction; Drug Priming; Drug Sensitization and Drug Abuse; Drug Withdrawal – Motivational View; Hallucinogens; Molecular Neurobiology of Addiction; Neural Basis of Working Memory; Neurobiology of Opioid Addiction; Nicotine; Psychostimulants; Rewarding Brain Stimulation; Transition to Addiction; Vulnerability Factors in Addiction Disorders; Δ9-THC.

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Cellular Plasticity in Cocaine and Alcohol Addiction

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Glossary

Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) – One subclass of glutamate receptors.

Dopamine (DA) – A neuromodulator implicated in regulation of learning and memory, motivated behavior, and other neural functions.

GluR – A protein subunit (GluR1-4) of the AMPAR.

Long-term depression (LTD) – In some cases a persisting decrease in AMPAR function after repeated stimulation of glutamate release.

Long-term potentiation (LTP) – In many cases a persisting increase in AMPAR function after repeated stimulation of glutamate release.

LVGCC – L-type voltage-gated calcium channels which in some cases provide calcium needed for LTD or LTP induction.

Metabotropic glutamate receptors (mGluRs) – Receptors activated by glutamate that do not link directly to a Na^+ - or Ca^{2+} -permeable ion channel.

Nucleus accumbens (NAcc) – A brain region critical for regulating many motivated behaviors.

N-methyl-D-aspartate receptors (NMDARs) – One subclass of glutamate receptors.

NR2B – A subunit of the NMDAR.

SK – The calcium-activated SK potassium channel

Ventral tegmental area (VTA) – A major source of dopamine in the brain.

The mesolimbic dopamine system, formed in part by the ventral tegmental area (VTA) and nucleus accumbens (NAcc), is an integral part of the brain's natural reward circuit. The VTA is a major source of dopamine (DA) for the brain circuit involved in encoding of reinforcement and learning, and the NAcc is a critical node that integrates limbic and motivational input (including DA signals from the VTA) to influence behavioral output. Thus, these brain regions, in concert with other areas such as the prefrontal cortex, thalamus, and amygdala, are considered to play a critical role in the control of motivated and goal-directed behaviors, including the development and expression of addiction. In addition, recent studies suggest that addiction is a form of maladaptive learning, where neural links can be formed between the action of taking the drug and the drug-related reward or withdrawal-related negative states. For this reason, this article, in part, addresses the consequences of passive versus

active exposure to drugs. Repeated passive exposure to a given drug can enhance or sensitize the locomotor-activating effects of that drug, called behavioral sensitization. Since locomotor sensitization can be very long-lasting and can enhance subsequent drug self-administration, sensitization has been considered a model of enhanced drug seeking during abstinence. Drug-related sensitization has been observed in humans, and can contribute to enhancement of psychoses with repeated psychostimulant exposure. However, although pharmacological effects through passive drug exposure can produce enduring plastic changes, human drug intake is typically active and voluntary, and associative learning between drug taking and reinforcing or negative consequences may be a critical component in the development of addiction.

Overall, this article seeks to address the interesting possibility that drugs of abuse produce persistent changes in neuronal function that may drive drug seeking after periods of abstinence, especially prolonged abstinence. The ability of drugs to alter neuronal function was originally investigated predominantly through biochemical methods such as Western blot, which allow one to determine changes in protein levels or phosphorylation state of a given receptor or channel subunit. More recently, large-scale screening for changes in protein levels (using proteomics) or mRNA levels (using DNA microarrays) have shown great promise for indicating potential changes in receptor or channel function. Importantly, *ex vivo* electrophysiological techniques in brain slice have allowed direct examination of functional changes in excitatory synaptic strength or ion channel activity after drug exposure. Electrophysiological studies are particularly critical because they provide detailed information about the functional state of a given receptor or channel, which can occur without concurrent alterations in total protein or mRNA.

Thus, we examine evidence that abused substances can cause long-term changes (after 1 day or more of withdrawal) in glutamate receptor and ion channel function in the VTA and NAcc. Furthermore, we focus here on cocaine and alcohol, although interesting studies have also been performed in relation to other abused drugs such as morphine, nicotine, and amphetamine.

Ionotropic Glutamate Receptors

Ionotropic glutamate receptors can be broken into two distinct classes: alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), which

generate fast depolarization to contribute to neuronal firing, and N-methyl-D-aspartate receptors (NMDARs), which are voltage activated and allow passage of cations including calcium. Thus, NMDAR currents both depolarize the neuron and supply calcium, which is critical for induction of synaptic plasticity in many systems. Also, NMDAR-dependent (and NMDAR-independent) synaptic plasticity often involves enduring changes in AMPAR function, although plasticity can also occur in the probability of glutamate release, intrinsic ion channel activity, or other aspects of neuronal physiology.

AMPARs are typically composed of four subunit proteins (GluR1-4), which can form hetero- or homomeric complexes. In both the VTA and NAcc, AMPARs are thought to exist as heteromeric complexes containing both GluR2/3 and GluR1 subunits in the basal state, before any changes in synaptic strength occur, and reflect a state where synaptic strength is low. In several cases, changes in AMPAR function during glutamatergic plasticity are associated with altered trafficking of particular AMPAR subunits to the plasma membrane or the post-synaptic density. Importantly, there are several powerful techniques which allow delineation of changes in AMPAR subunit composition during plasticity, including sensitivity to subunit-selective inhibitors and analysis of the rectification index. The latter measure is widely used, since a decrease in the rectification index, due to a reduced AMPAR current measured at +40 mV versus -70 mV, indicates an increased plasma membrane occurrence of GluR2-lacking receptors, primarily through increases in GluR1. This is important because only GluR2-lacking AMPARs can pass calcium, which may facilitate calcium-dependent plasticity.

Synaptic Plasticity in the VTA Following Cocaine Exposure

DA neurons of the VTA are crucial for many aspects of goal-directed behavior. Midbrain DA neurons exhibit two patterns of firing activity: a pacemaker firing responsible for tonic DA levels in target regions, and burst firing,

likely responsible for phasic DA changes in midbrain target regions. Burst firing in particular may indicate salience of important stimuli, which can serve to initiate goal-directed behavior as well as act as a teaching signal. Thus, phasic activation of VTA DA neurons is thought to promote behavior, especially in relation to conditioned stimuli. Importantly, modeling studies have shown that changes in AMPAR number or function can alter firing of VTA DA neurons, perhaps by indirectly facilitating NMDAR activity and burst firing. In this regard, excitatory synapses onto VTA DA neurons are highly plastic, exhibiting NMDAR-dependent long-term potentiation (LTP), long-term depression (LTD), as well as short-term plasticity. Thus, it is hypothesized that glutamatergic plasticity could modify AMPAR function in response to the environmental context and demands on the organism, and could significantly alter generation of firing in VTA DA neurons during goal-directed behavior. Indeed, learning in relation to behavioral sensitization to cocaine as well as cocaine and morphine-induced conditioned place preference can both be blocked by VTA NMDAR antagonists.

Interestingly, Ungless *et al.* were the first to report that, 24 h after a single exposure to cocaine, the ratio of AMPAR-mediated current to NMDAR-mediated current (termed the AMPAR–NMDAR ratio) is significantly elevated at excitatory synapses in the VTA relative to saline-injected controls (Table 1). The increase in the AMPAR–NMDAR ratio after cocaine exposure occludes further LTP induction, suggesting that these synapses are already in an LTP-like state. Importantly, LTP of VTA glutamatergic synapses is observed after a single exposure to many other drugs of abuse, demonstrating a convergence of cellular effect within the VTA by many abused drugs. The mechanism underlying LTP of excitatory synapses onto VTA DA neurons appears to be mediated by the insertion of GluR2-lacking AMPARs. This insertion of GluR2-lacking AMPAR subunits is thought to be transient, so that the GluR1 subunits are eventually replaced by newly locally synthesized GluR2/3 after many hours. Further, the insertion of GluR2-lacking AMPARs can be reversed

Table 1 Estimated consequence of repeated cocaine or alcohol on VTA and NAcc function

	Cocaine VTA	Cocaine NAcc	Alcohol VTA	Alcohol NAcc
Early withdrawal (6–24 h)	↑	↓	↓	↑
Protracted withdrawal (weeks)	↑ ^a	↑ ^b	↑	↑
Neuro-adaptation important for relapse/reinstatement?	n.d.	Yes	n.d.	Yes

^aVoluntary intake only.

^bIn some cases can be reversed by cocaine re-exposure.

We should note that multiple neuro-adaptations can occur simultaneously with opposite effects on excitability, especially during early withdrawal, making the integrated effect more difficult to determine, n.d. not determined.

by activation of the metabotropic glutamate receptor 1 (mGluR1), which induces replacement of GluR2-lacking receptors with GluR2-containing receptors. This is hypothesized to readjust synaptic strength back to basal levels and therefore prevent behavioral changes that could contribute to development of addiction.

Thus, LTP changes in VTA DA neurons after a single cocaine exposure are transient, exhibiting potentiated AMPAR currents 5 but not 10 days after cocaine exposure. The same time course for potentiated VTA AMPAR currents is also observed following repeated cocaine injection, suggesting that increased cocaine exposure does not *per se* increase the duration of the VTA AMPAR enhancement. Also interesting is that a single cocaine injection produces changes in opiate conditioned place preference and aversion with a similar time course (altered conditioning 5 but not 10 days after cocaine exposure), and these effects of cocaine were prevented by inhibition of NMDARs in the VTA during cocaine exposure. Since LTP often requires NMDARs, this is strong evidence that AMPAR LTP in the VTA after cocaine exposure could enhance Pavlovian conditioning and perhaps other reward-related learning.

Importantly, because pathologic drug use and seeking in humans is believed to involve associative learning mechanisms, it is critical to examine plasticity changes under conditions where animals voluntarily self-administer cocaine. Indeed, a recent study examined plasticity changes in the VTA in rats during abstinence from self-administered cocaine. Unlike previous experiments where animals passively received experimenter-administered cocaine, enhanced glutamate function in VTA DA neurons from cocaine self-administering rats remained significantly elevated for up to 3 months of abstinence (Table 1). Importantly, control experiments in this same study showed that rats given yoked cocaine infusions (noncontingent delivery of cocaine in a similar pattern and dosage as cocaine self-administering rats), or that self-administered food or sucrose, exhibited short-term but not LTP-like changes in VTA DA neurons. This study illustrates the importance of the role of associative learning and memory mechanisms in the generation of long-term plasticity after drug exposure. Finally, although there are some mixed results in biochemistry experiments, the majority of studies show an increase in GluR1 expression level after withdrawal from cocaine self-administration (Table 1). Changes in the VTA NMDAR levels have also been observed during cocaine withdrawal, although electrophysiological studies have not been performed to corroborate these results. Taken together, these data suggest that a number of behavioral conditions can produce short-term plasticity in the VTA (passive cocaine exposure, self-administration of natural reinforcers), but the combined outcome of cocaine's pharmacological effect

with the animal's volition to take cocaine can produce a long-lasting potentiation of glutamate transmission onto VTA DA neurons.

Nucleus Accumbens Glutamate Receptors Following Cocaine Exposure

The NAcc is another integral part of the brain's reinforcement circuit, and many studies concur that synaptic plasticity in the NAcc can be altered by cocaine exposure. LTP of glutamatergic transmission onto medium spiny neurons, the main output neurons of the NAcc, was first observed following passive, experimenter-administered cocaine injections (Table 1). However, unlike the VTA where LTP is elicited after a single cocaine exposure, NAcc synaptic plasticity was observed only after 5 daily cocaine injections. After repeated cocaine exposure, mice show both cocaine-induced behavioral sensitization and LTP *ex vivo* in NAcc shell but not core. Further, cocaine injection 24 h before recording converts the cocaine-associated LTP back to the basal state of LTD. Thus, the history of cocaine exposure can readily change the direction of synaptic plasticity in the NAcc. In addition, unlike the VTA, AMPAR potentiation in the NAcc following experimenter-administer cocaine was not attributed to changes in AMPAR subunits, since analyses of rectification index revealed no rectification before or after cocaine exposure. This is also consistent with the result that blocking constitutive recycling of GluR2-containing AMPAR prevented NAcc LTD and reduced expression of cocaine-induced sensitization, perhaps indicating a role for NAcc LTD in the enhanced locomotor activation by acute cocaine after sensitization.

Paralleling studies in the VTA, NAcc plasticity induced following cocaine self-administration revealed differences between passive and voluntary cocaine self-administration. In cocaine self-administering rats, LTD in the core and shell of the NAcc was inhibited after 1 day of abstinence. However, after 21 days of abstinence, LTD induction remained occluded in the NAcc core, but could be readily induced in the NAcc shell. This result suggests that voluntary cocaine self-administration induces long-lasting glutamatergic neuroadaptations exclusively in the NAcc core, a region associated with control of behavior by drug-related stimuli and relapse. In support of this finding, a number of studies have observed increased NAcc GluR1 levels after cocaine self-administration (Table 1). Importantly, a recent study demonstrated an increase in GluR1 subunits in the NAcc core, but not the NAcc shell, during abstinence from cocaine self-administration. Increased NAcc core GluR1 levels are associated with an increase in rectification in AMPAR currents, as would be expected from plasma membrane insertion of GluR1-containing AMPARs. Importantly, to demonstrate

that addition of GluR1-containing AMPARs plays a significant role in cocaine relapse, this study showed that intra-NAcc injection of antagonists that block only GluR1-containing AMPARs significantly reduces cocaine reinstatement.

In addition to altered AMPAR function, recent work has shown the importance of changes in the Homer proteins, scaffolding proteins that bind directly to mGluRs and indirectly to NMDARs, as critical neuroadaptations that can drive cocaine seeking. Repeated cocaine and abstinence is associated with reduced NAcc protein levels of Homer1b/c and Homer2a/b isoforms and group I mGluRs (mGluR1 and mGluR5) (**Table 1**). Activation of group I mGluRs with the NAcc can increase NAcc glutamate levels and produce locomotor activation. However, mGluR enhancement of NAcc glutamate levels and locomotor activation is blunted after 3 weeks but not 24 h of withdrawal from repeated cocaine injection, in agreement with reduced mGluR and Homer protein levels. No reductions in mGluR or Homer levels are observed after 3 weeks of withdrawal from cocaine in any other brain region, including the VTA. Also, under some models of cocaine exposure, the time course of changes in NAcc mGluR and Homer differ, but whether this is due to active versus passive cocaine exposure or other methodological details is unclear.

Alcohol and Glutamate Receptors

Alcohol is both highly addictive and legal to obtain, and alcohol abuse is considered the third leading preventable cause of human death; thus, alcoholism extracts enormous social and economic costs relative to other drugs of abuse. For this reason, neuroplastic changes that contribute to the pathological, compulsive alcohol seeking and relapse are of particular interest, especially because alcohol seeking and intake occurs even in the face of detrimental consequences, and represents a significant clinical hurdle when attempting to overcome alcohol use disorders.

A number of studies concur that, early in withdrawal from alcohol, there is increased NMDAR function in several brain regions, including the NAcc (**Table 1**). The exact changes in particular NMDAR subunit levels vary among brain regions, and may depend on whether alcohol intake is continuous or intermittent. Some studies have also observed increased NMDAR function without changes in subunit levels, for example, through changes in subcellular localization. In the NAcc, increases in both NR1 and NR2B have been observed during early withdrawal from alcohol, as well as increased levels of mGluR1 and Homer 2b (see below). One study also observed that long-term alcohol strongly upregulates the NAcc NR1-2 isoform, which lacks the C1 cassette of NR1 and thus is inefficient in

trafficking or anchoring NMDARs to the proper PSD sites. In any case, a general hypothesis is that increased NMDAR function during withdrawal from alcohol will enhance neuronal excitability and drive withdrawal symptoms and resumption of alcohol consumption. Since negative affects can strongly promote alcohol seeking, antagonism of NMDARs is an attractive clinical intervention to reduce early withdrawal symptoms.

In the VTA, increased NMDAR subunit levels are observed after chronic forced alcohol intake. In addition, a recent study found increased VTA AMPAR function after alcohol self-administration. Although enhanced glutamate receptor function would be predicted to increase VTA excitability during withdrawal, decreased activity of VTA neurons and decreased DA release in terminal regions is observed during early withdrawal (**Table 1**), and this hypoactivity is reversed by systemic inhibition of NMDARs. Thus, NMDAR activity in a region other than the VTA may predominate in terms of control of VTA activity during early withdrawal from alcohol as well as other drugs of abuse. Also, one study found reduced NMDAR excitation of VTA firing *ex vivo* during withdrawal from alcohol. Importantly, reduced DA levels in VTA target regions likely contribute to alcohol seeking, since self-administration of alcohol during withdrawal continues until NAcc DA levels are normalized. We should also note that VTA DA neurons may exhibit hypoactivity during withdrawal from alcohol, but it is also possible that enhanced NMDAR and AMPAR function after chronic alcohol could facilitate VTA neuron activity while the animal is consuming alcohol, especially since alcohol exposure can facilitate firing of VTA neurons.

During longer withdrawal from alcohol, the NAcc shows a different pattern from cocaine in terms of glutamate receptor levels, with increases in NR2B, mGluR1, and Homer2b (instead of the decreases associated with cocaine) (**Table 1**). These changes are observed after 2 weeks withdrawal from 3 months alcohol drinking, with an increase only in Homer2b after 2 months withdrawal. Also, changes in all three proteins are observed after shorter-term passive exposure or binge drinking, and thus the time course of glutamate receptor changes may depend on the duration and route of alcohol exposure. Interestingly, viral overexpression of Homer2b in the NAcc increases alcohol preference and alcohol-related place preference, suggesting that mimicking the drinking-induced increase in Homer2b is sufficient to enhance alcohol preference and reinforcement. Furthermore, NAcc Homer2b overexpression increases alcohol-induced NAcc glutamate and DA release, a phenotype that has been associated with increased alcohol preference and has been linked to mGluR1/5 regulation of glutamate release. Thus, enhanced NAcc Homer2b levels, acting through mGluRs, could facilitate the

reinforcing effects of alcohol after long-term intake and thus promote alcohol seeking.

Altered Intrinsic Excitability after Alcohol or Cocaine

In order for glutamate receptor activation to generate action potential firing, the synaptically generated excitatory postsynaptic currents (EPSCs) must travel along dendrites to reach the area where action potentials are generated, which is in the spike-initiation zone of the axon hillock in many neurons, and in a primary dendrite in the VTA. Although passive cable properties defined by the dendritic geometry can greatly filter EPSCs, numerous ion channels can amplify or degrade passing EPSCs. Thus, alterations in ion channel function could greatly alter EPSC translation into action potentials, as well as modulate the ion flux within synapses which impacts generation of LTD or LTP. Of particular interest is the possibility that persistent changes in ion channel regulation of NAcc or VTA firing could dramatically alter the ability of relapse-inducing stimuli to drive firing of these brain regions and to facilitate cravings and relapse during abstinence.

L-type voltage-gated calcium channels (LVGCCs) are particularly interesting, since LVGCC misregulation could represent a common mechanism underlying early withdrawal from many abused drugs, including alcohol. Chronic alcohol upregulates LVGCC levels in several brain regions, although mixed results have been observed in the few studies from the striatum. Furthermore, systemic administration of LVGCC antagonists decreases alcohol withdrawal symptoms as well as the depression in DA levels associated with withdrawal from alcohol and other drugs. There is also considerable evidence from human preclinical trials suggesting that LVGCC antagonists may ameliorate withdrawal, tolerance, and cravings in human alcoholics and addicts. Also, in the striatum, LVGCCs are necessary for induction of LTD, and NAcc LTD is necessary for the expression of behavioral sensitization to cocaine. Thus, like NMDARs, antagonism of LVGCCs may represent a clinical intervention to reduce early withdrawal symptoms, although the brain regions in which LVGCC alterations mediate withdrawal symptoms have not been identified.

Changes in NAcc ion channel activity after repeated cocaine injection and 3 days withdrawal are well studied. During withdrawal, there is increased potassium channel activity, decreased N- and R-type VGCC activity, and decreased sodium channel activity, which overall depress NAcc neuron excitability (**Table 1**). Decreased calcium channel activity might also depress induction of glutamatergic plasticity, neurotransmitter release, and perhaps calcium-dependent activation of kinases or other regulatory molecules. Interestingly, some cocaine-associated changes in channel function may be secondary to altered

tonic activity of intracellular signaling molecules. Increased tonic protein phosphatase activity and increased tonic PKA activity could underlie the reduced VGCC and sodium channel function, respectively. Psychostimulant-related changes in intrinsic excitability have also been identified in the prefrontal cortex and subiculum.

VTA DA neuron firing after alcohol exposure is also well studied, with a reduction in firing during the first 1–6 days of withdrawal from alcohol (**Table 1**). We should note that the possible role of ion channel changes in this decreased VTA neuron excitability is poorly understood, with even mixed results regarding whether VTA firing is reduced following alcohol exposure in the *ex vivo* brain slice preparation. Nonetheless, also of interest are changes in channel function that persist beyond the early withdrawal period. A recent study found that calcium-activated SK potassium channel (SK) function is significantly reduced in VTA DA neurons after 7 days withdrawal, with a small reduction also in the I_h current (which was also observed after one day of withdrawal in a different study). Although baseline pacemaker firing is not altered after alcohol exposure, NMDA receptor activation increases firing rate in control animals but leads to burst firing in alcohol animals. This is in agreement with *in vivo* and *ex vivo* studies showing that SK inhibition facilitates glutamate-induced bursting in midbrain neurons. Since bursting is associated with increased DA release in VTA terminal regions, loss of SK could enhance DA release in response to alcohol-related stimuli and thus facilitate alcohol seeking.

Most critical are neuroadaptations present after even longer periods of abstinence from alcohol, since these could anchor the increased propensity for relapse in human alcoholics apparent after even months or years of abstinence. An early study showed that protracted abstinence from long-term alcohol intake was associated with a reduced afterhyperpolarization in the dentate gyrus; the SK makes a strong, although not unique, contribution to the afterhyperpolarization. Thus, these electrophysiological data, as well as work from biochemistry and gene chip studies, identify changes in SK as well as other ion channels and neurotransmitter receptors as critical neuroadaptations in the NAcc and other brain regions that may facilitate alcohol seeking during protracted abstinence from alcohol.

Conclusions and Future Directions

Although there are tantalizing clues toward the effect of chronic drug exposure on glutamate receptor and ion channel function, many questions remain. Few studies have examined excitability changes beyond the period of early withdrawal, although recent studies of glutamate receptor and ion channel function have begun to address this question after cocaine, alcohol, or heroin exposure.

Identifying long-term changes is particularly critical since neuroadaptations persisting after weeks or months of abstinence may mediate long-term susceptibility to cravings and relapse. Importantly, these long-term neuroadaptations may also correlate with structural changes in spine density or dendritic architecture that can occur in the NAcc after passive or active cocaine administration. It is also clear that self-administration and passive administration can differentially alter gene expression and protein function, perhaps due to learned cues that come to be associated with the act of voluntary self-administration and that can reinforce further alcohol or drug seeking in a conditioned, involuntary manner. Fortunately, the field possesses a battery of powerful techniques including traditional Western blot protein analyses, larger-scale genomic and proteomic methods, local knockdown or overexpression of particular signaling molecules during behavior, and *ex vivo* brain slice electrophysiology from animals that have learned to self-administer cocaine or alcohol. Although laborious and technically challenging in adult animals, we argue that *ex vivo* electrophysiology is especially critical for uncovering and defining which neuroadaptations might persist during abstinence, in part because potent functional changes can occur without altered total protein or mRNA levels. In addition, exact delineation of the molecular alterations apparent after abstinence could allow one to utilize very precise antagonists to reverse that molecular change (e.g., reversing the plasma membrane trafficking of particular AMPAR subunits), and perhaps provide a novel therapeutic intervention for addiction and alcoholism.

See also: Basal Ganglia; Drug Addiction; Molecular Neurobiology of Addiction; Motivation; Neurophysiology of Drug Reward.

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Comorbidity – Depression

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Glossary

Ahedonia – An inability to experience pleasure from normally pleasurable activities.

Dysthymia – A chronic mood disorder with symptoms similar to, although less severe than, major depressive disorder.

Dysphoric – Having an unpleasant mood state which may include sadness, irritability, and anxiety.

Major depressive disorder (MDD) – A chronic, severe mood disorder.

Selective serotonin reuptake inhibitors (SSRIs) – This class of drug includes fluoxetine, paroxetine, sertraline, citalopram, and escitalopram, and is commonly used to treat depression and anxiety.

disorder, depression occurring as part of a bipolar illness, dysthymia, depression directly secondary to a general medical condition, and the variety of syndromes falling under the category of “depressive disorder not otherwise specified.” There is also depression secondary to the effects of a substance, which can be exceedingly difficult to separate from true comorbidity.

In North America, depression affects 8–10% of the population in a given 1-year period, with lifetime rates between 15% and 20%. Rates are higher (up to 25% lifetime incidence) in women. Onset can be anywhere from childhood to the end of life. Risk factors include family history of depression, medical illness or chronic pain, high levels of life stressors, social isolation, and low socioeconomic status.

Substance Use Disorders

Between 3% and 10% of Americans over age 12 use illicit substances (not including alcohol or nicotine) yearly, with higher use among men and rate of use being inversely proportional to education, level of employment, and socioeconomic status. Over half of those using illicit substances are younger than 30 (which may be one reason substance use precedes depression in a majority of patients with both illnesses). The most commonly abused drugs are prescription drugs, including anxiolytics and opiates, followed by marijuana.

Almost half of Americans over age 12 describe themselves as current alcohol drinkers, and a similar number have engaged in heavy drinking (more than five drinks on one occasion). Binge drinking most commonly occurs in the late teens and early 20s, and is more common among men. Approximately 30% of Americans over age 12 consider themselves to be regular smokers, and well over half report that they have tried to quit smoking and failed at least once.

Suicide

Approximately 1 million people per year commit suicide, with women being more likely to attempt but men being more likely to complete. The two biggest risk factors for suicide are depression and substance use, making patients with comorbid depression and substance use an especially high-risk combination. Up to 40% of people attempting or completing suicide are intoxicated at the time of the attempt; these numbers are higher in younger people.

Epidemiology

Depression

There are several distinct syndromes which fall under of the category ‘depression;’ these include major depressive

Lifetime risk of suicide in patients with alcohol dependence is tenfold that of the general population, and both chronic alcohol use and acute intoxication are associated with a higher risk of suicide attempts and completions. Opiate dependence is also linked to increased risk of suicide attempts and completions, particularly in patients who have also had accidental overdoses. Higher-than-average suicide risk has also been shown in patients dependent on cocaine, amphetamines, and many other substances.

Comorbidity

Patients with depression have an approximately 33% lifetime prevalence of problematic alcohol use. Patients with higher alcohol intake after a stressful event are more likely to develop depression than those with lower intake. In addition, patients with depression are less likely to attain sobriety and more likely to relapse if they do.

Abuse or dependence on opiates, including heroin, opium, and prescription narcotics, is also correlated with depression. As is the case with alcohol, patients with both depression and opiate dependence are less likely to attain sobriety and more likely to relapse than nondepressed opiate-dependent patients.

Stimulant abuse, including cocaine and amphetamines, is highly correlated with depression, both during and after the period of dependence. Several studies have also shown a correlation between cigarette smoking and a higher incidence and severity of depressive symptoms; it is unclear whether this is cause or effect.

There is also significant correlation between early-onset heavy or problematic marijuana use and later development of depression and suicide attempts; this correlation persists after controlling for possible confounding variables. Patients who develop depression have not been consistently shown to be at increased risk for marijuana use or dependence, calling the self-medication theory into question; there are, however, some smaller studies which do support an antidepressant effect of cannabis. There is clearly a correlation between lifetime cannabis dependence and lifetime incidence of major depressive disorder (MDD); it is still not clear how much of this is due to confounding variables. It is also not clear if recent cannabis use has a significant effect on, or is affected by, depression. One study suggests that depressed patients who use marijuana are less likely to experience euphoria during use and are more likely to experience anxiety, depression, and dysphoria than nondepressed users.

Ecstasy, or methyl-d-methamphetamine (MDMA), is a semisynthetic amphetamine which inhibits serotonin reuptake and induces release of norepinephrine and dopamine. Some studies have shown that people with depressive disorders are more likely to use MDMA, and use it more frequently than nondepressed people, possibly

as a means of self-treatment; other studies suggest that those who use MDMA infrequently have the same incidence of depression as the general population. Depression precedes MDMA use more often than the other way around, although acute withdrawal from MDMA often includes dysphoria, and there is some evidence that heavy MDMA use can lead to longer-lasting depressive symptoms.

Few studies exist on comorbidity of depression and benzodiazepine dependence. Many patients with depression are prescribed benzodiazepines, either as a mood stabilizer, an augmenting antidepressant, or a treatment for comorbid anxiety. Benzodiazepine overdose is a common form of suicide attempt, although generally benzodiazepines are only lethal if combined with alcohol or other gamma aminobutyric acid (GABA)ergic drugs.

There are also few studies on the relationship between barbiturates and depression; most of those that have been done examine the effect on mood when barbiturates are used to treat epilepsy or as part of a course of electroconvulsive therapy, rather than on depression in barbiturate-dependent patients. This may be in part due to the relatively uncommon occurrence of barbiturate dependence. The studies that do exist present conflicting data; some studies have shown that epileptic patients treated with barbiturates are more likely to be depressed, whereas others show that when depressed epileptic patients are treated with barbiturates, their depression improves.

Symptoms

Depression

The core depressive symptoms are depressed mood and loss of interest in activities that were formerly pleasurable (anhedonia). Other significant symptoms include changes in sleep (hypersomnia or insomnia), changes in appetite (either increased or decreased), poor concentration, low energy or fatigue, feeling guilty, feeling worthless, feeling hopeless, psychomotor slowing, and suicidal thoughts. Many of these symptoms also occur with substance intoxication or withdrawal, which makes it very difficult to distinguish between a depression secondary to substance use and a depression comorbid with substance use. Some of these symptoms are mitigated by substance intoxication, leading to the theory that some patients may begin or continue using substances in an effort to self-medicate their depression. This self-medication is described most commonly in middle-aged women, and after chronic use the medications themselves will induce depressive symptoms during either intoxication, withdrawal, or both.

Substance Use Disorders

Intoxication

Many substances have acute effects which may relieve depressive symptoms. Cocaine, amphetamines, and other stimulants, as well as MDMA, have a euphoric high, while opiates, alcohol, and anxiolytics may relieve comorbid anxiety, temporarily improve sleep, or allow for an escape from depression. For obvious reasons, few drugs of abuse consistently cause depressive symptoms during intoxication, although several, including stimulants and hallucinogens, may at times cause dysphoria, irritability, or panic in certain individuals.

Acute withdrawal

The acute withdrawal from most substances induces effects directly opposite of those induced by the high. Most drugs which involve a euphoric, elevated, or activated high can mimic depression during the acute withdrawal period. Depression, often severe, is a hallmark of acute cocaine withdrawal. There is some evidence that this withdrawal period is worse with crack use than with intranasal or intravenous use. Symptoms during this period may include severely depressed mood, suicidality, hypersomnia, hyperphagia, fatigue, and inability to concentrate – all symptoms of depression. Acute withdrawal from amphetamines and other stimulants is similar. Depressed mood may also occur with acute withdrawal from nicotine, hallucinogens, MDMA, alcohol, and opiates. Patients often relapse as a result of this dysphoric mood.

Protracted withdrawal

Some drugs cause long-lasting, or even permanent, brain changes which may result in depressive symptoms lasting for years after sobriety is achieved. Long-term cocaine users can develop chronic anhedonia, which may persist even after years of sobriety; this is thought to be related to permanent changes in dopamine neurotransmission from downregulated postsynaptic D2 dopamine receptors. In animal studies, high doses of MDMA have been shown to destroy serotonergic axons, which may explain why heavy MDMA use can lead to a depressive syndrome lasting years after the last use. Inhalants and solvents cause nonspecific brain damage, which can at times include a depressive syndrome.

Chronic use

Current chronic use of many substances may cause depressive symptoms; this is due both to the direct neurochemical effects and to the lifestyle and social consequences of chronic use. Patients who are currently abusing or dependent upon alcohol often have depressed mood, anhedonia, insomnia, poor appetite, difficulty concentrating, feelings of guilt about their drinking, feelings

of hopelessness and worthlessness, and suicidality – basically the entire complement of depressive symptoms. This is also true of patients who are currently using opiates or anxiolytics, and is true of those using stimulants when they are between highs. Current smokers have also reported higher levels of depressive symptoms and anxiety than do nonsmokers or former smokers.

Neurobiology and Genetics

Neurobiology

The three neurochemicals thought to be most strongly related to depression are serotonin, norepinephrine, and dopamine. When drugs of abuse act directly on these circuits, as with cocaine, for example, it is easy to see how comorbidity (and also substance-induced depression) can occur. However, many drugs of abuse do not act primarily or directly on these circuits – nicotine acts on nicotinic and cholinergic circuits, alcohol acts widely, opiates and cannabinoids act on their own specific receptors, and anxiolytics tend to act on the GABA system. While the neurobiology of these three amines certainly contributes to understanding the comorbidity of substance-use disorders and depression, it is not fully explanatory. Important findings in MDD have included that GABA brain activity is significantly downregulated in depression as well as most forms of drug abuse. The role of cholinergics, cannabinoids, and opiates in mood regulation has also become evident in the last 20 years.

Depressive disorder rates in stimulant-dependent individuals are substantially higher than community rates. The comorbidity of these disorders may reflect shared neurochemical alterations in the function of serotonin, dopamine, and peptide systems, such as corticotropin-releasing factor and neuropeptide Y. These alterations are observed in humans and in animal models of depression and stimulant dependence, particularly in limbic brain structures. Since this shared neurobiology does not seem to result from significant shared heritability or genetic linkage, it is thought that stimulants induce changes in neurobiology that are similar to those found in depression; these changes might provide a future therapeutic target. Stimulant-dependent patients with a depressive disorder may be a specific subpopulation for antidepressant trials, and they might reduce their stimulant abuse when treated with antidepressants. Concomitant dependence on alcohol or opioids may influence this response; antidepressants appear to be more effective for depression in combined stimulant and opioid dependence than in combined stimulant and alcohol dependence.

Many patients experience depressive symptoms while quitting smoking; this may be partially due to a substance in tobacco which acts as an MAO inhibitor rather than the

nicotine itself; smoking may serve to treat a primary depression, and the withdrawal of the MAO inhibitor then precipitates a relapse. Developing serious major depression within 6 months after stopping smoking is significantly more likely in patients with a history of MDD than in those without an MDD history.

Depressed patients have neurobiological abnormalities which persist even after treatment with antidepressants has been completed and remission achieved. One such abnormality is a decrease in cortical GABA; it is currently not clear if the low GABA levels precede the depression or are caused by it. Use of antidepressants leads to changes in the GABA system, and GABAergic drugs have been shown to have some mood-stabilizing and antidepressant effects.

Genetics

Genetic predispositions have been demonstrated for both depression and substance use disorders. Having a family history of either depression or substance-use disorders raises the individual's risk for both. The major forms of substance dependence, including cocaine, alcohol, nicotine, and opiate dependence, are all heritable to some degree – 50–60% by estimates obtained from large family, twin, and adoption studies. In the case of comorbidity, it may be that a single gene predisposes to both depression and substance-use disorders, or that a gene which predisposes to depression is linked to a gene which predisposes to substance use disorders.

The S and L types of the serotonin transporter have been shown to be related to depression, with homozygous S individuals being more prone to depression than heterozygotes, who in turn are more prone to depression than homozygous L individuals. The S allele has also been linked to higher rates of nicotine dependence in some studies, although other studies contradict this.

Some studies have suggested that alcohol dependence and mood disorders co-occur more frequently than would be expected by chance alone, while other studies suggest that they vary independently. Studies involving the mu opiate receptor polymorphism have shown one mu receptor form which predicts alcohol dependence responsive to naltrexone therapy; non-alcohol-dependent female relatives of these subjects had higher-than-average rates of depression. A study done in non-alcohol-dependent men showed that the men with a positive family history of alcohol dependence felt a lower subjective level of intoxication for a given blood alcohol level than did men who had no family history of alcohol dependence; this subjective insensitivity is itself a risk factor for alcohol dependence. Linkage studies have identified several potential loci which may influence the risk of alcohol dependence; many of these are in the alcohol dehydrogenase gene cluster on the long arm of chromosome 4.

Candidate genes include genes related to alcohol metabolizing enzymes, the mu opioid receptor, the GABA_A receptor, and the muscarinic acetylcholine receptor. There are also controversial studies which suggest a serotonin transporter subtype coded for on chromosome 17 may be a candidate. The A1 allele of the D2 dopamine receptor is another controversial candidate.

A large female twin study showed that a family history of depression was predictive of cigarette smoking; this association is thought to be due to genetic factors which may predispose an individual both to smoking and to depression. Heritability of nicotine dependence is estimated to be 60% or higher. Linkage studies have shown candidate loci for genes related to nicotine dependence on at least 12 chromosomes; one candidate gene is dopa decarboxylase, and others code for cholinergic receptors, parts of the cytochrome P450 system (with those who metabolize nicotine more slowly less likely to be addicted; linked genes in this area may have important implications for treatment) and the A1 allele of the D2 dopamine receptor.

A large Australian study done with same-sex twins discordant for cannabis dependence or for early onset heavy cannabis use found that cannabis-dependent twins were two to three times more likely to have suicidal ideation or a suicide attempt. Those who began marijuana use prior to age 17 had a 3.5 times higher risk of suicide attempt, but were not at increased risk of MDD or suicidal ideation. Fraternal, but not identical, twins had an increased risk of MDD if they were cannabis-dependent as compared to their non-cannabis-dependent twin. Fraternal twins were also more likely to become cannabis dependent if they developed MDD or suicidal ideation; this did not hold true for identical twins.

Twin studies done on the heritability of cocaine abuse and dependence have found heritabilities as high as 65–80%. Several candidate loci have been identified. Candidate loci for opioid dependence have been found on chromosome 17.

Treatment

Antidepressants

Successful treatment of underlying depression modestly improves outcomes for treatment of alcohol abuse and dependence, in contrast to previous theories that addiction must be treated first. Clinical and preclinical research since the 1980s has demonstrated an inverse relationship between serotonergic activity and alcohol use; however, SSRIs are not consistently effective as treatment for alcohol abuse or dependence in heterogeneous groups. They do show promise in specific groups of alcoholics, including specific genetic groups, early- or late-onset alcoholics, and alcoholics with comorbid anxiety or depression.

There is evidence to suggest there may be distinct subtypes of alcoholism which may be distinguishable by the type and complexity of the serotonergic dysfunction. Response to serotonin uptake inhibitors varies greatly by individual, with drinking reductions ranging from 10% to 70% in some studies. A major challenge is treatment matching to predict which patients will respond to which treatment.

In contrast to alcohol dependence, treatment with antidepressants in opiate-dependent patients does not have good evidence for improving outcomes of either depression or opiate dependence; however, attaining sobriety (whether by pharmacologic or behavioral means) typically leads to a significant decrease in depressive symptoms. It is possible that this indicates an opiate-induced depression rather than a true comorbidity, at least in a majority of patients. An important caveat here is that both methadone and buprenorphine stabilization and maintenance clearly reduce depressive symptoms. The distinction in chronic effects of dysphoria and depression between these treatments and abuse of short-acting opiates such as heroin or most prescription opiates is that short-acting opiates lead to intermittent withdrawal, then relief and sometimes euphoria with a sufficiently large dose of opiate in chronic abusers with significant opiate tolerance. This intermittent pattern of symptoms in the abuser of short-acting opiates seems critical to induction of depression. Further studies are needed to fully address this question.

Several studies have indicated that treating cocaine dependence and depression simultaneously may be more successful than sequential treatment. Preclinical studies have suggested that serotonin plays an important role in the dopamine reward pathway activated by cocaine use, suggesting a possible role for SSRIs in the treatment of cocaine dependence; however, most clinical studies have failed to show an effect. SSRIs may be less useful in this population than other agents, particularly desipramine and bupropion.

Bupropion is also commonly used for patients with comorbid depression and nicotine dependence, although this may not be the ideal choice for patients who are already on antidepressants or who have high levels of anxiety.

Table 1 summarizes recent data for antidepressant medications used in the treatment of comorbid substance-use disorders and depression.

Other Medications

Aside from antidepressants, the two major categories of pharmaceutical treatments for patients with comorbid depression and substance-use disorders are medications (which are FDA-indicated for treatment of substance use

disorders) and medications (which are used off-label for the treatment of one or both illnesses).

Patients treated for opiate dependence with methadone or buprenorphine maintenance therapy typically show improvement with regard to depressive symptoms, at least initially; it is unclear how much of this is a pharmacologic effect and how much may be related to improvement of life circumstances with treatment. There are few data supporting improvements in depressive symptoms with naltrexone, disulfiram, or acamprosate.

Nicotine replacement therapy does not appear to mitigate the depression associated with smoking cessation, although it does improve the dysphoria and anxiety experienced during the first several weeks of abstinence. Varenicline showed early promise, but concerns have now arisen about possible worsening of depression and suicidality.

Anti-epileptic drugs have been used off-label (except in the case of those approved for use in bipolar depression) in patients with comorbid depression and substance use with varying degrees of success. These drugs have the benefit of reducing the risk of seizures during acute withdrawal from anxiolytics or alcohol, and those which act as mood stabilizers may help improve irritability as well as depressive symptoms. Valproic acid has been studied in patients with comorbid substance abuse and bipolar disorder; it shows the most promise in patients whose drug of choice is alcohol, as it relieves symptoms of alcohol withdrawal and may help prevent relapse to drinking.

Table 2 summarizes recent data for medications used in the treatment of comorbid substance use disorders and bipolar disorder.

Behavioral Treatments

Pharmacologic treatment is generally just one part of the overall plan in patients with either depression or substance-abuse disorders; this is also the case for patients with comorbidity. There are several behavioral treatments which have excellent data to support their use in depression, including cognitive behavioral therapy (CBT), insight-oriented therapy, 12-step facilitation, motivational enhancement therapy (MET), group therapy, behavioral activation, and, in severe cases, residential treatment. All of these modalities have also been used for patients with substance-use disorders, with varying degrees of success. Self-help groups such as Alcoholics Anonymous, Narcotics Anonymous, and similar groups have limited systematic data but many testimonials of helping patients maintain sobriety. These groups may also help improve depressive symptoms by improving social connectedness, although there are currently few studies on the topic. Motivational interviewing and contingency management strategies also have good data to

Table 1 Current literature on antidepressant treatment in depressed substance abusers, (all studies are double-blind placebo controlled)

Author, date	Drug (s)	Diagnoses	Effect size	Test stat	Comments
Cornelius, 1997	Fluoxetine	MDE; EtOH dep	-6.0 (9.6) Fluoxetine -2.0 (13.3) PBO	F = 4.17; p < 0.05	DSM-III-R Change HAMD 24
Petrakis, 1998	Fluoxetine	Opioid dep on MM; depressive syndrome	Mean diff HamD-17: -6.0 (5.3) fluoxetine -7.7 (6.2) PBO	NS	DSM-III-R No med effect on UDS or HamD
Cornelius, 1998	Fluoxetine	MDE, EtOH and Coc dep	No diff between active and PBO; coc dep group did worse than etoh group on all measures	NS	DSM-III-R; compared 17 coc users to 34 coc + etoh users
Schmitz, 2001	Fluoxetine	MDE; cocaine dep	No medication diff in HamD scores	NS	DSM-IV
Dean, 2002	Fluoxetine	Methadone Maint with Beck > 21	No medication effect	NS	
Pettinati, 2001	Sertraline	EtOH dep with and without Lifetime depres	No med effect in lifetime depressed group	NS	DSM-III-R; current depression not required
Roy, 1998	Sertraline	MDE; EtOH dep	Mean HamD: 12.7 (9.1) sertraline 16.3 (7.5) PBO -10 Nefazodone -7 PBO	F = 10.15, p = 0.0031	DSM-III-R Change HamD 21
Roy-Byrne, 2000	Nefazodone	MDE; EtOH dep	-10 Nefazodone -7 PBO	Wk 8 p = 0.03; Wk 12 p = 0.07	No med effect on EtOH use
Nunes, 1998	Imipramine	Opioid dep on MM; depressive syndrome	Mean HamD: 8.0 (7.4) Imipramine 13.6 (6.4) PBP	F = 18.0; p < 0.001	DSM-III-R; no diff in UDS
Wilson, 1982	Doxepin	MM pts with HamD-24 > 18	Mean diff Hamd-24 -8.0 Doxepin -4.4 PBO	p < 0.05	
McGrath, 1996	Imipramine	EtOH dep; current depressive syndrome	End point HamD-21 9.4 (7.7) Imipramine 12.4 (9.7) PBP	p < 0.03	DSM-III-R; no drug effect on subst abuse
Kleber, 1983	Imipramine	Opiate dep on MM or MDE	Diff in HamD -8.3 PBO -10 imipramine	NS	DSM-II

Table 2 Medications for concurrent bipolar disorder and substance-use disorder

Category	Drug	Affective disorder eff.	SUD efficacy	Neural effects	Selected references
Antipsychotic	Olazapine	↓ psychosis	↓ cocaine use; no effect	DA antag & 5HT agonist	Littrell 2001 Kampman 2003
	Clozapine	↓ psychosis	↓ cocaine effects	DA antag & 5HT agonist	Farren 2000
	Risperidone	↓ psychosis	↓ cocaine effects	DA antag & 5HT agonist	Roy 1998 Grabowski 2000
Mood stabilizers	Valproate	↓ bipolar symptoms	↓ relapse to alcohol and cocaine	GABA agonist	Malcolm 2001
	Gabapentin	↓ bipolar symptoms	↓ relapse to cocaine	GABA agonist	Sokolski 1999 González 2007
Depression	Lamotrigine	↓ depression and psychosis	↓ cocaine craving	Glutamate antagonist	Brown 2003
	Bupropion	↓ depression	↓ smoking	DA and NE reuptake blocker	Hurt 1997 Hughes 1999
Augmenters	Venlafaxine	↓ depression	↓ cocaine use	5HT & NE reuptake blocker	McDowell 2000
	Baclofen	↓ depression	↓ cocaine use	GABA agonist	Ling 1998
	Tiagabine	↓ depression	↓ cocaine use	GABA agonist	González 2003 Somoza 2007
ADHD/ adolescents	Topiramate	↓ bipolar	↓ alcohol use ↓ cocaine use	GABA agonist	Johnson 2003 Kampman IP
	Naltrexone	↓ depression	↓ alcohol use	Opiate antagonist	Salloum 1998
	Buprenorphine	↓ depression	↓ opiate use	Partial mu opiate agonist	Kosten 1990
	Lofexidine		↓ opiate craving	NE agonist	Sinha 2003
	Pemoline	↓ ADHD	↓ cocaine use	DA agonist	Riggs 2001
	Fluoxetine	↓ depression	↓ cocaine use	SSRI	Deas 2001
	Paroxetine	↓ depression	↓ cocaine use	SSRI	Lohman 2002

support their use in patients with substance-use disorders, but may not specifically target the depression.

Summary

The comorbidity of depression and substance abuse has dire consequences such as much higher rates of suicide than the community rates or than either disorder alone and is not due to a simple causation of one disorder leading to the other. The interaction of these disorders is based on shared genetics, neurobiology, environmental precipitants, and social supports. Their treatments can often be overlapping and patients have the best outcomes when both disorders are treated simultaneously. Treatments include medications such as bupropion, SSRIs, and tricyclic antidepressants, as well as medications focused on the substance abuse itself such as methadone and buprenorphine for depressed opiate-dependent patients. Behavioral interventions can be critical and a wide range of options is available for treating the substance abuse and the depression.

See also: Acute Dependence; Alcoholism; Animal Models of Behavior: Alcohol Addiction; Depression; Drug Addiction; Drug Withdrawal – Motivational View; Hallucinogens; Neurobiology of Opioid Addiction; Nicotine; Psychostimulants; Δ9-THC; Transition to Addiction.

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Drug Addiction

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Glossary

Behavioral sensitization – A progressive increase in a behavioral response following repeated administration of a drug at a constant dose.

Conditioned stimulus – A previously neutral stimulus that through association with an unconditioned stimulus elicits a response similar to that of the unconditioned stimulus.

Negative reinforcement – An increase in the probability of a response when the response is followed by removal of a stimulus.

Positive reinforcement – An increase in the probability of a response when the response is followed by presentation of a stimulus.

Common Themes

In the subsequent discussion around specific models, there are some common themes that are relevant to the use and interpretation of all animal models of drug addiction. As a result, a more general introduction to these themes is warranted. It should be noted that the purpose of raising these themes is not to say that they are problematic or specific to the animal models, since their impact may be just as significant on the drug addict; in fact, some aspects are likely to be crucial to understanding addictive behavior in man. Instead, the aim is to highlight the potential importance and impact of such variables on both the animal models and clinical manifestations of drug addiction.

The first of these is the impact that learning and conditioning can have on models of drug addiction. By its very nature and definition, drug addiction is intimately tied to both positive and negative reinforcement. The definition of a positive reinforcer as a stimulus that strengthens the behavior that precedes it parallels the clinical observation that the use of drugs of abuse (the positive reinforcer) increases the probability of subsequent drug use. Similarly, the removal of a stimulus that strengthens the behavior that precedes it, known as a negative reinforcer, can describe at its most basic level, the avoidance of a negative affective state such as that induced by drug abstinence. While these parallels between the definition of reinforcement and the basic tenets of drug addiction are admittedly simplistic, they serve to highlight the key role of reinforcement in drug addiction. One key aspect of such primary reinforcers is that through conditioning they routinely become associated with conditioned stimuli (CSs) that previously held neither significant motivational value nor possessed any ability to influence behavior relevant to drug addiction. However, following the formation of conditioned associations, CSs acquire key roles in drug-taking and -seeking behavior, to the extent that some behaviors can be completely absent when the stimuli are removed. For some models, this aspect is used to our advantage and is indeed a core principle of the assay, while for others it is a potential variable that should be considered. Therefore, an understanding, or at the very least an appreciation, of the role of conditioning in animal models of drug addiction is critical if these models are to be used and interpreted correctly.

A second point to consider, again common to many models, is how one can dissociate between effects specific

General Principles

Drug Addiction

While specific definitions of drug addiction (or substance dependence) vary, there is general agreement regarding the factors that are typical of the addicted or dependent individual. Definitions typically require the act of compulsive or obsessive drug seeking, a progressive increase in the amount of drug taken over time, and the occurrence of a negative affective state following withdrawal of the drug. These three components can also be viewed as describing aspects of craving, loss of control over drug intake, and physical dependence, respectively. While it is not possible to study or model drug addiction in any single animal model, by breaking down the complex disorder of drug addiction into its more basic components, it becomes possible to model specific aspects of addictive behavior. For example, one can develop animal models to study aspects of drug taking or drug seeking, while separately investigating the development of physical dependence and the occurrence of negative affect during withdrawal from chronic drug administration. As with all complex disorders of the brain, the value of animal models of drug addiction really lies in understanding the psychological and neurobiological mechanisms, which underlie specific aspects of the disorder, and the subsequent application of such learning to the clinical situation.

to drugs and those that have more general application to other nondrug reinforcers, such as food, water, and sex. Many of the models described below can be applied effectively to study the positive-reinforcing effects of food, or the ability of food-paired stimuli to influence behavior such as the seeking of positive reinforcement. To understand the specificity of mechanistic investigations to drug reinforcement, researchers will often run parallel studies with nondrug reinforcement. However, the lack of specificity to drugs does not preclude outcomes from being relevant to the study of drug addiction. While the specific actions and effects of drugs may be different to those of many natural reinforcers, the underlying psychological processes and neurobiology of these effects may share many commonalities. As such, an understanding of these common mechanisms may be an important first step to understanding the specific nature of drugs of abuse as highly addictive stimuli. Taking such an approach allows the drug addiction researcher to tap into the wealth of knowledge obtained in complementary fields of positive and negative reinforcement.

Finally, while breaking drug addiction down into its component parts may enable study of the disorder in a manageable way; it is important to remember that each of the models described here still relies on the assessment and analysis of behavior, which, by its very nature, is the complex output reflecting a number of underlying psychological and neurobiological processes. A decrease in the number of responses emitted by an animal to obtain a drug could in theory result from effects of the drug on motor output, muscle relaxation, attention, learning, memory, or motivation. In reality, it probably reflects a combination of these domains. The aim is not to say that such models are impossible to interpret, but rather to highlight that the dependent variables studied in animal models of behavior are not always what they seem. The use of appropriate controls and a broader appreciation of complimentary research fields can help tie down effects to particular domains.

Positive Reinforcement Models

Of the animal models of behavior developed to study drug abuse, the most frequently used have focused on the assessment of positive reinforcement. Drugs of abuse typically act as positive reinforcers, meaning that the administration of these drugs increases the probability of the behavioral output, which resulted in drug delivery. It is easy to see the parallel between this description of positive reinforcement and the clinical manifestation of drug abuse. The ability of drugs of abuse to act as reinforcing stimuli can be studied in animals using both the drug self-administration model and the conditioned place preference (CPP) model. In addition, the ability of drugs to

modulate the neuronal pathways that underlie reinforcement can be studied using the intracranial self-stimulation (ICSS) model.

Drug Self-Administration

The drug self-administration model is founded on the principle that an animal will learn the contingency between the performance of an operant response, such as a lever press or nose poke, and the administration of the drug (**Figure 1**). By virtue of the drug's ability to act as a reinforcer, the probability of the operant response being repeated is therefore increased and the behavior maintained. Drug self-administration has been demonstrated in animals for the majority of drugs that have high abuse potential in humans. Typically, drugs are administered via the intravenous route to allow rapid delivery of the drug to the brain and a closer temporal contingency between response and the drug effect. However, self-administration has also been demonstrated via other routes, principally oral, mimicking, for example, alcohol self-administration in humans. To increase the understanding of the neurobiological substrates of drug self-administration, studies have also been performed demonstrating self-administration of drugs directly into discrete brain regions.

The self-administration model is itself a valuable tool in understanding the mechanisms that underlie the ability of an addictive drug to act as a positive reinforcer. However, by manipulating experimental variables, it is also possible to use this model to understand more regarding the nature of the reinforcing properties of drugs of abuse. For example, by altering the schedule of reinforcement, it is possible to build up a picture of the reinforcing profile of the drug. Animals are typically trained to respond under a fixed-ratio (FR) schedule of reinforcement, where a fixed number of responses are required to obtain a single administration of drug. One limitation of such schedules is that response rates are frequently influenced by factors such as satiation and pharmacokinetics, whereby response rates actually decrease with higher unit doses of drugs, reflecting a potential titration of drug intake by the animal (**Figure 2**). Interestingly, a similar effect has been reported in humans where, for example, smokers switching to cigarettes containing lower levels of nicotine have been shown to partially compensate by increasing the number of cigarettes smoked or altering their smoking behavior to increase nicotine intake. As a result, the rate of responding is not necessarily considered a good predictor of reinforcing value and may not differentiate between drugs with a high and low propensity for abuse. One method frequently employed to address the question of relative reinforcing efficacy is the use of progressive-ratio (PR) schedules of reinforcement (**Figure 3**). Under PR schedules, response requirements

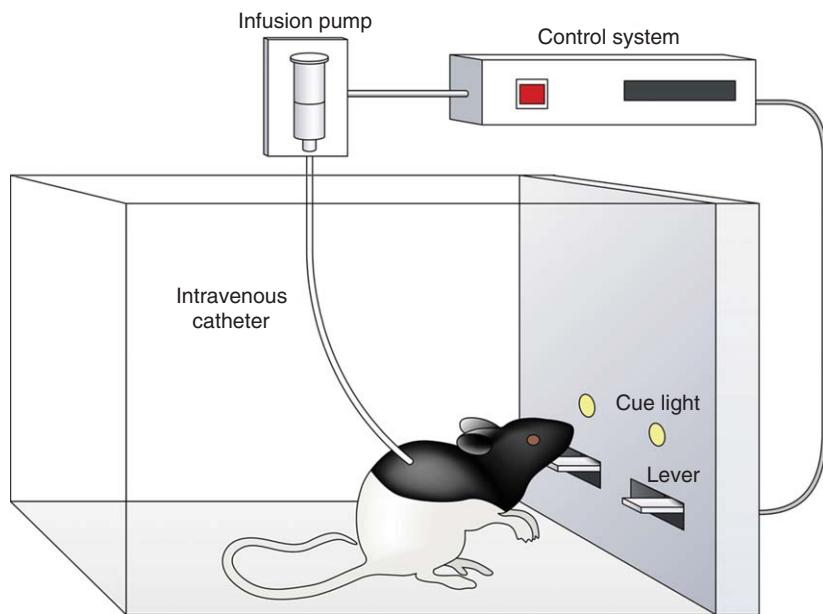


Figure 1 Schematic of the intravenous self-administration procedure in an operant chamber. The rat, with an indwelling intravenous catheter externalized to a port, is connected to an infusion pump containing the test solution. Typically, two levers are presented, and responding on one of these (termed the 'active' lever) results in the delivery of drug. Responding on the alternative 'inactive lever' has no consequence and this is frequently viewed as a measure of response generalization or motor disruption. Cue lights are often included and can be used to signal drug availability or delivery, increasing the speed with which the operant contingency is acquired, and permitting the study of the effects of drug associated conditioned stimuli.

for successive drug administrations gradually increase until the animal fails to continue responding. The point at which responding ceases or drops below a predetermined level, termed the 'break point,' can then be used as an estimate of reinforcing strength. It should be noted that this methodology has its limitations, principally regarding comparisons across drug classes and also regarding the effects of stimulant and sedative drugs on motor output.

Second-order schedules of reinforcement warrant discussion as an example of the potential value that the self-administration model brings to understanding the importance of CSs in reinforced operant responding. Second-order schedules of reinforcement are those in which responding is not only maintained by the drug, but also by response-contingent drug-associated CSs. The principles of such schedules are that the CS, by virtue of its association with the primary reinforcer, attains reinforcing value of its own (termed a conditioned reinforcer) and is therefore capable of supporting an operant response. Typically, responding for the drug is maintained on a high-demand schedule, during which performance of a lower-demand schedule produces multiple presentations of the CS. For example, responding under a FR 15 (FR 10:S) schedule produces presentation of the CS following each set of 10 responses and administration of the drug after 150 responses (15 sets of 10). This schedule provides two distinct advantages. First, the ability of the CS to act as a reinforcer can be examined in

the absence of the drug during the first drug-free interval. This is particularly a key aspect since it is well established that many drugs of abuse can alter cue-maintained behaviors. For example, a range of drugs of abuse has been shown to increase response rates for conditioned reinforcers. Second, second-order schedules allow a direct assessment of the role of the drug-paired CS in drug-reinforced behavior. There is little doubt that the presence of both discrete CSs and environmental drug-paired cues is critical in influencing self-administration behavior in animals and in influencing subjective state and craving in drug users, and therefore the study of the mechanisms underlying such associations are likely to be of importance in improving our understanding of the role of reinforcement in drug addiction.

A final demonstration of the utility of self-administration models comes from studies attempting to mimic an aspect of addiction describing the progression from controlled to uncontrolled drug taking. Historically, self-administration sessions with cocaine have often comprised of daily limited-duration sessions lasting less than 3 h. This method results in stable patterns of responding that show little variability from day to day. However, when rats are given access to cocaine for 6 h or more per day, response rates begin to change over time, to the extent that drug intake progressively increases. Furthermore, increases in the reinforcing efficacy of cocaine are observed as estimated from break points

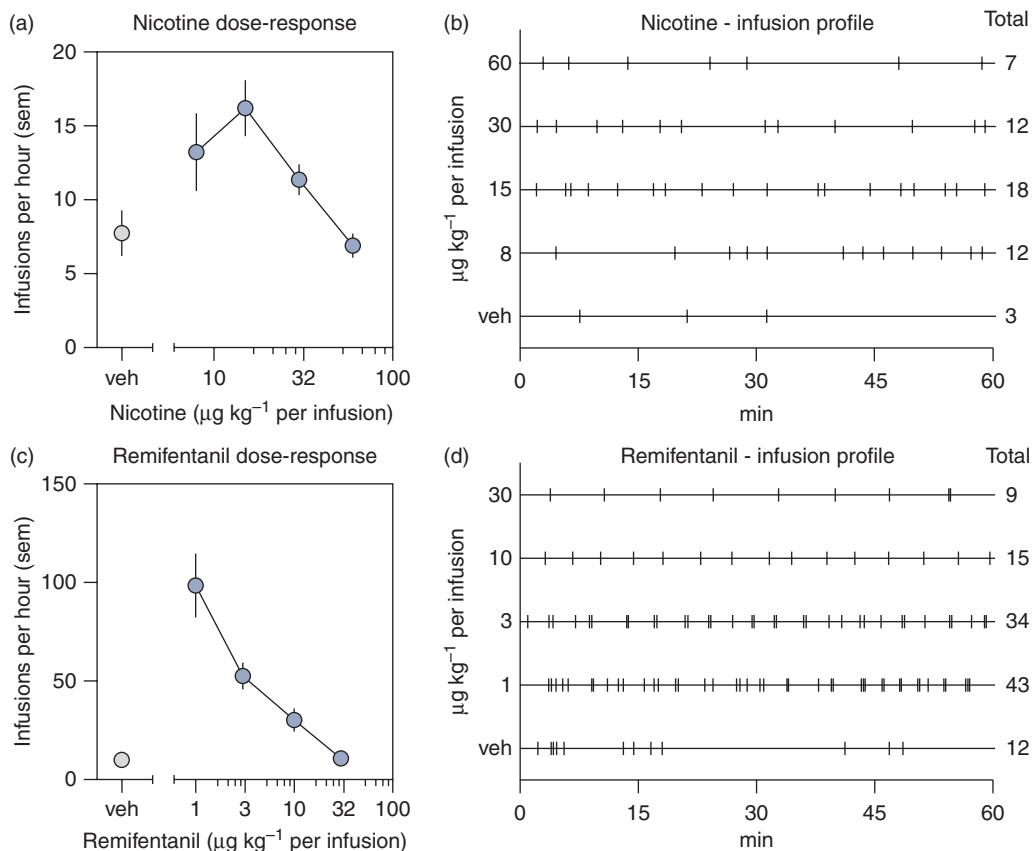


Figure 2 Dose–response relationship and response profiles under an FR schedule of self-administration. Effects of varying the unit dose of nicotine (a) or remifentanil (c) on the response rates of rats under a fixed-ratio 3 schedule of reinforcement. As the unit dose increases, the response rate decreases reflecting titration of drug intake. Panels on the right depict representative response profiles demonstrating the dose-dependent changes in infusion frequency with either nicotine (b) or remifentanil (d). Both examples highlight the importance of assessing response profiles in addition to total response rates. While total infusion numbers are lower for a $30\mu\text{g kg}^{-1}$ infusion dose of remifentanil than for vehicle, the response profile for remifentanil suggests regular inter-infusion intervals characteristic of self-administration of a reinforcing drug under a fixed-ratio schedule. In contrast, the profile of responding for vehicle is far more sporadic. Source: EC O'Connor, D Parker, AN Mead, unpublished results.

under PR schedules. This incremental change has been interpreted as representative of an increased drive to obtain the drug similar to that observed in humans following the transition from social/intermittent drug use to dependency and addiction.

Conditioned Place Preference

The conditioned place preference (CPP) model has been used extensively for the assessment of the reinforcing properties of drugs of abuse. In the CPP model, the animal receives experimenter-administered drug in one compartment of a multicompartment apparatus and the vehicle in a different compartment; the two compartments typically differing in visual and tactile domains. Through repetitions of this contingency, the environment in which the animal receives drug becomes associated with the drug through Pavlovian conditioning. Subsequently, the animal is given a choice test, whereby

it is given free access to both compartments and the relative time spent in the drug and vehicle-paired compartments is viewed as representative of its preference (or aversion) for the drug. Comparisons are often drawn between CPP and the self-administration model, largely on the basis that both models are sensitive to the majority of abused drugs and are based on the principles of positive reinforcement. However, rather than comparing the advantages and disadvantages of the two models, it is probably more constructive to assess the value that the two models provide independently. CPP, by its nature reflects the approach of an animal toward an environment previously associated with the drug. In theory, this approach could represent an expectation or anticipation of drug delivery, the receipt of reinforcement from a conditioned stimulus or an instinctive autoshaping approach to a CS. While it is not entirely clear which of these psychological processes underlies the expression of a CPP, all three interpretations require that the drug acts

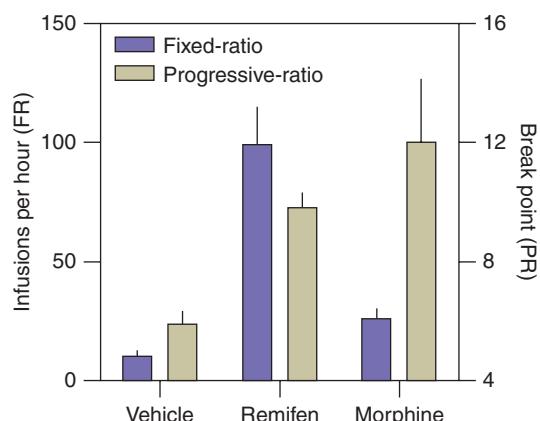


Figure 3 Importance of schedule choice for self-administration studies. Comparison between the assessment of two μ -opioid agonists under either a fixed-ratio schedule or a progressive-ratio schedule of reinforcement. Both the typical μ -opioid agonist morphine and the ultra-short acting μ -opioid agonist remifentanil reinforce self-administration behavior relative to vehicle, but the conclusions regarding reinforcing strength would differ according to the schedule of reinforcement. Under a fixed-ratio schedule, remifentanil maintains higher rates of responding than does morphine, in part due to its shorter half-life. Under a progressive-ratio schedule, both μ -opioid agonists maintain similar levels of responding, as assessed by the 'break point'. (Note: full dose-response functions were generated for both drugs, but only data from the unit dose resulting in the highest response rate or break point for each compound are shown for clarity) Source: EC O'Connor, AN Mead, unpublished results.

as a positive reinforcer during conditioning sessions, and therefore the model is well structured to assess the positive-reinforcing properties of addictive drugs.

Intracranial Self-Stimulation

The ICSS model takes advantage of the fact that animals will perform an operant response for direct electrical stimulation of specific neuronal pathways or brain regions. It is believed that the pathways that support ICSS are those responsible for mediating the response to natural reinforcers. By altering the frequency and intensity of stimulation, it is possible to generate response curves giving an estimation of sensitivity thresholds for a particular brain region. The potential utility of this model for drug addiction research has been supported by the fact that many drugs of abuse reduce reinforcement thresholds for ICSS. This is interpreted as a selective effect on reinforcement sensitivity by controlling for other drug-induced behavioral changes, such as increased or decreased locomotor activity, and demonstrating dissociation between the two. Therefore, ICSS is an example of a model where it is possible to begin to understand the direct effects of drugs of abuse on the systems that underlie positive reinforcement.

Drug Seeking and Relapse

While it can be argued that both the self-administration and CPP models assess aspects of drug-seeking behavior, the dependent variables are not typical tested after a period of protracted abstinence. Clearly, the maintenance of drug self-administration requires an element of drug seeking; however, with the exception of perhaps extinction testing where drug access is replaced with vehicle access, this is most frequently studied in the presence of drug taking. In addition, while second-order schedules provide an insight into drug seeking in the absence of drug taking, this is restricted to the first interval of any session. Similarly, the expression of a CPP may represent drug seeking, though, as discussed previously, other explanations for CPP exist, and normally testing is conducted within a few days of conditioning sessions. As a result, there has been an increasing effort around the development and characterization of animal models of drug seeking and relapse following protracted abstinence.

Reinstatement and Renewal of Drug Seeking

Reinstatement and renewal models of drug seeking are typically centered on the ability of stimuli to reinitiate a previously extinguished operant response for drug. The term 'reinstatement' is used to describe the ability of a discrete CS, drug stimulus, or stressful stimulus to reinitiate responding, while 'renewal' is preferred when referring to the ability of a contextual cue to reinitiate responding. Both variations have key concepts in common and provide an assessment of the ability of stimuli to increase the probability of drug-seeking behavior. As a result, these models are frequently viewed as analogous to relapse in the clinical situation, whereby exposure to certain stimuli has been associated with an increase in the probability of reinitiating drug taking. This comparison highlights one important difference between the reinstatement/renewal models and the clinical relapse situation: in the animal model, it is purely a measure of drug seeking, as the drug is never available during tests, while the clinical situation is also influenced by drug taking. Of course, by excluding drug availability in the animal model, one can be more confident that the behavior observed is driven by the inducing stimulus and not by the primary reinforcing or pharmacological properties of the drug.

There are three general forms of the reinstatement/renewal models of drug seeking. These are more commonly performed in an operant box following a phase of drug self-administration and subsequent extinction of this response. However, similar effects have been demonstrated in reinstatement versions of the CPP model, where the preference for the drug-paired chamber is

reinstated following extinction. In the drug-prime induced reinstatement model, responding is reinstated by a non-contingent priming injection of a drug prior to the test session. In the stress-induced reinstatement model, responding is reinstated by the presentation of a noncontingent stressful stimulus such as a footshock or food deprivation. In all other ways, the test session for both versions of reinstatement is no different to previous extinction sessions. In contrast to these models, cue-induced reinstatement is typically assessed using one of the two methods. During initial self-administration training, a discrete CS such as a light or tone is paired with drug delivery. During extinction sessions, this CS is no longer presented but is instead reintroduced for a reinstatement test session following extinction to assess the ability of the contingent CS to reinstate responding. Renewal studies are typically performed in a similar way, except that rather than removing the drug-paired CS during extinction the contextual cues in the operant chamber are altered prior to being restored for the renewal test. A variation of the cue-induced reinstatement model involves the noncontingent presentation of the CS prior to and/or during the reinstatement session. Both methods have been shown to reliably reinstate responding following extinction, though it is reasonable to assume that the mechanisms underlying one may differ from those underlying the other, so equivalence should not be assumed.

Following extensive research during the past 25 years, a significant amount has been learned for a number of different drugs regarding the neurobiological mechanisms underlying the different forms of reinstatement and renewal. At face value, the classes of stimuli that reinstate responding in reinstatement models appear to translate well to the stimuli which are frequently associated with relapse in abstinent drug addicts. However, due to the limited availability of pharmacological interventions for relapse prevention in human, it has been difficult to assess the predictive validity of such models. At a more basic science level, there is no doubt that such models have helped increase our understanding of the mechanisms that underlie the reinstatement of drug-seeking behaviors following the presentation of different stimuli, and the commonalities in mechanisms among the different forms of reinstatement may help elucidate the neural substrates that underpin reinitiation of drug-seeking behavior *per se*.

One of the more recent developments in the field of animal models of drug relapse has been the description of the incubation of drug seeking following extended withdrawal. The term ‘incubation’ has been used to describe a time-dependent increase in the level of drug seeking following presentation of reinstating stimuli during abstinence, and it has been argued that this increase parallels a similar temporal increase in propensity to relapse in abstaining addicts. By integrating behavioral endpoints of relapse with cellular and molecular changes, there

have been significant strides in our understanding of the mechanisms underlying drug-seeking behavior.

Alcohol Deprivation Effect

The alcohol deprivation effect (ADE) describes the observation that, following a period of abstinence, animals which had previously demonstrated consistent levels of alcohol intake transiently consume significantly greater amounts of alcohol. This effect has also been demonstrated for other drugs of abuse, including stimulants and opioids. It has been argued that the increase in drug intake described by the ADE represents a shift toward uncontrolled drug intake, and that it therefore represents a useful model for the progression to addiction. However, an alternative explanation should also be considered. Similar reports of increased intake have also been observed for natural reinforcers, suggesting that the increased intake may simply represent a general response to a reinforcing stimulus following a period of unavailability, rather than being specific to drugs of abuse (and therefore symptomatic of the move toward the addictive state). Second, when viewed alongside the incubation effect observed in reinstatement models (an effect that has also been demonstrated for natural reinforcers), it is perhaps not surprising that increased consumption of a positive reinforcer would occur at times when levels of reinforcer seeking are apparently increased. While it is not clear whether either the ADE or the incubation effect describes the phenomenon critical for, or specific to, the addictive process, or whether they represent the general properties of reinforced behavior, understanding such effects will be important to understanding the mechanisms that underlie increased drug consumption or seeking following periods of protracted abstinence.

Physical Dependence and Negative Affect

Tolerance and Withdrawal

The study of negative affect during abstinence following chronic drug administration has played a key role in increasing the understanding of the role that negative reinforcement processes may play in addictive behavior. Typically, drugs are administered at either a constant or increasing dose over a period of approximately 1–4 weeks, and monitoring of behavioral and physiological parameters during dosing can frequently reveal tolerance to drug effects. Withdrawal states are subsequently examined following either cessation of dosing (spontaneous withdrawal) or administration of an antagonist at the primary pharmacology of the test drug (precipitated withdrawal). Precipitated withdrawal has the advantage of producing a more immediate and often more severe withdrawal syndrome. Withdrawal behavior can be

assessed using a number of methods. For drugs that induce severe withdrawal symptoms such as opioids, it is often sufficient to monitor behavioral signs and symptoms, food intake, and body weight. However, for detection of more subtle withdrawal signs, a number of other endpoints have been studied. Particularly sensitive endpoints include operant responding for food (under various schedules of reinforcement) and ICSS, which are frequently viewed as measures of anhedonia and therefore representative of negative affect. A variation of the CPP model has also been employed where, instead of pairing a drug effect with one compartment of the apparatus, the assumed aversive state of withdrawal is associated with a compartment to allow the assessment of a conditioned place aversion (CPA). More recently, and with improved telemetry technology, analysis of sleep architecture has also been introduced with success for drugs with milder withdrawal syndromes. This effort was largely driven by the observation that withdrawal from most drugs of abuse in humans results in sleep disturbances, and while the impact of sleep disorders on propensity for relapse and drug use is far from clear, is an excellent example of the power of translational endpoints in drug addiction research.

Neuroadaptive Models

Behavioral Sensitization

In relation to drug addiction, the term behavioral sensitization describes a progressive increase in a behavioral response following repeated administration of a drug at a constant dose. Typically, this is measured through assessment of locomotor activity, as the majority of drugs of abuse will induce changes in locomotor activity. Evidence has also grown for the sensitization of other reinforced behaviors, such as responding for conditioned reinforcement (responding for a CS previously paired with a primary reinforcer such as food), primary reinforcement and Pavlovian-to-Instrumental Transfer (the enhancement of responding for a primary reinforcer during noncontingent presentation of a CS). Behavioral sensitization has received increased focus in the last two decades due to its core role in some recent theories of drug addiction, where it is proposed that behavioral sensitization is representative of a sensitization of the neural systems that underlie the attribution of incentive value to drug-paired stimuli, and therefore the wanting of these drugs. Behavioral sensitization of locomotor activity has been demonstrated for most drugs of abuse, and the conditions for its development have become increasingly well understood. For example, sensitization generally only occurs following intermittent administration of drugs and is largely dependent on conditioning to environmental cues. Similarly, our understanding of the

mechanisms underlying behavioral sensitization has increased greatly, with major focus being placed on the mesocorticolimbic system. The dependency of drug addiction on the development of behavioral sensitization is, like most current theories of disorders of the brain, a topic of intense debate. In addition, key questions remain unanswered around aspects such as the evidence for incentive sensitization in animals and humans, as opposed to sensitization of the locomotor response. However, as a model of drug-induced neuroadaptation, the study of behavioral sensitization has, at the very least, contributed significantly to our understanding of drug-induced plasticity in the neural substrates common to many drug-induced effects and has demonstrated that repeated drug exposure can alter the subsequent response to drugs and drug-paired cues.

Subjective Effects

Drug Discrimination

The drug discrimination model has been used extensively to understand the mechanisms underlying the potential subjective properties of drugs of abuse. The model is based on the ability of the animal to discriminate between the presence and absence of an interoceptive cue induced by a training drug. Assessment of discrimination is made by making the delivery of reinforcement (normally food or water) contingent on an operant response; following drug administration, the animal is required to perform one response, while, following vehicle administration, it is required to perform an alternate response. Once the animal has learned this discrimination, the similarity of the interoceptive cue induced by other drugs can be examined by studying the distribution of responses across the two response options (**Figure 4**). During these generalization test sessions, either both or neither response is reinforced to provide an unbiased measure of preference. While the role of the drug-induced interoceptive cue in processes leading to addiction is not clear, the drug discrimination model generally demonstrates good class specificity (i.e., drugs of a common class/mechanism will show cross-generalization to each other) and as such is a powerful model for understanding drug–drug similarities (**Figure 5**), and the impact of potential therapeutics on drug-induced interoceptive cues.

Conclusions

The purpose of this article is not to provide a thorough account of all behavioral models relevant to the study of drug addiction; this is beyond the scope of an

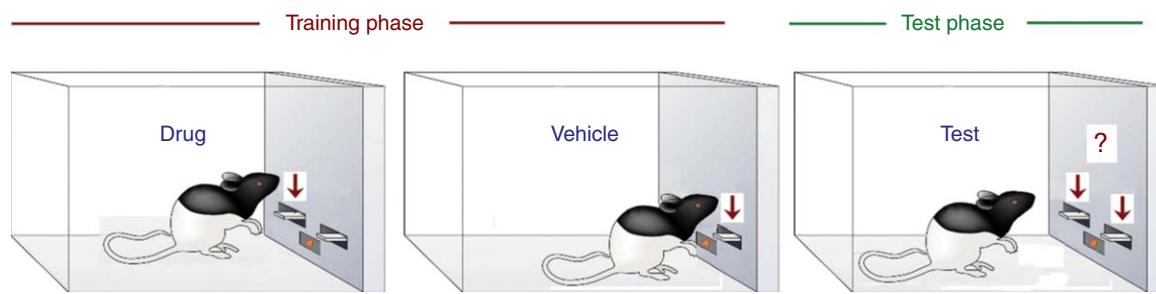


Figure 4 Schematic and the principles of the drug discrimination model. During the training phase, the rats are required to respond on the left lever to obtain food if given a drug injection prior to the session. Alternatively, following a vehicle injection, they are required to respond on the right lever to obtain food. Once rats have successfully acquired this discrimination, a test compound is given and responding on both levers is reinforced by food delivery. The proportion of responses on the drug-paired lever compared to the vehicle-paired lever gives an indication of the similarity of the test drug interoceptive cue to that induced by the training drug.

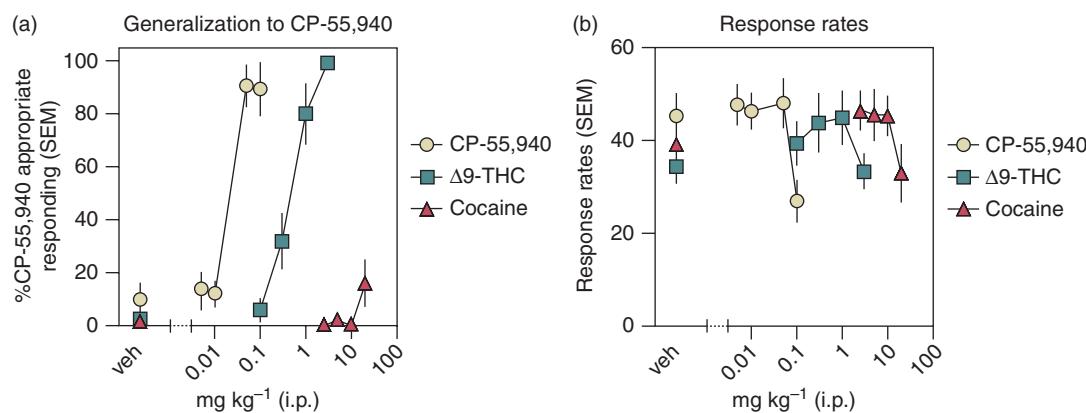


Figure 5 Example of the class specificity of the drug discrimination model. Rats trained to discriminate between the cannabinoid agonist CP-55,940 (0.05 mg kg^{-1}) and vehicle demonstrate generalization to the cannabinoid $\Delta 9$ -tetrahydrocannabinol ($\Delta 9$ -THC) but not the dopamine-reuptake inhibitor cocaine. Generalization is dose dependent demonstrating that the interoceptive cue is dose specific as well as class specific. Comparison of response rates is often used to assess disruption of performance with both sedative and stimulant drugs, resulting in reduced response rates when doses are increased sufficiently. Source: S Staveley, E Barrow, AN Mead, unpublished results.

overview, and all of the models described have additional levels of complexity and detail, which warrant consideration prior to study design or interpretation. Instead, the aim is to provide a top-level view of the range of models, which can be applied to answering questions about specific aspects of addictive behavior. If there is one take-home message, it should be that it is simply not possible to model drug addiction in an animal model (this is really no different to any other disorder of the brain). Instead, a more focused approach should be taken to understand specific aspects of the addictive process, whether this is positive reinforcement, negative reinforcement and affect, the progression to uncontrolled drug intake, behavioral sensitization, or drug-seeking behavior. By taking this approach, our understanding of the neurobiological mechanisms and psychological processes of drug addiction has improved dramatically over the past few decades. The challenge moving forward is to

continue asking and answering these focused questions, and then to start putting the information back together again to understand how drug addiction can be treated effectively.

See also: Alcoholism; Animal Models of Behavior: Alcohol Addiction; Brain Stimulation and Addiction; Cellular Plasticity in Cocaine and Alcohol Addiction; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Drug Priming; Drug Sensitization and Drug Abuse; Hallucinogens; Drug Withdrawal – Motivational View; Hormonal Contributions to Arousal and Motivation; Incentive Motivation and Incentive Salience; Motivation; Neural Basis of Working Memory; Neurobiology of Opioid Addiction; Neurophysiology of Drug Reward; Nicotine; Psychostimulants; Stress and Drug Craving; $\Delta 9$ -THC; Transition to Addiction; Vulnerability Factors in Addiction Disorders.

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Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction

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Glossary

Appetitive behavior – Behavior occurring early in a natural behavioral sequence and serving to bring the organism into contact with a particular stimulus eliciting that behavior.

Conditioned reinforcer – A stimulus that becomes an effective reinforcer because of its association with a primary or unconditioned reinforcer (also termed 'secondary' reinforcer).

Conditioned stimulus (CS) – A previously neutral stimulus that does not elicit a particular response but, as a result of becoming associated with an unconditioned stimulus, eventually comes to do so.

Consummatory behavior – Behavior that serves to bring a natural sequence of behavior to consummation and completion.

Discriminative stimulus – A stimulus that controls the performance of instrumental behavior because it signals the availability of a reinforcer.

Extinction – In classical conditioning, the procedure of repeatedly presenting a CS without the unconditional stimulus (US), leading to reduction of a learned response because the CS is no longer paired with the US. In instrumental conditioning, the procedure of no longer reinforcing the instrumental response, leading to reduction of this response because it is no longer followed by the reinforcer.

Instrumental response – A response (also termed 'operant response') that is defined by the consequences it produces in the environment. For example, any sequence of movements that results in depression of a lever in an operant-conditioning chamber that, in turn, results in delivery of a reward represents an instance of that particular operant.

Occasion setting – A contingency in which one cue (e.g., a discriminative stimulus) designates when another will be reinforced.

Positive reinforcement – Positive reinforcement is an instrumental conditioning procedure involving a positive contingency between the instrumental response and a reinforcing stimulus. In this context, a subject receives a positive or pleasant stimulus if a correct response is performed, increasing the frequency and/or future probability of the response.

Reinforcement contingency – Causal relationship between a response and a reinforcer, measured in terms

of the probability of getting reinforced for performing a response as compared to the probability of getting reinforced in the absence of the response. Often refers to the particular schedule of reinforcement that is in effect in studies of learning and conditioning.

Reinforcer – A stimulus whose delivery shortly following a response increases the probability of this response in the future.

Renewal – Recovery of excitatory responding to an extinguished stimulus produced by removal from contextual cues that were present during extinction. Renewal typically occurs when a subject is returned to the environment in which the response was previously reinforced following extinction of this response in a different environmental context.

Schedule of reinforcement – A rule that determines under what specific conditions (i.e., the reinforcement contingency) the occurrence of a response is followed by the delivery of a reinforcer.

Introduction

The conditioning of both the positive reinforcing actions of drugs of abuse and the aversive aspects of drug withdrawal states with environmental stimuli represents an important mechanism for the addictive potential of drugs of abuse. Early studies, pioneered by Abraham Wikler and, subsequently, Goldberg and Schuster, have focused on the classical conditioning of the opiate withdrawal syndrome whereby environmental stimuli paired with morphine withdrawal elicit withdrawal symptoms in opiate addicts. Interest, later, rapidly shifted toward the role of conditioned stimuli in drug-seeking and self-administration associated with the positive reinforcing effects of opiates and psychostimulants. Studies by Davis and Smith, in rats, and by Schuster and Woods, in squirrel monkeys, provided original evidence that conditioning factors influence the initiation, maintenance, and extinction of drug-seeking and -taking behavior. It has since been firmly established that drug-related associative learning, whereby environmental stimuli repeatedly paired with drug consumption acquire incentive-motivational value or set the occasion to engage in drug-seeking behavior, is a critical factor in maintaining or modifying ongoing drug use and in eliciting drug desire during abstinence, precipitating relapse.

Conditioned Locomotor Activity and Sensitization

Drug-paired CSs produce drug-like responses, such as conditioned locomotor activity and increases in mesolimbic dopamine activity when animals are exposed to the same environment where the drug had been administered, an effect that is most prominent with psychostimulants. Cocaine-paired cues have also been shown to produce conditioned increases in the rewarding effects of electrical brain stimulation, eliciting a state similar to that produced by the drug itself, a finding that is consistent with functional brain imaging data that cocaine cues elicit striatal dopamine release in the humans. These conditioned consequences of drug-cue exposure are thought to reflect the incentive motivational properties of drug-associated cues that underlie drug seeking and recidivism in dependent individuals. When animals are repeatedly exposed to drugs of abuse, they show enhanced (i.e., sensitized) locomotor activity and reactivity of mesolimbic dopamine neurons in response to later challenge-administration of the drug. This, often long-lasting, enhancement in behavioral and neural activity has been implicated in enhanced drug-directed appetitive behavior and sensitization of the incentive salience of drug of abuse. It is well established that the expression of behavioral and neural sensitization is strongly influenced by conditioned contextual stimuli in that drug challenges administered in an environment not previously paired with the drug fail to show sensitization and such environment-specific effects have been reported with psychostimulants and opiates. Thus, stimuli conditioned to the availability or subjective effects of drugs are associated with the expression of sensitized neurobehavioral actions of drugs of abuse such that the presence or absence of these stimuli may be linked to fluctuations in susceptibility to relapse and enhanced incentive salience attached to drug-related cues, both during ongoing drug use and during periods of abstinence.

Initiation, Maintenance, and Reacquisition of Drug Seeking

Drug-paired CSs can produce responses that resemble the effects of the unconditioned drug stimulus, such as conditioned locomotor activation, by acting as secondary reinforcers maintaining responding under second-order schedules of reinforcement (Box 4), and reinstating extinguished drug seeking similar to the effects of a small dose of the drug itself. Conditioned responses, therefore, must be assumed to also influence the initiation, maintenance and extinction of drug self-administration. Several lines of evidence support this prediction. For example,

environmental contexts associated with the subjective effects of noncontingent ethanol administration maintain higher levels of responding for ethanol. In other words, an environmental context signaling the effects of ethanol maintains a higher response rate when ethanol is subsequently offered as a response-contingent reinforcer. Moreover, exposure to ethanol-associated contextual cues during extinction and abstinence, but not abstinence alone, reduced the resumption of ethanol intake after abstinence. Importantly, reacquisition of ethanol self-administration was reduced in proportion to the number of abstinence-associated cues present during abstinence, suggesting that ethanol consumption is governed by ethanol-associated cues. In the case of cocaine, discriminative stimuli and, in particular, independently established cocaine-predictive discriminative stimuli (i.e., a tone and a light cue) presented in compound have been shown to increase both response rates and intake of cocaine in rats, suggesting that previously neutral stimuli can come to have profound effects on drug self-administration. In the case of nicotine, it has been established that nicotine-associated cues consisting of a compound, response-contingent, drug-paired cue and a discriminative stimulus signaling availability of the drug exert particularly strong control over nicotine-directed behaviors. These stimuli were found to be as important as nicotine itself in sustaining a high rate of responding once intravenous nicotine self-administration is established, in the degree of resistance to extinction they induce when nicotine is withdrawn, and in the reacquisition of nicotine self-administration after extinction. The role of drug-associated environmental stimuli in the initiation and maintenance of drug self-administration has not gone unchallenged. For example, a discrete cocaine-paired stimulus (CS) did not modify the reinforcing effects of cocaine although it effectively reinstated extinguished cocaine-seeking behavior. It is likely that these discrepancies are related to differences between the conditioned effects of discrete and discriminative/contextual stimuli discussed below. Importantly, in the case of ethanol, the learning of associations between the self-administration context and the subjective effects of ethanol is essential due to the delayed nature in the onset of ethanol's pharmacological actions, whereas this is not necessary in the case of intravenous drug reinforcement where the pharmacological actions of the drug are contiguous both with the operant response and the presentation of the CS. Therefore, ethanol reinforcement may be more sensitive to changes both in drug-predictive contextual and discrete reward-paired cues than in intravenous drug reinforcement. To the extent that a large body of literature supports a role of drug-related learning in the initiation and maintenance, it will be important to consider the implications of these findings both with regard to understanding increases in drug intake or drug

desire as well as for the design of extinction procedures that may be effective in preventing relapse.

Conditioned Reinstatement, Craving, and Relapse

Drug addiction is a chronically relapsing disorder. Long-lasting vulnerability to relapse is a core feature of addiction and an issue of critical importance for the treatment of drug addiction (see **Figure 1**). A major factor precipitating craving and relapse, as recognized both in the clinical and experimental literature, comprises learned responses evoked by environmental stimuli or events that have become associated with the subjective actions of drugs or alcohol. Exposure to such stimuli elicits craving and drug desire that often leads to the resumption of drug or alcohol use in abstinent individuals. Drug-related stimuli can also elicit automatic responses that lead to drug seeking and relapse without the intervention of conscious desire or distinct feelings of craving.

One reason for the enduring, perhaps lifelong, persistence of relapse risk is that craving associated with drug-cue exposure does not readily extinguish. For example, in dependent drinkers, alcohol craving induced by ethanol cues shows little habituation with repeated cue exposure although conditioned responses such as autonomic

reactivity become attenuated. In addition, in humans, the severity of craving induced by drug cues is positively correlated with the history and degree of drug or alcohol dependence. One explanation for this relationship is that experience of the drug during withdrawal – an inevitable event associated with a history of dependence – modifies an individual's reinforcement history to include learning about the subjective effects of drug consumption during withdrawal. This experience introduces a novel motivational dimension that enhances the incentive value of the drug, such that the drug comes to function as a qualitatively different and more effective reinforcer, particularly during subsequent withdrawal experiences.

The role and mechanisms by which drug-associated stimuli contribute to and control addictive behavior in the human is addressed in greater detail elsewhere in this encyclopedia. The focus of this article is on experimental studies in animals. As described below, systematic studies in animals on the role of conditioning factors in drug seeking and relapse have confirmed that environmental stimuli associated with the reinforcing actions of drugs or alcohol – either by means of classical conditioning or by acting as discriminative or contextual stimuli signaling drug availability – reliably reinstate drug seeking in animals in which this behavior had been previously extinguished. These studies have also elucidated the neurocircuitry and signaling mechanism that regulate specific

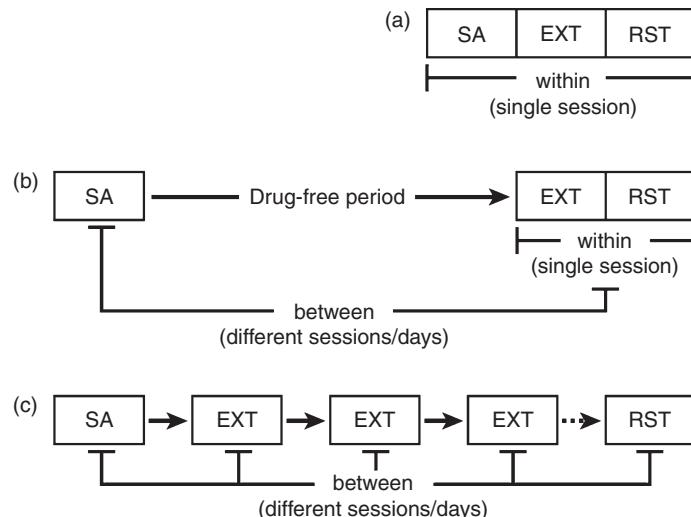


Figure 1 In models of conditioned reinstatement, extinction and reinstatement tests can be conducted according to a 'within-session,' 'between-session,' and 'within-between session' sequence. In the 'within-session' procedure (a), a single extinction session is conducted, followed immediately by the reinstatement test. A limitation of this method is that it does not include an abstinence period and, therefore, does not mimic relapse as traditionally understood. Moreover, animals in this procedure are not drug-free because testing occurs within one hour of the last drug administration. In the 'between-session' procedure (c), extinction sessions are conducted daily and reinstatement tests commence typically 1 day after an extinction criterion is reached. This procedure 'adds' a drug-free (i.e., abstinence) period to the mere extinction of drug-reinforced responding and, thus, more closely resembles relapse in the human where resumption of drug use occurs beyond the acute withdrawal phase. In the 'between-within' procedure (b), no extinction sessions are conducted after rats acquire drug-reinforced responding. Instead, an abstinence period is imposed after which extinction and reinstatement sessions are conducted in a within-session manner. This model is particularly useful for studying the relationship between the duration of abstinence and degree of reinstatement. The great majority of contemporary applications of the extinction-reinstatement model employ 'between-session' or 'between-within-session' procedures.

aspects of conditioned drug seeking, and provided important leads with regard to promising neural targets for pharmacotherapeutic relapse prevention.

Animal Models of Conditioned Drug Seeking and Relapse

Conditioned drug-seeking responses can be demonstrated in a wide range of animal models implemented to investigate specific aspects of the motivational impact of drug-associated environmental stimuli. The most prominent among these is the extinction-reinstatement procedure. Reinstatement, in the learning literature, refers to the recovery of excitatory responding to an extinguished stimulus produced by noncontingent exposure to the unconditioned stimulus (US). Conditioned reinstatement is a term largely confined to the addiction literature and refers to the resumption of responding at a previously drug-paired lever produced by exposure to drug-associated environmental stimuli (see **Box 1** and **2**).

The original demonstration, that conditioned cues previously paired response-contingently with drug infusions reinstate cocaine or morphine seeking following extinction of drug-reinforced instrumental responding in the absence of these cues, was made in the late 1960s and mid-1970s – in rhesus monkeys, by Schuster and Woods and Goldberg and colleagues, and in rats, by Davis and Smith. In contemporary applications, the degree of responding maintained by the contingent presentation of the drug-paired CS in the absence of further drug availability provides an operational measure of craving or relapse (see **Box 1**). A more recently employed set of extinction-reinstatement procedures utilizes drug-related discriminative or contextual stimuli, present non-contingently during drug availability, measuring the recovery of extinguished drug seeking by noncontingent presentation of these stimuli (see **Box 2**). Noncontingent exposure to contextual cues readily elicits reinstatement, whereas reinstatement produced by a discretely drug-paired CS requires response-contingent presentation. Once initiated, however, both types of stimuli maintain behavior at a previously active operandum. These two variants of associative learning likely occur simultaneously during drug use but may be coded, at least in part, through different neural circuitries or activation patterns.

An important consideration concerning the significance of learning factors for drug addiction concerns the role of discrete drug-paired versus discriminative or contextual stimuli. The latter category of stimuli signals the availability of a reinforcer and thereby sets the occasion to engage in behavior that brings the organism into contact with the reinforcing substance. A condition often associated with drug craving in the human is cognitive awareness of drug availability. It has been argued, therefore, that the manner in which drug-associated contextual cues attain their

Box 1 Conditioned reinstatement: discrete cues.

One of the original and most widely employed methods to study the effects of drug cues on the recovery of extinguished drug-seeking behavior. In this procedure, animals are trained to respond at a lever (or other operandum), with the completion of each response requirement (as determined by the schedule of reinforcement in effect) resulting in delivery of an intravenous (e.g., opioids or psychostimulants) or oral (e.g., ethanol) dose of the drug. Each response producing the drug reinforcer is contingently paired with a brief presentation of an environmental stimulus (e.g., a tone or cue light) that, by virtue of these repeated pairings, comes to serve as a conditioned stimulus (CS). Both drug administration and presentation of the CS are contingent upon a response by the animal (i.e., response-contingent). Once reliable drug self-administration is acquired, drug-reinforced instrumental responding is extinguished by ceasing to reinforce responses by drug delivery and withholding presentation of the CS. Extinction sessions are then conducted until responding is substantially lower than that maintained by delivery of the drug and deemed incidental rather than motivated by expectation of obtaining the drug. Subsequently, reinstatement tests are conducted in which the degree of recovery of responding at the previously drug-paired lever, now maintained by response-contingent presentation of the CS only (i.e., without further drug delivery), is operationally defined as a measure of craving or relapse. Extinction and reinstatement tests can be conducted in different sequences as shown in **Figure 1**.

incentive properties is likely to involve the predictive nature of these stimuli (as utilized in contextual reinstatement and the runway model) rather than only classically conditioned stimulus-response associations as modeled with reinstatement procedures utilizing discrete drug-paired CSs. Moreover, by virtue of their presence during drug consumption, contextual cues also become associated with the rewarding effects of the drug and, thus, acquire incentive-motivational value (i.e., elicit memories of previous drug euphoria and the magnitude or value of the rewarding effect of drug consumption). Because of this dual action, these stimuli are particularly powerful in eliciting drug seeking and reinstatement, and this action is likely to underlie their powerful potent and persistent motivating actions both in humans and animals. Most contemporary applications of the reinstatement procedure utilize contextual or discriminative stimulus models.

Another contextual model of drug seeking is the renewal of conditioned place preference (CPP) (see **Box 2**). This procedure evolved from the traditional CPP procedure, one of the original and most widely employed tools for studying conditioned reinforcement. Place-conditioning procedures permit examination of neural substrates involved in the acquisition and expression of the conditioned reinforcing effects of drugs of abuse. Manipulations that specifically alter the expression of CPP, once acquired, provide information on the neural basis of conditioned drug seeking, whereas interference with the acquisition of CPP is relevant for the understanding

Box 2 Conditioned reinstatement: contextual cues.

To study the effects of environmental context on the recovery of drug seeking. Drug availability is conditioned to stimuli (i.e., olfactory, auditory, tactile, or visual cues) present in the self-administration environment, and these stimuli are not paired contiguously with drug infusions nor contingent upon a response. Owing to their predictive nature for drug availability, these stimuli set the occasion for engaging in reward-seeking (i.e., lead to the initiation of responding). Except for using context or noncontingent discriminative stimuli as cue manipulations, these models are identical to the discrete cue (CS) model in terms of the training and experimental sequence, with conditioning followed by extinction in the absence of the cue and, subsequently, reinstatement tests in the presence of the drug-associated cues. Multiple contextual reinstatement procedures exist:

The basic model utilizes differential reinforcement of behavior in the presence of discriminative stimuli. In this procedure, during self-administration learning, responses at the operandum are reinforced by the drug only in the presence of this stimulus. In the absence of the stimulus (or, as more frequently employed, presence of a distinctly different cue) responses remain nonreinforced.

A second frequently employed contextual conditioning model – pioneered by Bouton and Schwartzrenuber in the early 1990s to study how the context influences extinction and resumption of learned behavior – utilizes distinct environments that provide compound contextual cues (i.e., concurrent presence of olfactory, auditory, tactile, and visual cues). Here responding is reinforced by a given drug reinforcer in one context. Drug-reinforced responding then is extinguished in a second context. Subjects subsequently tested in the second context show low drug seeking because the behavior has been extinguished in this context. In contrast, animals tested in the first (drug-paired) context show reactivation or renewal of responding at the previously active operandum.

A variant of the contextual reinstatement procedure above is based on learning of a conditioned place preference (CPP) for an environment paired with experience of the drug. Following extinction of CPP, accomplished by pairings of vehicle rather than drug with the environment, re-establishment – technically termed renewal or reactivation of CPP – is produced by a drug injection. This model can also be employed to study reactivation of an acquired CPP following an imposed period of abstinence.

of mechanisms mediating the acute reinforcing effects of drugs or the learning of Pavlovian associations. The degree of preference for a previously drug-paired environment provides an index of the strength of drug seeking associated with the incentive-motivational effects of a drug-associated stimulus context. Traditional applications of CPP, however, do not involve imposition of abstinence and thus have limitations with respect to providing a tool for studying relapse. In contrast, the CPP renewal procedure has been successfully applied in conjunction with abstinence manipulations, following which drug injections or stress reactivate CPP. Nonetheless, while this procedure is advantageous in that it is easy to perform technically, it has other limitations.

Among these is that it does not provide a pure measure of conditioned reinforcement or reinstatement, but rather of interactions between contextual conditioning and the effects of small priming doses of the drug or stress. An issue to be considered as well is that CPP typically involves noncontingent administration of drugs such that the strength or nature of associations that are formed between drugs and environmental stimuli may differ in CPP versus self-administration procedures. Lastly, the number of learning trials in the CPP procedure is substantially lower than in reinstatement models utilizing self-administration procedures. Possibly due to these differences, the expression of CPP and conditioned reinstatement is sometimes differentially sensitive to pharmacological manipulation.

The operant runway model is a procedure in which a role of conditioning factors in drug seeking can also be readily demonstrated (see **Box 3**). The runway model dates back to the 1930s and has been widely employed to study goal-seeking behavior. It incorporates aspects of both second-order schedule and CPP procedures in that it permits examination of the animals' motivation to engage in drug seeking in a drug-free state before the drug reinforcer is applied and with responding (here runtime) unaffected by the performance-altering consequences of the drug. A unique finding with this procedure was the demonstration that rats receiving cocaine as a reinforcer exhibit approach-avoidance retreat behaviors, on the following day, before entering the goals box where drug administration occurs. This ambivalence is thought to reflect the presence of both positive and negative associations with the goals box, consistent with subjective reports of cocaine users that the initial euphoric effects of cocaine are often followed by an unpleasant state. A limitation of this model is that rats are receiving only a limited number of drug injections each day and under conditions that do

Box 3 Operant runway model of relapse.

A procedure in which run-time from a start-box to a goal-box where the drug is administered provides a dependent measure of drug seeking. In this procedure, a discriminative stimulus present in the start-box, runway, and goal-box is predictive of drug reward obtainable in the goal box, whereas a different discriminative stimulus predicts nonavailability of drug reward. Run-times eventually decrease in the presence of the drug-predictive discriminative stimulus, but not with the nonreward cue. Rats then are placed on extinction conditions under which both the discriminative stimulus and drug injections are absent, with the result that run-time increases progressively. During subsequent reinstatement tests, reintroduction of the drug-paired discriminative stimulus decreases the run-time for reaching the goal-box again. In addition, a drug infusion in the goal-box during extinction reduces run time on the subsequent drug-free day. This decrease in the latency to reach the goal-box as associated with these manipulations serves as a measure of relapse.

Box 4 Second-order schedules of reinforcement.

A reinforcement contingency by which a set of responses on a schedule of conditioned reinforcement is treated as a unit-response under a second schedule that is concurrently in effect. Completion of the unit-response for the first schedule component produces presentation of a CS, and completion of n unit-responses produces the CS accompanied by a single dose of the primary (drug) reinforcer. The contingency then reverts again to the non-drug-reinforced schedule component with response-contingent presentation of the CS only, until completion of n unit response requirements leads to the next drug injection. The principal measure of interest in this procedure is the degree of conditioned reinforcement maintained by the drug-paired CS, prior to drug delivery. Second-order schedules are highly effective for investigating the significance of conditioned reinforcement in ongoing drug seeking and taking.

not permit the animal to control or regulate drug intake. The procedure, therefore, models conditioned reinforcement processes associated with recreational drug use, but not the consequences of voluntary high-dose drug intake on conditioned drug seeking and relapse.

Widely employed also are second order and seeking-taking chained schedules of reinforcement where the measure of interest is conditioned reinforcement maintained by a drug-paired CS or context, leading to eventual availability of the drug reinforcer (see **Boxes 4 and 5**). These schedules produce very high rates of responding and have proven uniquely effective for investigating conditioning factors in ongoing drug seeking and taking and, most recently, in establishing a link between the trait of impulsivity and the reinstatement of cocaine seeking despite a history of footshock stress-punished seeking behavior. Investigation of the neural basis of conditioned drug seeking using these schedules is, however, somewhat constrained by administration of the drug that must follow completion of the CS-maintained component under these reinforcement contingencies. In other words, these schedules provide a narrow drug-free window during which behavior controlled by the CS alone can be studied unaffected by pharmacological effects of the drug on neural function or behavioral performance.

Characteristics of Conditioned Drug Seeking and Reinstatement

Both discretely conditioned and contextual stimuli produce robust reinstatement of drug or alcohol seeking following extinction and abstinence. This effect is reliable across different drug classes including cocaine, amphetamine, heroin, ethanol, and nicotine, and holds across variations in training, conditioning, and reinstatement test procedures. The response-reinstating effects, in particular, of drug-related contextual stimuli show remarkable persistence over repeated tests with nonreinforced

Box 5 Seeking-taking chained schedules.

Chained schedules employed in the context of studies of conditioned drug seeking typically consist of a seeking phase in which responses at a drug-seeking lever are initially required. Following completion of a response requirement or time interval in the first (i.e., seeking) link of the chained schedule, the second link is initiated by making available a drug-taking lever. Responses at this lever produce a drug-reinforcer and presentation of a CS, followed by a time out period, whereupon the seeking link of the chain is reinitiated. The degree of conditioned drug seeking or relapse is measured by the number of seeking responses during sessions in which responses at the taking lever produce only the CS but no drug injection.

In the area of alcohol addiction research, a variant of the seeking-taking schedule is often employed that dissociates ethanol-reinforced consummatory behavior (i.e., ethanol drinking) from appetitive ethanol-seeking responses (i.e., behavior induced and maintained by the incentive-motivational effects of ethanol-associated contextual cues present in the self-administration environment). In this procedure, rats must complete a set of responses at a lever operandum during which time ethanol is not available (appetitive phase). Completion of a response requirement within a specified time results in retraction of the lever and presentation of a sipper tube containing ethanol solution from which rats are then allowed to freely drink for a given amount of time (consummatory phase). Thus, responding during the appetitive phase in this model provides a measure of the day-to-day strength of animals' motivation to initiate and engage in ethanol-seeking behavior when exposed to the ethanol-predictive stimulus environment of the operant conditioning chamber.

exposure, and remain effective in eliciting drug seeking even after prolonged periods of abstinence. In fact, stimuli conditioned to cocaine during a single lifetime drug experience have been shown to elicit drug seeking for up to 1 year. Moreover, it can be demonstrated that the intensity of drug seeking shows time-dependent increases over the initial months of abstinence, a phenomenon referred to as incubation of craving. The persistence of the motivating effects of drug-associated stimuli in the animal literature resembles the long-lasting, compulsive-like nature of craving and relapse-risk associated with exposure to drug cues in humans, and provides experimental confirmation for the hypothesis that learned responses to drug-related stimuli are a significant factor in persistent vulnerability to relapse. Conditioned drug seeking in animals resembles compulsive-like drug seeking in the human not only in terms of persistence. Rats with a history of prolonged daily access to cocaine fail to show suppression of drug seeking maintained by a CS when concurrently presented with a conditioned stressor or exposed to a punishing stimulus, and this behavior appears similar to impaired impulse control. Thus, drug-related cues in rats can sustain drug-directed behavior despite adverse consequences, one of the hallmarks of substance dependence. Important for understanding the scope of drug-related learning in the relapse

process are findings that presentation of drug cues significantly exacerbates reinstatement of drug seeking produced by stress, another major risk factor for relapse. This has originally been demonstrated in rats concurrently exposed to alcohol-related cues and footshock stress. The existence of such interactive effects between drug cues and stress has been corroborated in the context of a pharmacological stress manipulation where yohimbine, a noradrenergic α_2 -receptor antagonist with anxiogenic action, significantly potentiated conditioned reinstatement of cocaine seeking. Lastly, evidence exists that opiate-dependent rats develop a CPP for an environment associated with relief from withdrawal after morphine administration. Thus, drug or alcohol self-administration during withdrawal may similarly convey conditioned incentive value to environmental stimuli associated with amelioration of the negative consequences of withdrawal, thereby exacerbating the effects of drug cues in tests of reinstatement. Consistent with this hypothesis, ethanol-dependent rats repeatedly allowed to self-administer alcohol during withdrawal and presented with an ethanol-paired CS several weeks following withdrawal (i.e., during what has been referred to as the protracted abstinence phase) showed greater conditioned reinstatement than dependent rats not given experience with ethanol during withdrawal.

Important for understanding the significance of drug-related maladaptive learning in addiction are differences that exist between the stimulus control of behavior motivated by cues conditioned to drugs of abuse as opposed to natural rewards essential for survival, well-being, and normal hedonic pursuits. For example, a single pairing of cocaine with drug-predictive discriminative stimuli elicited conditioned reinstatement for 1 year whereas the same discriminative stimuli – paired with a palatable sweet solution – remained effective for less than 3 months. Similarly, while seeking behavior for natural reward (e.g., sucrose) shows incubation, this effect is weaker and more short lived than incubation of conditioned cocaine seeking. Moreover, when exposed to punishing stimuli, animals typically show conditioned suppression of appetitive behavior whereas this is not the case with drug-directed conditioned behavior. Several mechanisms have been proposed by which neural signaling that regulates normal appetitive conditioning differs from that mediating conditioned behavior resulting from drug-related learning. First, neural circuits mediating the effects of drug cues may be distinct from those mediating the conditioned effects of natural reward. Second, neural circuits mediating the effects of drug cues may not be specific to addiction-related events, but normal circuits are activated to a greater degree, thereby creating new motivational states or tilting processes that normally govern responding for natural rewards toward drug-directed behavior. A related possibility is that information about drug versus nondrug cues is processed by distinct populations of neurons, but within

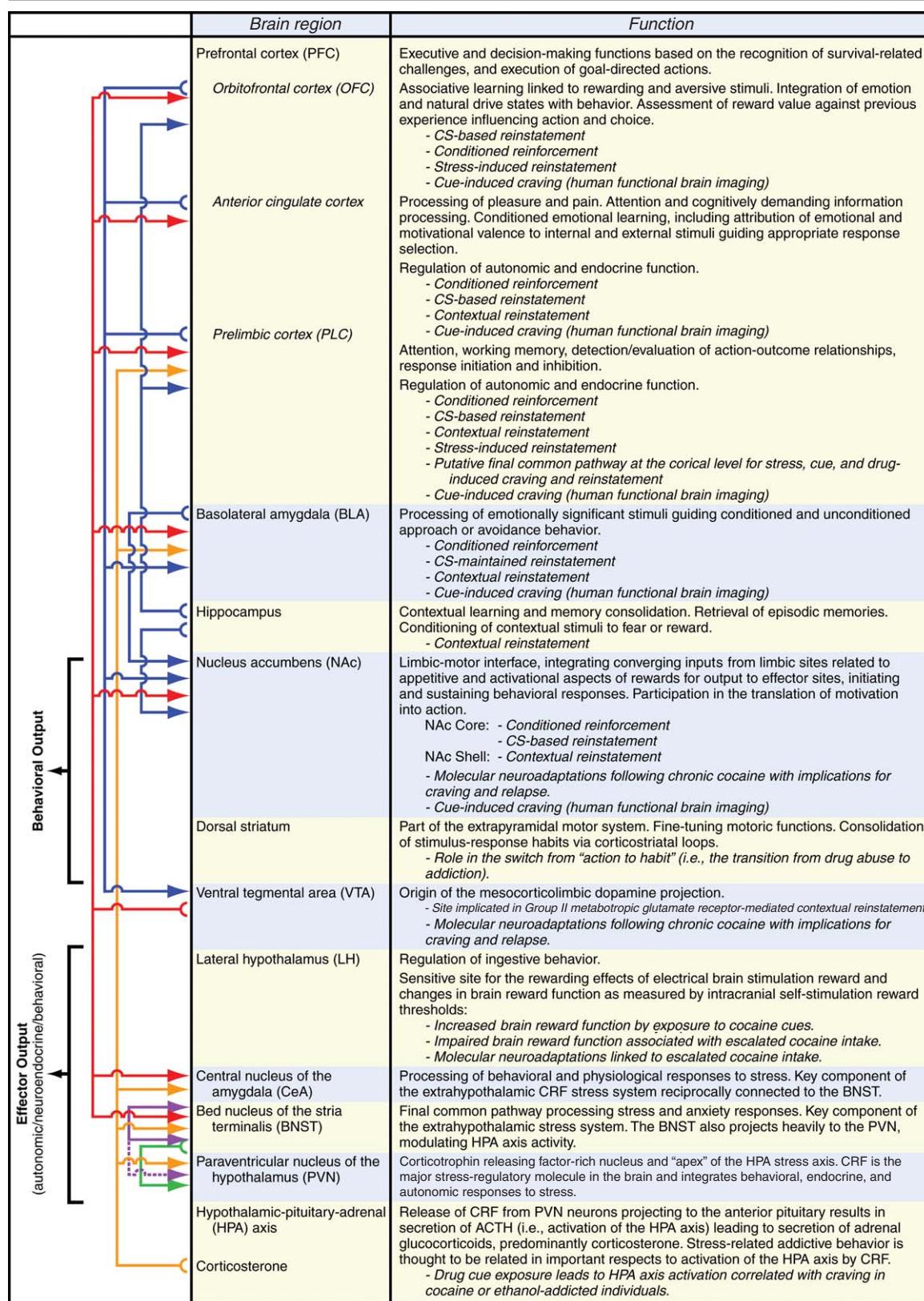
the same circuitry. Third, drug-induced neuroadaptation may provide a switch whereby a circuit not normally involved in drug seeking becomes responsive to drug cues. Some experimental support exists for all of these hypotheses such that it appears likely that several mechanisms may contribute to the differential control of behavior by stimuli conditioned to drug versus natural reward.

Neurocircuitry Mediating Conditioned Drug Seeking and Reinstatement

Drugs of abuse have diverse pharmacological profiles and produce differential behavioral effects. Nonetheless, their conditioned effects share the common feature of activating major components of the brain incentive-motive circuit. Specifically, neural mapping studies, targeted lesions and site-specific pharmacological manipulations in animals implicate a distributed set of brain regions connected by the corticostriatopallidal circuit in conditioned drug seeking and reinstatement. Major components of this circuit include the terminal regions of the mesocortical dopamine pathway that originates in the ventral tegmental area (VTA) and projects to the orbitofrontal, anterior cingulate, and prelimbic divisions of the prefrontal cortex, and of the terminal regions of the mesolimbic VTA dopaminergic projections that include the central and basolateral amygdala, hippocampus, nucleus accumbens (NAcc), as well as projections to the dorsal striatum. These brain regions, their function, and their neurochemical and neuroanatomical connectivity are described in **Table 1** and **Figure 2**.

Among brain regions associated with the mesolimbic dopamine pathway, the NAcc which has a critical role in the primary reinforcing effects of drugs of abuse also participates in mediating drug-directed conditioned responding. Current evidence favors a preferential role of the NAcc core subregion in CS-maintained second-order-schedule performance and reinstatement induced by discrete drug-paired cues, whereas drug seeking elicited by contextual stimuli is dependent on the NAcc shell. Direct evidence exists for a role of the amygdala (including both the central and basolateral nuclei) and the ventral hippocampus in discrete stimulus-reward associations relevant for conditioned drug seeking on a second-order schedule and reinstatement induced by discrete drug-paired CSs. The basolateral and central nuclei of the amygdala may, however, participate also in mediating the effects of contextual stimuli because these brain regions show neural activation in animals exposed to drug-paired contextual cues. The dorsal hippocampus has an important role in contextual memory retrieval and the occasion-setting actions of contextual stimuli, and evidence implicates the dorsal hippocampus in mediating the stimulus-stimulus associations relevant for drug-related contextual learning. The orbitofrontal

Table 1 Major function and role in addictive behavior (italicized) of brain regions implicated in mediating conditioned drug-seeking. Shown in highly simplified form are the major neurochemical pathways by which these brain regions are connected (Semicircles represent the region of origin and arrows the terminal fields of individual neural connections. The identity of the major neurotransmitter in each pathway is represented by different dotted line patterns: dopamine (---), glutamate (- - -), CRF/GABA (— · —), GABA (— · — · —), adrenal glucocorticoid (predominantly corticosterone) feedback (— · — · — · —). Additionally, the schematic indicates major effector output originating from addiction-relevant sites. Note that these are not direct, but polysynaptic pathways via projections to sites including the thalamus, hypothalamus, dorsal and ventral pallidum, and brainstem nuclei. [Abbreviations: ACTH = adrenocorticotrophic hormone, CRF = corticotropin-releasing factor, HPA = hypothalamic-pituitary-adrenal, mGluR = metabotropic glutamate receptor]



cortex and prefrontal cortex, brain regions associated with the mesocortical dopamine pathway, regulate cognitive and executive function as well as emotional responses. Chronic cocaine exposure, and likely exposure to other drugs of abuse, produces neuroadaptive dysregulation in prefrontal glutamatergic afferents to the NAcc that contribute to persistent addictive behaviors, including diminished cognitive and impulse control, heightened sensitivity to drug-associated stimuli, and, presumably, impaired ability to learn alternative responses to compete with drug-seeking behavior. Lastly, the dorsal striatum has been recognized as serving a critical role in consolidating stimulus-response habits associated with addictive behavior via the engagement of corticostriatal spirals. It is important to note that the findings on the neural regulation of conditioned drug-seeking responses in the animal literature are consistent with brain-activation patterns observed in functional brain imaging studies of cue-induced craving in the human – findings that support the validity of conditioned drug-seeking procedures as models of craving and relapse.

Neural mapping data show that, in addition to activation of the corticostriatopallidal circuitry, contextual cues conditioned to ethanol produce activation of brain sites not traditionally linked to conditioned drug seeking and reinstatement. These include the medial parvocellular and magnocellular

paraventricular nuclei (PVNs) of the hypothalamus. Activation of medial parvocellular PVN neurons is positively correlated with hypothalamic–pituitary–adrenal (HPA)-axis activation, suggesting that alcohol cues elicit a stress-like neuroendocrine response. Activation of the magnocellular PVN by ethanol cues represents an effect that is consistent with psychological stress, lending further support to the hypothesis that ethanol cues produce stress-like effects. In addition to influencing the HPA axis, activated PVN neurons, through descending brainstem projections, may influence autonomic responses associated with the anticipation of ethanol reward predicted by ethanol cues as observed in alcoholic subjects. Although not yet clearly documented, activation of these stress regions likely extends to the effects of cues conditioned to other drugs of abuse. This is so because, in the human, drug-cue manipulations associated not only with ethanol but also with cocaine induce a pattern of cue reactivity and craving similar to that induced by stress, and these effects are accompanied by increased HPA-axis activity and anxiety. Thus, subjective responses to drug cues include stress-like reactions and these may contribute to drug seeking (in animals) and resumption of drug use (in humans) elicited by these cues, given the well-established significance of stress as a risk factor for relapse.

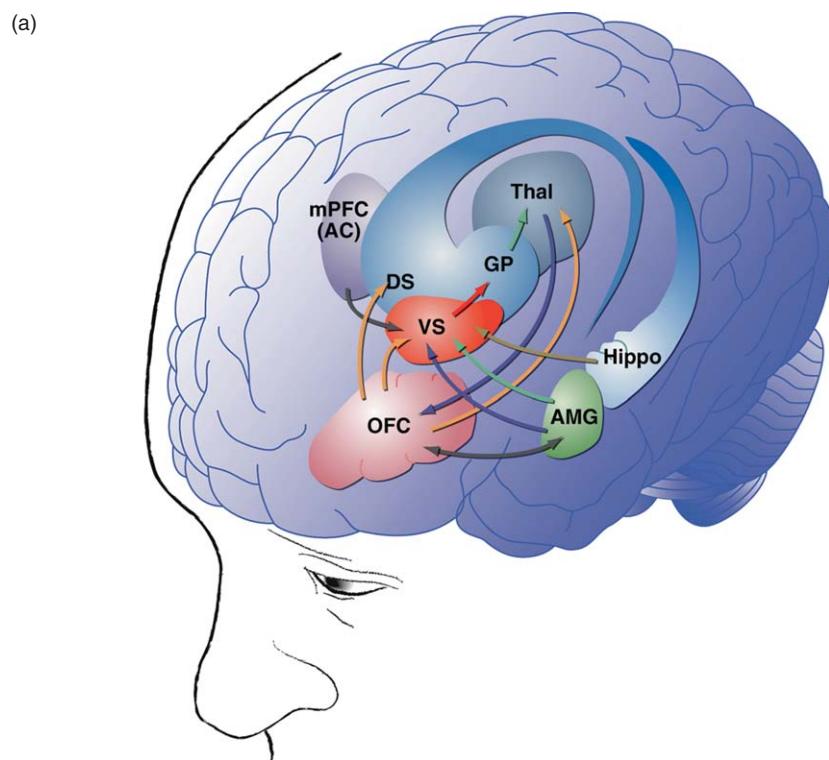


Figure 2 (Continued)

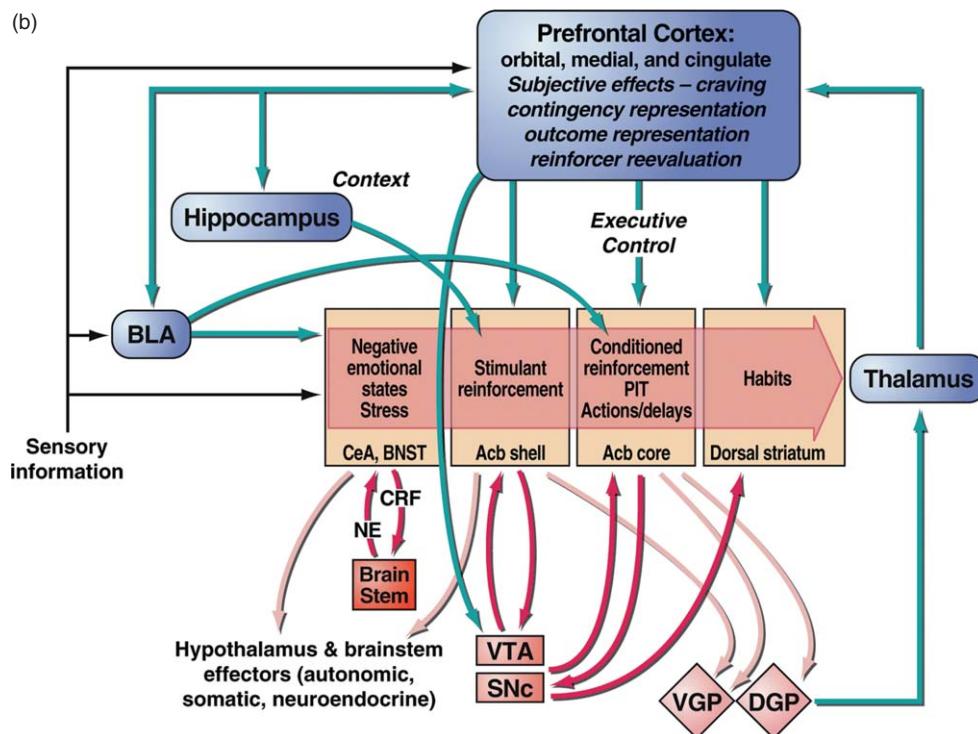


Figure 2 Representation of limbic cortical and associated striatal circuitry, with tentative localization of functions involved in drug addiction. (a) Key structures include the medial prefrontal cortex (mpFC), which also includes the anterior cingulate (AC), orbitofrontal cortex (OFC), hippocampus (Hippo), ventral striatum (VS), nucleus accumbens, dorsal striatum (DS), globus pallidus (GP), amygdala (AMYG), and thalamus (Thal). (b) Limbic corticostriatopallidal circuitry. (i) Processing of conditioned reinforcement and delays by basolateral amygdala and of contextual information by hippocampus. (ii) Goal-directed actions involve interaction of the prefrontal cortex with other structures, possibly including the nucleus accumbens and dorsomedial striatum. (iii) 'Habits' depend on interactions between the prefrontal cortex and dorsolateral striatum. (iv) 'Executive control' depends on the prefrontal cortex and includes representation of contingencies, representation of outcomes and their value, and subjective states (craving and, presumably, feelings) associated with drugs. (v) Drug craving involves activation of the orbital and anterior cingulate cortex and temporal lobe, including the amygdala, in functional imaging studies. (vi) Connections between dopaminergic neurons and the striatum reflect 'spirals' – serial interactions organized in a ventral-to-dorsal cascade. (vii) Reinforcing effects of drugs may engage stimulant, Pavlovian instrumental transfer and conditioned reinforcement processes in the nucleus accumbens shell and core and then engage stimulus-response habits that depend on the dorsal striatum. (viii) The extended amygdala is composed of several basal forebrain structures including the bed nucleus of the stria terminalis, the centromedial amygdala, and, more controversially, the medial portion (or shell) of the nucleus accumbens. Corticotropin-releasing factor is a major transmitter in the extended amygdala, which projects to the brainstem where noradrenergic neurons provide a major projection reciprocally to the extended amygdala. Activation of this system is closely associated with the negative affective state that occurs during withdrawal. Green/blue arrows: glutamatergic projections; orange arrows: dopaminergic projections; pink arrows: GABAergic projections; Acb = nucleus accumbens; BLA = basolateral amygdala; VTA = ventral tegmental area; SNC = substantia nigra pars compacta; VGP = ventral globus pallidus; DGP = dorsal globus pallidus; BNST = bed nucleus of the stria terminalis; CeA = central nucleus of the amygdala; NE = norepinephrine; CRF = corticotropin-releasing factor; PIT = Pavlovian instrumental transfer. (a) Redrawn from Zald and Kim (2001). (b) From Koob GF, Everitt BJ, and Robbins TW (2002). In Squire FG, Berg D, Bloom FE, Du Lac S, Gosh A, and Spitzer N (eds.) *Fundamental Neuroscience*, 3rd edn., pp 987–1016. Amsterdam: Academic Press.

Conditioned Withdrawal

As outlined in the introduction to this article, the investigation of conditioning factors in drug abuse has originated with studies on the role of classical conditioning in the opiate withdrawal syndrome. Anecdotal evidence has existed for some time that return to drug-related environments can elicit withdrawal-like symptoms in former opiate addicts, a conditioning phenomenon that may precipitate return to drug use. Using a peppermint-odor cue paired with administration of the opiate antagonist naloxone to

induce withdrawal in methadone-maintained heroin addicts, O'Brien and colleagues demonstrated that, following several conditioning trials, the peppermint odor alone elicited conditioned withdrawal reactions. These results lend credence to subjective reports of such conditioned withdrawal responses occurring naturally although their clinical significance for recidivism remains to be established. Such conditioned withdrawal reactions have also been demonstrated in the rat and monkey. In particular, CSs predicting the onset of heroin withdrawal produced an elevation in the threshold for rewarding electrical brain

stimulation in rats. Such reward deficit are thought to be reflective of the presence of negative affect (i.e., dysphoria, anhedonia, etc.), a state that is likely to contribute to relapse risk. Neuroanatomically, the basolateral amygdala is a critical neural substrate mediating conditioned opiate withdrawal in rats. Given that the basolateral amygdala also regulates the appetitive effects of drug-related stimuli, it appears that the amygdala mediates associative learning whereby both positive and aversive events become conditioned to specific environmental cues. As such, this brain site may be a key element within the corticostriatopallidal circuitry that sustains compulsive drug seeking and relapse associated with both conditioned positive reinforcement and the impact of conditioned withdrawal.

See also: Acute Dependence; Alcoholism; Animal Models of Behavior: Alcohol Addiction; Depression; Drug Addiction; Drug Withdrawal – Motivational View; Hallucinogens; Neurobiology of Opioid Addiction; Nicotine; Psychostimulants; Transition to Addiction; Δ9- THC.

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Drug Priming

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Glossary

Conditioned place preference – The preference animals show for a contextually distinct location that has previously been paired with a drug of abuse.

Drug self-administration – A model for drug addiction in which animals engage in a specific behavior in order to receive the drug of abuse.

Extrasynaptic glutamate – The glutamate located in extrasynaptic areas that is regulated by glial cells and provides activation of presynaptic glutamate receptors. It is regulated separately from synaptically released glutamate.

Locomotor sensitization – The increased locomotor response of an animal to an acute drug injection after a period of chronic drug administration, as compared to an acute injection in a naive animal.

Medial prefrontal cortex – The brain structure involved in executive function as well as decision making and part of a final common pathway in the reinstatement of drug-seeking behavior.

Nucleus accumbens (NAcc) – The part of the ventral striatum that acts as an interface between decision-making brain regions and behavioral output, known to be involved in motivation and reward, including drug reward.

Reinstatement – The return to drug-seeking behavior in animals after extinction training in the drug self-administration paradigm, frequently used as a model for relapse.

Relapse – The return to drug use after a period of withdrawal/abstinence.

Drug addiction refers to the end state of a drug-taking process in which individuals move from recreational drug use to chronic drug abuse to, finally, addiction itself, wherein the person finds it difficult-to-impossible to cease using the drug and the drug use impairs the person's ability to conduct normal life functions. After undergoing treatment for drug addiction, human drug users have great difficulty remaining drug-free and face high rates of relapse back to drug use. 'Relapse' can be defined as the return to drug taking following a period of abstinence. While some modest strides have been made in treating addiction and reducing the risk of relapse, no treatment has emerged as a true panacea. Thus, there is considerable

ongoing research to identify the neurobiological mechanisms underlying relapse.

Models of Drug Addiction

In order to investigate the neurobiology of drug addiction related to relapse, animal models have been developed. Such models include: (1) repeated noncontingent drug administration and the development of locomotor sensitization, (2) conditioned place preference, and (3) drug self-administration. In the locomotor sensitization model, animals are given repeated systemic administration of the drug of interest, typically over 1 week. Nearly all drugs of abuse induce changes in animals' locomotor activity. It has been found that repeated administration of drugs induces locomotor sensitization, which is defined as an animal's increased locomotor response to an acute administration of a drug following chronic drug administration, as compared to the locomotor response to the first acute administration of the drug. Although the sensitization model has enabled the examination of some of the neuronal adaptations induced by repeated drug administration, it lacks the learning components involved in human drug use associated with relapse. In the conditioned place preference model, rats are given drug injections and placed in a contextually distinct location, typically one chamber of a two-chamber apparatus. At other times, rats are given saline injections and placed in the other chamber in the apparatus. Through these pairings, rats learn to associate one chamber with the drug of interest. During a test day, rats are permitted to freely explore the entire apparatus and show a preference for that chamber paired with the drug of abuse, as indicated by time spent in that chamber. Conditioned place preference has been useful because it allows the investigation of the rewarding properties of the drug as well as the associated learning that occurs between the drug and the paired chamber. However, similarly to the sensitization model, conditioned place preference requires the experimenter to administer the drug of abuse, making it a significant departure from the human experience in which humans decide to consume the drug of abuse.

In the drug self-administration model, the animal engages in a particular behavior, such as pressing a lever, in order to receive a small amount of the drug of interest. The drug may be taken orally, through inhalation, or intravenously, and animals used in this model

have included pigeons, mice, rats, and nonhuman primates, thus enabling a variety of experiments to be performed. Studies examining self-administration have provided considerable knowledge about the reinforcing effects of drugs of abuse. Most commonly abused drugs increase mesolimbic dopamine levels throughout the forebrain. Dopamine normally serves as a neuromodulator that indicates the importance of external stimuli and reinforces the animal's learning about the stimuli. Dopamine is particularly important for inducing the neuroplasticity that underlies adaptive changes in the animal's behavior in response to external consequences of the behavior. A critical locus of dopamine's influence on the plasticity underlying reinforcement is the nucleus accumbens (NAcc). Natural rewards and drugs of abuse increase dopamine levels in the NAcc, and blockade of dopamine receptors impairs this reinforcement learning.

Reinstatement Model of Relapse

The drug self-administration model has provided considerable important insight regarding how drugs of abuse induce people to consume more of the drug in the future; however, it has not explained why people relapse to drug use, even after they have undergone treatment and extended abstinence. To model some aspects of the relapse associated with drug addiction, animals are trained to self-administer an addictive drug for a period of time (e.g., 2 weeks) followed by another period, termed 'extinction' training, in which the animal's responses no longer produce the drug reward. During extinction training, the animal's behavioral responses eventually decrease (extinguish) as the animal learns that drug reward is no longer achieved by pressing the lever. When the animals have reached a sufficiently low level of responding, relapse to drug-seeking is then induced in a reinstatement session. Reinstatement refers to the return to drug-seeking behavior (e.g., lever-pressing) even though the behavior is not rewarded with the drug. Thus, reinstatement serves as index of the animal's drug-seeking, not drug-taking, behavior and presumably reflects the animal's desire and drive to receive the drug of abuse.

Several triggers are known to induce relapse in humans, each of which has been modeled in rats. Human drug addicts frequently return to drug use after being exposed to the cues or places associated with the drug taking. This is modeled in 'cue- or context-induced reinstatement', in which cues or the context, previously paired with the drug infusions, are present again during the reinstatement test. Human addicts also return to drug use following a stressor, which is modeled in 'stress-induced reinstatement'. In this model, immediately prior to the reinstatement test, rats receive a stressor such as a

series of footshocks, or in an alternative method, an injection of the α_2 -adrenergic antagonist yohimbine. These stressors induce reinstatement of drug seeking. Another trigger occurs when people consume a small amount of the drug itself, believing that they can handle 'just one hit' or 'just one drink'. In addicts, this small amount of the drug then serves as a kind of reminder that induces a full return to drug taking and relapse. In 'drug prime-induced reinstatement', animals receive a noncontingent administration of the drug and are then tested for the reinstatement of drug seeking.

During the reinstatement test, animals significantly increase their responding, which serves as an index of drug-seeking behavior, but the animals never receive a drug infusion during the reinstatement test. The lack of a drug reward during the reinstatement test is critical for studying relapse, as it permits examination of the neurobiology of reinstatement without concern for the reinforcing effects of the drug of abuse on drug seeking (e.g., lever pressing). The focus of this article is drug-induced reinstatement/relapse to drug seeking.

Neurotransmitters Underlying Drug-Induced Reinstatement

As noted, most, if not all, drugs of abuse increase dopamine levels. Drugs such as cocaine and amphetamine do this directly through actions on the dopamine transporter located presynaptically on dopaminergic neurons. Other drugs, such as heroin, nicotine, and alcohol, do this indirectly by increasing activity in dopamine-producing neurons located in the ventral tegmental area (VTA). Thus, it is not surprising that drug-induced reinstatement depends on activation of dopamine receptors. Blockade of either D1 or D2 dopamine receptors by a systemic administration of dopamine receptor antagonists reduces reinstatement induced by cocaine, alcohol, and heroin. Similarly, dopamine reuptake inhibitors, which mimic cocaine's action, and D2 agonists induce reinstatement of cocaine seeking. Drugs that work on the dopamine transporter, including cocaine and amphetamines, also appear to have effects on the noradrenergic and serotonergic systems through their transporters. However, the role of those systems in drug-induced reinstatement is not as obligatory. Serotonin and norepinephrine reuptake inhibitors do not induce cocaine seeking in rats, but norepinephrine reuptake inhibitors induce cocaine seeking in squirrel monkeys, although with decreased efficacy compared to a dopamine reuptake inhibitor or cocaine itself.

Drugs that work through other systems and indirectly increase dopamine levels also require those other systems to induce reinstatement. Thus, heroin- or opiate-induced reinstatement is prevented by systemic blockade of

mu-opioid receptors, and nicotine-induced reinstatement is blocked by systemic administration of nicotinic antagonists. Although alcohol acts in part as an allosteric agonist at γ -aminobutyric acid (GABA) receptors, it has other pharmacological effects in the brain, making its mechanism of action relatively complex. Thus, it is not surprising that blockade of other systems, such as the mu-opioid receptors or the endocannabinoid CB1 receptors, inhibits reinstatement of alcohol seeking. Systemic CB1 receptor antagonist administration prevents reinstatement of cannabinoid seeking. The endocannabinoid system also appears to be involved in relapse to drug seeking for other drugs of abuse, as systemic blockade of the CB1 receptors also prevents relapse to alcohol-, nicotine-, cocaine-, and methamphetamine-seeking behavior.

The interactions between the endocannabinoid system and relapse to non-cannabinoid drugs of abuse raises the issue of cross-reactivity. ‘Cross-reactivity’ of drug-induced reinstatement refers to the ability of one drug to induce reinstatement in animals that originally self-administered a different drug. Intriguingly, the ability of one drug to induce reinstatement of seeking for another drug does not mean the reverse is true. For example, heroin administration induces reinstatement of cocaine seeking, but cocaine administration has no effect on the reinstatement of heroin seeking. Moreover, drug classes respond differentially to reinstatement by the same drug of abuse. For example, endocannabinoid agonists induce reinstatement of alcohol seeking but have no effect on the reinstatement of cocaine seeking. The neurobiology of cross-reactivity has not been extensively studied but may have clinical relevance as drug addicts frequently use multiple classes of addictive drugs.

Neural Circuitry of Drug-Induced Reinstatement

As noted, the reinforcing effects of drugs of abuse appear to depend on the dopaminergic system, particularly the dopamine projections from the VTA to the NAcc. Drug-induced reinstatement not only involves activity in those systems but also recruits additional brain circuits. This is not surprising, as drug-seeking behavior mimics relapse and reflects the animal’s drive to obtain the drug of abuse. Thus, structures involved in decision making, such as the medial prefrontal cortex (mPFC), are also necessary for drug-induced reinstatement. However, the actual array of structures necessary for drug-induced reinstatement depends on the drug of abuse.

Relapse to Cocaine Seeking

Local inactivation of brain structures, using either GABAergic agonists or sodium-channel blockade, has

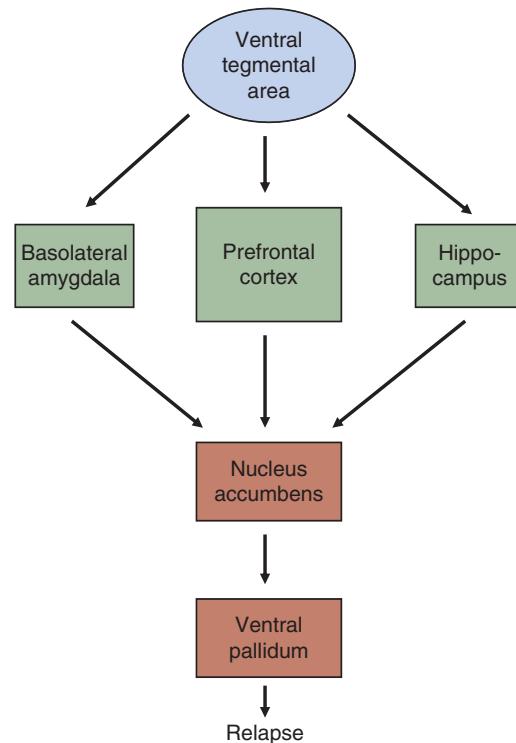


Figure 1 Schematic diagram of the neural structures underlying the relapse to drug-seeking behavior. The ventral tegmental area provides the dopaminergic input to most of the forebrain. The dorsomedial prefrontal cortex, basolateral amygdala, and hippocampus provide glutamatergic inputs to the nucleus accumbens, which sends GABAergic fibers to the ventral pallidum and regulates behavioral output. Although the basolateral amygdala and hippocampus are involved in cue- and context-induced reinstatement, the dorsomedial prefrontal cortex is required for cue-, context-, and drug-induced reinstatement, suggesting that it is a critical part of the final common pathway regulating drug-seeking behavior in rats.

been particularly useful in elucidating the structures responsible for relapse. Cocaine-prime reinstatement depends on activity in the VTA, the dorsal mPFC, the NAcc core, and the ventral pallidum (VP; see Figure 1). In contrast, inactivation of the substantia nigra, ventral mPFC, the NAcc shell, the basolateral amygdala, the central nucleus of the amygdala, or the medial dorsal thalamus has no effect on cocaine-induced reinstatement. Those areas involved in cocaine seeking are part of a circuit, in which the dorsal mPFC projects to the NAcc core, which in turn projects to the VP. Although the VTA projects to all three structures, blockade of dopamine receptors in the dorsal mPFC, and not the NAcc core or VP, prevents cocaine-induced reinstatement. This suggests that, during reinstatement, cocaine increases dopamine levels in the dorsal mPFC, thereby activating projections to the NAcc core. The NAcc core then integrates this information and controls behavioral output through the VP. Cue-, context-, and stress-induced reinstatement to cocaine seeking also

depend on activity in the dorsal mPFC, NAcc core, and VP, suggesting that these structures serve as a final common pathway for relapse to cocaine-seeking behavior.

Paradoxically, although inactivation of the NAcc shell has no effect on drug-induced reinstatement of cocaine seeking, blockade of dopamine receptors in the NAcc shell prevents reinstatement. It appears that the D1 and D2 class of dopamine receptors work together following a cocaine prime to induce reinstatement and that blockade of either class alone prevents such reinstatement. Moreover, recent work also indicates a critical role for the glutamatergic system in the NAcc shell during cocaine-induced reinstatement, as blockade of the α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptors in the shell prevents reinstatement. Continued work on the NAcc shell and core will elucidate their respective roles during drug-induced reinstatement.

It is not surprising that structures such as the NAcc and the mPFC are involved in cocaine-induced reinstatement. Evidence from studies with humans shows that presentation of drug-associated cues increases activity in those regions homologous to the dorsal mPFC in the rat. The dorsal mPFC is involved in decision making, and drug addicts have difficulty in decision making, frequently choosing drug-seeking and drug-using behavior despite the known negative consequences of those choices. Dysfunction in the dorsal mPFC and its outputs would, therefore, be expected as a consequence of chronic drug abuse. The NAcc serves as a critical node in the integration of information from the dorsal mPFC and memory- and emotion-related structures such as the basolateral amygdala and hippocampus. Through this integration, the NAcc then controls the behavioral output of the organism. Although the basolateral amygdala and hippocampus are not known to be involved in cocaine-induced reinstatement, activity in the basolateral amygdala or hippocampus is necessary for cue- or context-induced reinstatement, respectively. The animal and circuitry studies, therefore, support the hypothesis that enduring changes in the brain regions regulating decision making and behavioral output underlie the propensity to relapse.

Relapse to Heroin Seeking

Heroin-induced reinstatement appears to depend on a wider range of structures. It requires the same structures as cocaine-induced reinstatement but also requires activity in the basolateral amygdala, the central nucleus of the amygdala, the bed nucleus of the stria terminalis, and the substantia nigra. Although the mechanisms underlying the similarities and differences between heroin and cocaine reinstatement are not known, they clearly must depend on the pharmacological differences between the drugs. Heroin acts at mu-opioid receptors and disinhibits the dopaminergic neurons in the VTA through

mu-opioid receptors on the GABAergic neurons in the VTA, whereas cocaine acts directly on the dopamine system to block dopamine reuptake. One critical effect of this is that cocaine induces a much larger dopamine release than heroin does in structures such as the NAcc. In addition, as mu-opioid receptors are located throughout the forebrain, heroin influences neurons through direct effects on mu-opioid receptors in the structure as well as indirectly through the increase in dopamine release in the structure.

Continued work on relapse to heroin seeking as well as relapse for other drugs of abuse will provide critical knowledge on the neural circuitry and will, hopefully, confirm that structures such as the dorsal mPFC and the NAcc are involved in a final common pathway underlying relapse.

Glutamatergic System during Drug-Induced Relapse

Evidence strongly implicates that, following drug self-administration, the glutamatergic system, particularly in the NAcc, plays a critical role during drug-induced reinstatement. Much of the work on this issue has focused on cocaine-seeking behavior, but recent findings with heroin have bolstered support that alterations in the glutamatergic system underlie relapse in general. During cocaine- or heroin-induced reinstatement, increased dopamine levels activate dorsal PFC glutamatergic projections to the NAcc core, producing a significant increase in glutamate levels in the NAcc core. The necessity of this glutamate increase in the NAcc core has been demonstrated through studies showing that AMPA receptor blockade in the NAcc core prevents drug-induced reinstatement to cocaine or heroin seeking. Moreover, inactivation of the dorsal PFC prevents the glutamate increase in the NAcc core as well as reinstatement. Studies with stress-induced reinstatement of cocaine seeking and cue-induced reinstatement of heroin seeking confirm the critical role of the glutamatergic system in the NAcc core during drug seeking in general, as blockade of AMPA receptors in the NAcc core prevents reinstatement in those models. Intriguingly, blockade of the glutamatergic *N*-methyl-D-aspartic acid (NMDA) receptors in the NAcc core or shell induces reinstatement, suggesting that the AMPA receptors are the major contributors to the drive in the NAcc core that produces relapse but leaving unclear the role of NMDA receptors in the NAcc during relapse.

In contrast to rats with a history of self-administering cocaine or heroin, naive rats that receive a drug-priming injection do not have an increase in NAcc core glutamate levels. Rats that passively received cocaine or heroin also do not show the glutamate increase following a drug-priming injection. These findings strongly

suggest that chronically self-administering the drug of abuse, rather than simply repeated exposure to the drug, produces the critical alterations in the glutamatergic system that underlie relapse. Considerable work on cocaine-seeking behavior has elucidated the mechanisms underlying the increased glutamate release during cocaine-induced reinstatement. It appears that, following cocaine self-administration, basal glutamate levels in the NAcc core, as measured by microdialysis, are decreased compared to those found in naive rats. The basal levels are believed to reflect the extrasynaptic glutamate that is tightly regulated by glial cells. In naive animals, the basal (extrasynaptic) glutamate levels provide tonic activation of the group II metabotropic glutamate receptors (mGluR2/3s) located on the presynaptic glutamate terminals in the NAcc core that normally inhibit glutamate release. Thus, when a cocaine-priming injection is administered, activity in the dorsal mPFC projections and subsequent glutamate release in the NAcc core are quickly terminated by mGluR2/3s' activity. In contrast, rats that have undergone cocaine self-administration have decreased basal (extrasynaptic) glutamate levels in the NAcc core, thus

providing reduced tonic mGluR2/3 activation. When a cocaine prime is administered, increased dopamine levels activate the dorsal PFC projections to the NAcc core. Without the inhibitory effects of mGluR2/3 activation, the dorsal PFC projections produce a significant and sustained increase in glutamate levels that activate NAcc core neurons, leading to the drug-seeking behavior. **Figure 2** provides a diagram of how glutamate regulates the prefrontal cortical inputs to the NAcc.

The mechanism underlying the decreased basal glutamate levels in rats with a cocaine history depends, at least in part, on changes in the regulation of glutamate by glial cells. Extrasynaptic glutamate levels are controlled by glutamate transporters and the glutamate–cystine exchanger on glial cells. After chronic cocaine or nicotine self-administration, rats have reduced glutamate–cystine exchanger activity in the NAcc core, which contributes to the reduced basal glutamate levels. Confirmation of the critical role of this system in the glutamate dysregulation and drug seeking comes from studies using *N*-acetylcysteine, a cystine pro-drug. Systemic administration of *N*-acetylcysteine has been shown to restore basal glutamate levels to normal levels and to prevent

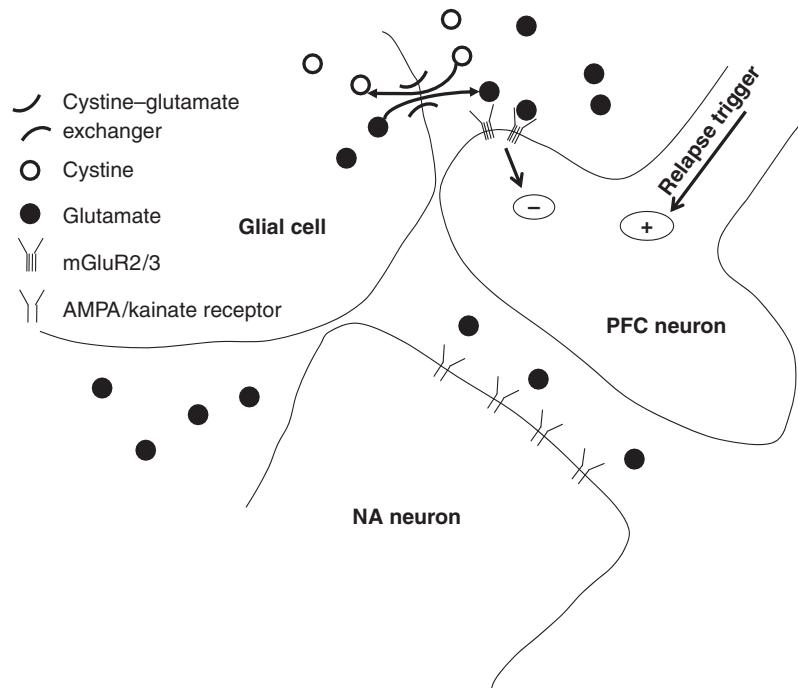


Figure 2 Schematic diagram of the regulation of glutamate in the nucleus accumbens (NA). In naive (non-drug treated) rats, prefrontal release of glutamate is inhibited by the extrasynaptic glutamatergic activation of mGluR2/3s that reside on the presynaptic neuron. Cocaine given to a naive rat, therefore, does not produce measurable increases in glutamate in the NA. In contrast, in rats that have experienced chronic cocaine, extrasynaptic (basal) glutamate levels are decreased due to reduced activity of the cystine–glutamate exchanger, thereby providing less tonic inhibition on the presynaptic glutamate release. When cocaine is administered to such a rat, as in a drug-prime reinstatement test, the cocaine-induced stimulation of the prefrontal neuron without the tonic inhibition by the mGluR2/3s results in increased glutamate levels and drives drug-seeking behavior. *N*-acetylcysteine restores the basal glutamate levels by activating cystine–glutamate exchange, and thereby increasing stimulation of the mGluR2/3s and inhibiting synaptic glutamate release and reinstatement.

cocaine- or heroin-induced reinstatement. The mechanism of *N*-acetylcysteine's action is through activating the glutamate–cystine exchanger, as administration of the exchanger inhibitor central pattern generator (CPG) reverses *N*-acetylcysteine's effects.

Concurrent mGluR2/3 blockade also prevents *N*-acetylcysteine's effects of reducing reinstatement, suggesting that restoration of glutamate levels inhibits reinstatement through its modulation of presynaptic activity through mGluR2/3 activity. Other work has shown that *N*-acetylcysteine also prevents heroin-seeking behavior in rats, suggesting that dysregulation of extra-synaptic glutamate may be involved in relapse in general. In fact, double-blind, pilot clinical trials have shown *N*-acetylcysteine to be effective at reducing cocaine craving and cocaine use in human drug addicts as well as at reducing the number of cigarettes smoked by human smokers. Thus, considerable evidence points to dysregulation of the glutamatergic system, at least in the NAcc core, as a major basis for relapse and as a potential therapeutic target in the treatment of drug addiction.

Conclusions

Considerable progress in our understanding of relapse has been accomplished through the use of reinstatement models of drug-seeking behavior, particularly drug-prime-induced reinstatement. This model has been particularly advantageous as animals initially learn to engage in an operant behavior to receive a drug reward. The reinstatement test permits the animal to engage in drug-seeking behavior without receiving a drug reward, thus enabling studies to separate the reinforcing effect of the drug of abuse from the neurobiology of drug seeking. Although the initial reinforcing effects of drugs of abuse clearly depend on an increase in mesolimbic dopamine, the neurobiology underlying relapse involves a more complicated brain circuit in which dopamine plays a critical, but not exclusive, role. In particular, long-term drug use induces a glutamatergic dysfunction in the dorsal mPFC and the NAcc core. Evidence suggests that, for cocaine-induced reinstatement, increased dopamine levels in the dorsal mPFC activate efferents to the NAcc core that, in turn, produce a significant increase in glutamate that drives NAcc core activity and reinstatement behavior. As a result of the cocaine prime, the glutamate increase in the NAcc core occurs because the chronic cocaine-induced reduction in extra-

synaptic glutamate levels result in less inhibitory regulation of glutamate release through mGluR2/3s. The reduced basal levels of glutamate appear to be the result of dysfunction in how glial cells regulate extra-synaptic glutamate. This neurobiological mechanism has resulted in novel glutamatergic therapeutic agents with a potential for treating addiction.

See also: Acute Dependence; Animal Models of Behavior; Alcohol Addiction; Alcoholism; Basal Ganglia; Brain Imaging and Addiction; Brain Stimulation and Addiction; Cellular Plasticity in Cocaine and Alcohol Addiction; Drug Addiction; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Drug Sensitization and Drug Abuse; Drug Withdrawal – Motivational View; Ethanol and Nicotine Interactions; Hormonal Contributions to Arousal and Motivation; Incentive Motivation and Incentive Salience; Molecular Neurobiology of Addiction; Neural Systems of Motivation; Neurobiology of Opioid Addiction; Neurophysiology of Drug Reward; Nicotine; Psychostimulants; Rewarding Brain Stimulation; Stress and Drug Craving; Transition to Addiction; Vulnerability Factors in Addiction Disorders.

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Drug Sensitization and Drug Abuse

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Glossary

Incentive – A stimulus that elicits approach. Incentive motivation refers to a central motivational state that enhances the salience and effectiveness of incentives. The incentive-sensitization view of Robinson and Berridge argues that sensitization in the mesoaccumbens dopamine (DA) pathways underlies the enhanced motivation to seek and self-administer drugs.

Sensitization – In drug-sensitization experiments, refers to the enhancement of a drug response that results from previous exposure to the drug. A descriptor preceding this term refers to the effect sensitized, as with a behavioral (behavioral or locomotor sensitization) or a neurochemical response (DA or glutamate sensitization). Sensitization of psychological processes has also been proposed as in incentive sensitization to refer to the enhancement of neuronal drug effects mediating the incentive valence of drug stimuli.

Test for sensitization – In drug-sensitization experiments, a test that assesses the ability of a drug to elicit a response that is greater than that observed when it was administered for the first time. Animals are challenged with the drug on a test conducted some time after they were repeatedly administered the drug. Sensitization can be assessed by comparing responding to the first and last drug injections in the same animals (within subject) or by comparing responding to the challenge drug injection in animals previously exposed to the drug or saline (between subject).

The term sensitization has a long history of use in several fields, including those focusing on the development of allergies, the induction of psychotic states, the effects of repeated exposure to analgesic and other drugs, and the formation of conditioned associations. It is used to describe the finding observed in many biological preparations that presentation of a strong stimulus produces a long-lasting increase in the subsequent response to the same or a weaker stimulus. Thus, sensitization has been used to describe increased reflex responses following their repeated elicitation as well as increased behavioral and biochemical responding to stressors and increased responsiveness of the immune system to antigens following repeated exposure. The last four decades have also

witnessed a growing interest in sensitization of the behavioral and biochemical responses to drugs. This has been driven, in part, by the need to understand the consequences of prolonged exposure to agents with central nervous system effects, either when these are used therapeutically – as in the case of opiates to control pain – or when they are used recreationally as in the case of abused drugs such as ethanol, nicotine, morphine, amphetamine, cocaine, and others. In the latter case, delineation of the neuronal systems underlying the behavioral effects of these drugs, including their ability to support self-administration, has helped focus the search for molecular, cellular, and systems-level mechanisms underlying sensitization and to understand the contribution of these neuroadaptations to the enhanced drug seeking and drug taking that is characteristic of drug abuse. This is illustrated below for the psychostimulants amphetamine and cocaine.

Behavioral Sensitization

Drugs typically produce multiple effects not all of which may exhibit sensitization. For example, the anorectic and lethal cardiovascular effects of amphetamine show tolerance or diminish with repeated exposure while its locomotor stimulant or stereotypic effects show sensitization. Similarly, in the biphasic locomotor response to moderate to high doses of morphine, the initial hypolocomotor response shows tolerance while the subsequent hyperlocomotion shows sensitization. Thus, it is important to note that sensitization is a phenomenon associated with specific drug effects rather than to drugs themselves. Whether sensitization is observed can also be influenced by other factors including the intensity of the drug-exposure regimen (e.g., drug dose, number, intermittency of injections), the time of testing following exposure, and the presence during testing of stimuli previously paired or unpaired with the drug. Notably, these factors can interact. Exposure to high doses of amphetamine, cocaine, and other drugs generally leads to enhanced responding when animals are tested long (i.e., weeks), but not soon (i.e., days later), after exposure. In addition, intermittent rather than continuous exposure to moderate doses of amphetamine has been reported to produce enhanced responding at different withdrawal periods; although it is possible that sensitization following continuous exposure to

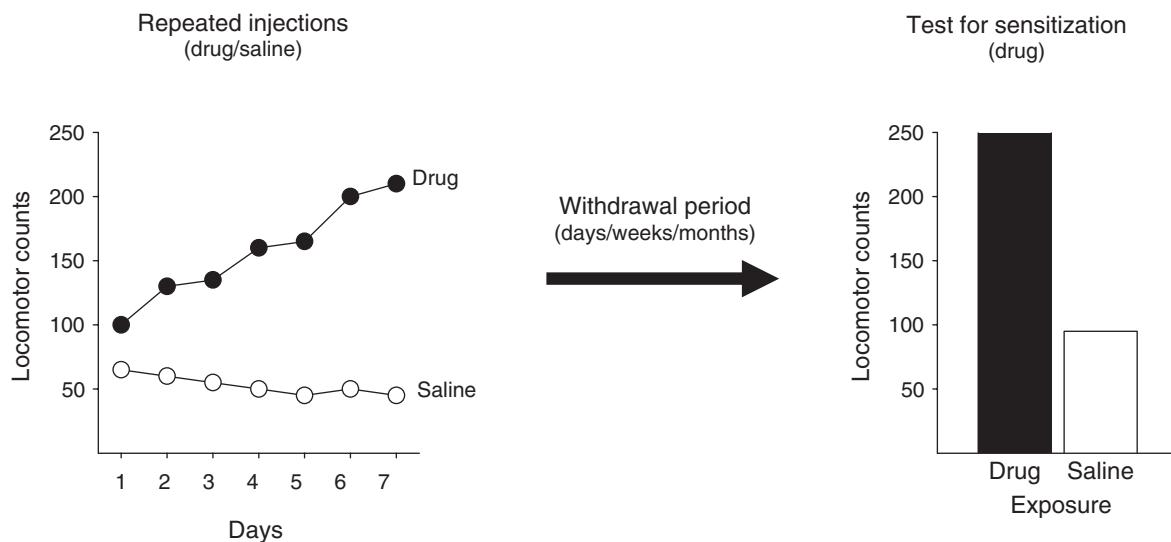


Figure 1 Illustration of procedures commonly used to produce and assess locomotor sensitization. Here, rats were administered seven daily injections of saline or drug and tested following a drug-challenge injection 3 weeks later. When locomotion is monitored during the exposure phase (left), a progressive enhancement in locomotor responding from the first to the last drug injection is often (but not always) observed, permitting within-subject assessment of sensitization. Comparing responding to a drug-challenge injection (right) in animals previously exposed to saline or the drug permits between-subject assessment of sensitization following a withdrawal phase.

amphetamine may also be observed but at significantly longer, not-yet-tested withdrawal periods.

The most studied response to amphetamine and cocaine in rodents is locomotion. Both drugs increase locomotion acutely across low to moderate doses and this response becomes enhanced with repeated injections. Similar findings have been obtained with other responses as well, including rotational behavior in unilateral 6-hydroxydopamine (6-OHDA)-lesioned animals and different stereotyped behaviors following moderate to high doses. Because stereotypies and locomotion constitute opposing behavioral outputs, it is important to note that development of sensitization in one will interfere with expression of sensitization in the other. Locomotor sensitization has been demonstrated using either within-subject comparisons of responding to the first and to the last drug injection or between-subject comparisons of responding in animals previously exposed to saline or to the drug (Figure 1). A notable characteristic of sensitization of drug effects is that it is enduring; locomotor sensitization has been observed up to 1 year after drug exposure in the rat. Such findings indicate that long-lasting neuroadaptations are produced that underlie this enhanced behavioral output. Because drug exposure also produces short-term transient neuronal effects, it remains unclear whether the sensitization observed within-subjects during drug exposure reflects the same neuronal changes underlying the sensitization observed between subjects after a long withdrawal period.

Importantly, exposure to psychostimulants also enhances the subsequent self-administration of these drugs. Indeed, exposure to a number of drugs leads to

enhanced conditioned place preference, facilitates acquisition of drug self-administration, and enhances motivation to obtain the drug, once the behavior has been acquired (Figure 2). As outlined below, sensitization of drug-induced locomotion and drug self-administration are produced by the same drug-exposure regimens via actions in the same nuclei, are prevented by the same pharmacological interventions, and are accompanied by the same neuroadaptations, providing strong support for the proposition that the enhanced expression of complex motivated behaviors can represent yet another manifestation of behavioral sensitization.

Anatomical and Neuronal Substrates of Behavioral Sensitization by Psychostimulants

While amphetamine and cocaine interact with serotonin-, norepinephrine-, and dopamine (DA)-containing neurons, it is recognized that they increase locomotor activity and support self-administration primarily by binding to DA transporters and by increasing extracellular levels of DA in forebrain sites. The mesocorticolimbic DA pathways, particularly those projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc), are known to be critical for the production of these effects (Figure 3). Both effects are blocked by DA-receptor antagonists or 6-OHDA lesions of DA neuron terminals in the NAcc. Sensitization of the ability of a drug such as amphetamine to increase locomotion, NAcc

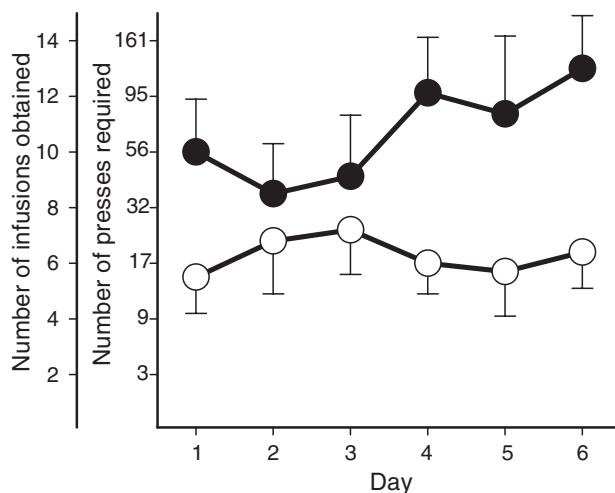


Figure 2 Previous exposure to amphetamine promotes the pursuit of the drug in rats. Data are shown as group mean (\pm SEM) number of amphetamine infusions (200 μ g/kg/infusion) obtained on each of 6 days of testing on a progressive ratio (PR) schedule of reinforcement. The number of presses required to obtain the successive infusions is also shown. Rats previously exposed to amphetamine (filled circles) worked more and obtained significantly more amphetamine infusions on these PR tests than rats previously exposed to saline (open circles). PR testing was conducted 2–3 weeks following exposure to the drug ($5 \times 1.5 \text{ mg kg}^{-1}$ amphetamine or saline, intraperitoneally (IP)). Adapted from Vezina P, Lorrain DS, Arnold GM et al. (2002) Sensitization of midbrain dopamine neuron reactivity promotes the pursuit of amphetamine. *Journal of Neuroscience* 22: 4654–4662.

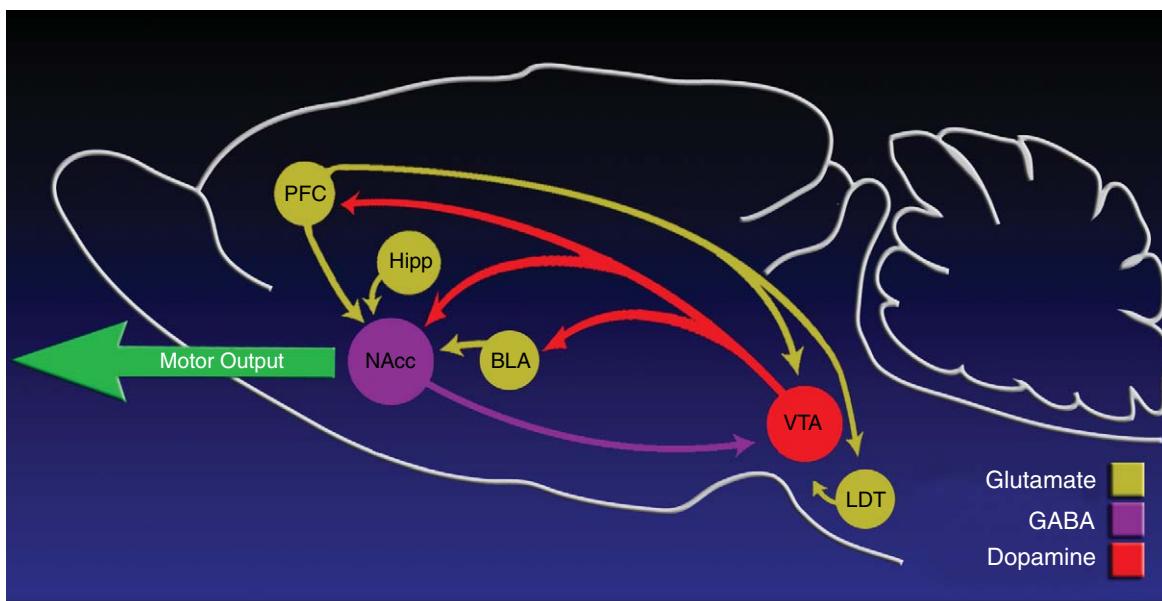


Figure 3 The mesoaccumbens DA projections are imbedded in a circuit of pathways in the basal ganglia with nuclei topographically interconnected to promote the flow of information from limbic areas to pyramidal and extrapyramidal motor systems. The NAcc is a major interface of this circuit because it receives rich sensory DA and glutamate projections from the VTA and the indicated forebrain regions and, in turn, sends projections to nuclei connected to motor systems. BLA = basolateral amygdala; Hipp = hippocampus; LDT = laterodorsal tegmentum; PFC = prefrontal cortex.

DA overflow, and to support self-administration is produced by infusions of amphetamine into the VTA but not into a number of DA-neuron terminal fields, including the NAcc. Thus, it is likely that the induction of sensitization of these effects is produced by a cascade of neuronal events initiated by the acute increase in extracellular levels of DA

in the VTA and that its eventual expression requires the manifestation of neuroadaptations in forebrain sites including the NAcc.

A number of neurotransmitters in the VTA have been implicated in the induction of sensitization by psychostimulants. This site is richly innervated by several afferent

projections using several neurotransmitter systems from several forebrain and hindbrain regions. In addition to somatodendritically derived DA, these include acetylcholine, serotonin, peptides, and inhibitory and excitatory amino acids. There is some evidence that, during exposure to psychostimulants, γ -aminobutyric acid (GABA)-mediated inhibition of VTA DA neurons is decreased while glutamate-mediated excitation is increased. Indeed, a number of findings indicate that during drug exposure there is a transient increase in the activation of DA neurons in this site – an effect also supported by other transient changes, including decreased DA D₂ autoreceptor sensitivity and increased α -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptor function. The critical role of glutamate is underscored by the finding that activation of all three glutamatereceptor subtypes (*N*-methyl-D-aspartic acid (NMDA), AMPA, and metabotropic) is required for the induction of locomotor sensitization and the enhancement of drug self-administration by amphetamine. Activation of ionotropic glutamate receptors promotes calcium influx into dopamine neurons through AMPA and NMDA receptors as well as L-type calcium channels leading to the recruitment of second messenger pathways involving a number of protein kinases. Evidence from pharmacological activation and inhibition studies supports a role for calcium, calcium- and calmodulin-dependent protein kinase (CaMK), mitogen-activated protein kinase (MAPK), as well as protein kinase A (PKA) and C (PKC), although the nature of their contribution to sensitization remains unknown. It is conceivable that phosphorylation of some of their target proteins by these kinases leads to long-term alterations such as changes in the synthesis of candidate proteins and their transport to forebrain sites like the NAcc where amphetamine is known to produce enhanced responding in sensitized rats. Notably, inhibition of protein synthesis in the VTA blocks the induction of locomotor sensitization by psychostimulants.

The adaptation most consistently associated with the expression of locomotor sensitization by psychostimulants is the enhancement in the ability of these drugs to increase extracellular levels of DA in the NAcc. As with the sensitization of drug-induced locomotion and drug self-administration, this adaptation is produced by amphetamine in the VTA, its induction requires activation of D₁ DA receptors in this site, and its expression is dependent on CaMK-II in the NAcc. These findings suggest a close relation between the sensitization of the NAcc DA-activating effects of psychostimulants and behavioral manifestations of sensitization such as enhanced locomotion and drug self-administration. It has been argued that activity in mesoaccumbens DA neurons encodes the incentive valence of a drug. Thus, these findings also support the resulting argument that enhanced reactivity in

these neurons should similarly enhance this encoded valence and, as a result, enhance the incentive to pursue and self-administer the drug. Interestingly, some of the first reports of sensitized DA release were from studies using striatal slice preparations, suggesting a similar association between enhanced extracellular levels of striatal DA and the expression of sensitized stereotypies.

A number of other long-lasting adaptations have also been reported to occur in forebrain following repeated exposure to psychostimulants. These include alterations in dendritic morphology, increased glutamate overflow, functional upregulation of AMPA receptors, and changes in synaptic strength at cortico-accumbens glutamate synapses, as well as changes in the AMPA receptor-subunit expression and membrane insertion. Interest in these adaptations has been fueled in part by the finding that activation of NAcc-AMPA receptors is required for the expression of acute and sensitized psychostimulant-induced locomotion. However, the nature of the contribution of AMPA-receptor-mediated glutamate transmission in the NAcc to the expression of psychostimulant sensitization remains controversial. It remains a point of debate, for example, whether increased or decreased medium spiny neuron activity maps onto increased behavioral output. Importantly, terminals releasing DA and glutamate form synaptic contacts on the same dendritic spines of these neurons in the NAcc, suggesting that glutamate-DA interactions contribute to the expression of behavioral sensitization. However, the nature of the relation between altered pre- and postsynaptic glutamate and DA transmission remains to be determined. Delineation of adaptations in a number of postreceptor pathways in medium spiny neurons and the afferents they receive may ultimately provide some answers to these questions. Indeed, a number of proteins have been implicated in the effects observed following repeated exposure to psychostimulants, including CaMK-II, PKA, casein kinase, Darpp-32, cyclic adenosine monophosphate (cAMP) response-element-binding protein (CREB), extracellular signal-regulated kinase (ERK), and immediate early gene Fos-related antigens like delta FosB.

Sensitization in the Human

The assessment of the effects of acute and repeated psychostimulant exposure on neurotransmission in the human has been aided, over the course of the last two decades, by the development of functional neuroimaging techniques – such as positron emission tomography (PET) – that use radioactively labeled benzamide ligands for DA D₂/D₃ receptors and that can be coupled to magnetic resonance imaging (MRI). The resulting studies of the effects of

different drugs on DA reactivity in the human forebrain have recently achieved sufficient spatial resolution to allow assessment of different striatal subregions. As established in rodents, a number of reports have now shown that, when acutely administered, psychostimulants similarly increase extracellular levels of DA especially in the ventral subregions of the human striatum. Notably, these effects correlate with positive mood states and craving as well as novelty and sensation seeking. Despite such findings, the evidence for sensitization of DA overflow in the human was originally more equivocal than that observed in rodent studies. As a result, it has been argued that drug sensitization as a mechanism for drug abuse and other forms of pathology does not extend to the human case and is, thus, of limited value. Indeed, a number of imaging studies using PET and functional MRI (fMRI) techniques appear to support this argument. These generally report a reduced, rather than an augmented, striatal DA response to a psychostimulant challenge in cocaine-addicted patients, compared to controls. More recent evidence has emerged, however, demonstrating that drug sensitization does, in fact, occur in the human.

Sensitization to a number of drug effects is observed in the human when sufficiently high amphetamine concentrations are administered to nondrug-dependent subjects. These include potentiated indices of vigor and energy levels as well as potentiated eyeblink and mood-elevating responses. The latter sensitized drug-induced elevation in mood has been found to correlate positively with the personality trait of novelty seeking. In addition, some of the measures assessed (e.g., increases in vigor) were

observed a full year following exposure to the drug. Importantly, these studies did not typically observe sensitization of drug liking. This finding is consistent with evidence indicating that NAcc-DA is not associated with the pleasure derived from drug intake but rather with the motivational salience of the drug and the cues associated with it. Interestingly, cocaine-dependent abusers develop tolerance to the euphoric effects of psychostimulants despite the fact that they show enhanced drug seeking.

Similarly, significantly greater amphetamine-induced DA release has been observed in the ventral striatum 2 weeks and again 1 year following the administration of amphetamine in studies conducted in nondrug-abusing human subjects (Figure 4). Again, the extent of the DA sensitization observed was found to correlate positively with sensitization of energy level and eyeblink rate as well as the personality trait of novelty seeking. In contrast, the opposite effect has been reported in experiments conducted in detoxified patients with a history of cocaine dependence: less, rather than more, striatal DA release is observed in response to a psychostimulant challenge.

The results of these studies reveal that a number of factors can influence whether sensitization is observed in the human. These include the measures assessed, the challenge dose of the drug administered on the test, and the subject population studied. Indeed, at least two additional significant differences can be denoted between studies conducted in healthy subjects and those in drug-abusing patients that might account for the different results reported. Unlike healthy subjects, drug-abusing subjects have, by definition, been exposed to

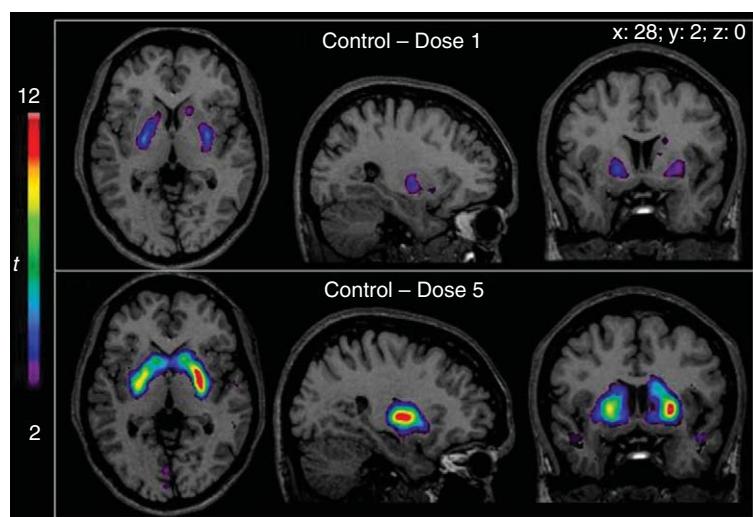


Figure 4 Previous exposure to amphetamine sensitizes ventral striatal DA release in the human. Shown are *t*-statistical maps of [¹¹C]raclopride binding potential change illustrating a decrease in binding potential (increase in DA release) after the first, and a greater effect following the fifth, amphetamine administration relative to a drug-free control condition. The fifth administration was administered approximately 1 year following the first. Amphetamine was administered by mouth (0.3 mg kg⁻¹). Colored *t*-maps ($>t = >\text{DA release}$) are overlaid on an averaged MRI of all the participants. Adapted from Boileau I, Dagher A, Leyton M, et al. (2006) Modeling sensitization to stimulants in humans. *Archives of General Psychiatry* 63(12): 1386–1395.

substantial amounts of drug and it is likely that even in detoxified patients the intensity of this exposure may interfere with the subsequent expression of sensitization. This possibility is supported by findings from the preclinical rodent literature showing that enhanced drug-induced NAcc DA overflow is not observed in the days following drug exposure but rather weeks to months later. The withdrawal period necessary to observe sensitization may be longer in the human, and longer still following prolonged intense drug exposure. Another critical difference between studies conducted in these two groups involves the various environmental stimuli surrounding exposure to the drug and those constituting the testing conditions. Again, an accumulating preclinical rodent literature indicates that stimuli that predict the availability of the drug promote sensitized responding whereas stimuli that predict its absence inhibit the expression of sensitization. In studies conducted in the human, it is likely that the constellation of stimuli afforded by the PET-testing environment exerts different effects in individuals that have received the drug only in their presence (healthy subjects) compared to individuals that have generally associated these cues with the absence of drug (drug-abusing patients). Thus, the results of the human studies described above suggest that the presence of cues predicting drug availability is associated with sensitized responding while the absence of these cues or the presence of stimuli predicting the absence of drug is associated with the absence of sensitized responding. Clearly, such cues capable of affecting the expression of sensitization can regulate the salience that is attributed to drug cues and, in so doing, influence predisposition to drug addiction and relapse susceptibility.

Other Factors Affecting Drug Responding

The evidence reviewed above indicates that long-term neuroadaptations produced by previous exposure to psychostimulants could strongly influence liability to initiate or resume drug use. However, it is important to remember that other adaptations are capable of augmenting drug intake in the absence of sensitization. For example, escalation of drug intake is observed in rats given long (6-h) rather than short (1-h) access to cocaine in a self-administration paradigm. It has been argued that these findings reflect tolerance rather than sensitization of the appetitive (locomotor and DA activating) properties of the drug because escalation of intake is not accompanied by sensitized responding in tests conducted during or soon after the escalating phase of the experiments. Importantly, sensitization is known not to be observed in the days immediately following exposure to intense psychostimulant-exposure regimens but rather to manifest at later time points weeks or months later. Thus, escalation of drug intake during intense drug exposure or in the days immediately following this exposure may be due to factors other than sensitization such as the incremental recruitment of opponent processes or tolerance to the psychostimulant's aversive or suppressive effects. On the other hand, enhanced responding long after drug exposure likely reflects the expression of sensitization of the drug's incentive effects. In this case, sensitization could exert its impact on behavior during self-administration testing in individuals previously exposed to the drug, as well as long after exposure to the drug in these sessions.

These findings indicate that different adaptations can show different temporal profiles (Figure 5) and, as such, can potentially influence responding differently at different times following drug exposure. Although not all cases

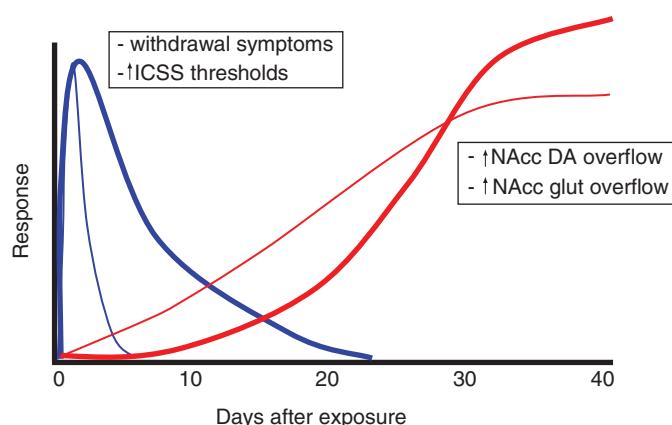


Figure 5 Different adaptations observed following drug exposure show different temporal profiles. Shown in this illustration are the duration and manifestation times of adaptations known to result from drug exposure. Withdrawal symptoms and blunting of reward – associated with increases in intracranial self-stimulation thresholds – appear soon after discontinuing drug exposure, while sensitization of NAcc DA and glutamate overflow, associated with enhancement of the appetitive properties of drugs, appears later. Increasing the intensity of the drug-exposure regimen prolongs the duration of the former and may delay the expression of the latter (thicker lines).

have been evaluated, findings reported to date suggest that the intensity of the drug exposure regimen can influence the duration and time of appearance of the adaptations observed, with more intense exposure regimens leading to longer-lasting manifestations of withdrawal symptoms and delayed (and perhaps longer-lasting) expression of sensitized responses. It remains unclear whether these corresponding shifts in the temporal manifestation of these effects following increasingly intense drug exposure reflect parallel but unrelated neuroadaptations or the competing influence of some effects over others.

Acknowledgment

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See also: Cellular Plasticity in Cocaine and Alcohol Addiction; Drug Addiction; Incentive Motivation and Incentive Salience; Motivation; Transition to Addiction.

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Drug Withdrawal – Motivational View

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Glossary

Allostasis – Stability of physiological systems through change in set points.

Binge/intoxication stage – Stage of the addiction cycle characterized by active drug taking, often to excess.

Compulsivity – Elements of behavior that result in perseveration in responding in the face of adverse consequences or perseveration in the face of incorrect responses in choice situations.

Corticotropin-releasing factor – Neuropeptide that mediates hormonal, sympathetic, and behavioral responses to stressors.

Dependence – The manifestation of withdrawal symptoms.

Extended amygdala – Basal forebrain macrostructure comprising the bed nucleus of the stria terminalis, the central medial amygdala, and a transition zone in the posterior part of the medial nucleus accumbens (i.e., posterior shell).

Impulsivity – A predisposition toward rapid, unplanned responses to internal and external stimuli without regard for the negative consequences of these responses to oneself.

Motivational symptoms of withdrawal – Symptoms of withdrawal associated with negative emotional states.

Negative emotional state – Unpleasant state associated with dysphoria, irritability, pain, and decreased reward processing.

Opponent process – Theoretical construct in which the initial hedonic response to a stimulus is followed by the opposite hedonic response. The opposite hedonic response is slower in onset and slower to resolve than the initial hedonic response.

Preoccupation/anticipation stage – Stage of the addiction cycle that follows the withdrawal/negative affect stage and precedes the binge/intoxication stage and is characterized by preoccupation with obtaining the drug (craving).

Protracted abstinence – Persistence of motivational symptoms of withdrawal during the period after acute withdrawal from chronic drug use.

Somatic symptoms of withdrawal – Physical/body symptoms associated with drug withdrawal.

Withdrawal – Abstinence or removal from chronic drug use, usually characterized by signs and symptoms opposite to the acute effects of the drug.

Withdrawal/negative affect stage – Stage of the addiction cycle after cessation of drug use characterized by motivational signs of withdrawal.

Stages of the Addiction Cycle

Conceptual Framework: Motivational View of Addiction

Drug addiction is a chronically relapsing disorder characterized by (1) compulsion to seek and take the drug, (2) loss of control in limiting intake, and (3) emergence of a negative emotional state (e.g., dysphoria, anxiety, and irritability) reflecting a motivational withdrawal syndrome when access to the drug is prevented (defined here as dependence with a ‘little d’). Withdrawal from drugs of abuse is one symptom of what is defined symptomatically as addiction and is historically focused on what were defined as the physical symptoms of withdrawal and reflected signs and symptoms of a physical nature usually opposite to the acute effects of the drug itself. For example, with opioids, pupillary dilation was a telltale sign of opioid withdrawal, whereas pupillary constriction was a telltale sign of opioid intoxication. Similarly for alcohol, sympathetic-like responses, such as hyperthermia, reflected withdrawal, whereas hypothermia characterized acute intoxication. However, to be discussed below, these somatic measures are basically a red herring for the more motivational measures of withdrawal from the perspective of negative reinforcement, drug-seeking, and craving associated with acute and protracted abstinence. Nevertheless, the somatic signs of withdrawal are an index of dependence with a ‘little d’ and provide a quantifiable measure by which to assess the level of dependence and to relate to more motivational measures.

Motivation is a state that can be defined as a “tendency of the whole animal to produce organized activity” (Hebb, 1972), and such motivational states are not constant but rather vary over time. Early work by Wikler stressed the role of changes in drive states associated with dependence. In dependent subjects, the positive reinforcing effects of opioids remain, but subjects who become dependent describe withdrawal changes as a hunger or primary need, and the effects of morphine on such a state as

satiation or gratification of the primary need (Wikler, 1952). The concept of motivation was linked inextricably with hedonic, affective, or emotional states in addiction in the context of temporal dynamics by Solomon's opponent process theory of motivation. Solomon and Corbit postulated that hedonic, affective, or emotional states, once initiated, are automatically modulated by the central nervous system with mechanisms that reduce the intensity of hedonic feelings, and these are manifested as withdrawal symptoms but in the emotional domain. Positive hedonic responses occur shortly after presentation of a stimulus, correlate closely with the intensity, quality, and duration of the reinforcer, and show tolerance. In contrast, negative hedonic responses appear after the positive hedonic process has terminated, are sluggish in onset, slow to build up to an asymptote, slow to decay, and get larger with repeated exposure. The thesis here is that opponent processes begin early in drug-taking, reflect changes in the brain reward and stress systems, form the negative emotional state that constitutes motivational withdrawal, and form one of the major motivations for compulsivity in drug-taking.

Thus, dependence or manifestation of a withdrawal syndrome after removal of chronic drug administration is defined in terms of motivational aspects of dependence such as emergence of a negative emotional state (e.g., dysphoria, anxiety, and irritability) when access to the drug is prevented, rather than on the physical signs of dependence. Indeed, some have argued that the development of such a negative affective state can define dependence as it relates to addiction: "The notion of dependence on a drug, object, role, activity or any other stimulus-source requires the crucial feature of negative affect experienced in its absence. The degree of dependence can be equated with the amount of this negative affect, which may range from mild discomfort to extreme distress, or it may be equated with the amount of difficulty or effort required to do without the drug, object, etc" (Russell, 1976).

Drug addiction has been conceptualized as a disorder that involves elements of both impulsivity and compulsivity, in which impulsivity can be defined behaviorally as a predisposition toward rapid, unplanned responses to internal and external stimuli without regard for the negative consequences of these responses to oneself. Compulsivity can be defined as elements of behavior that result in perseveration in responding in the face of adverse consequences or perseveration in the face of incorrect responses in choice situations. These elements are analogous to the symptoms of Substance Dependence outlined by the American Psychiatric Association: continued substance use despite knowledge of having had a persistent or recurrent physical or psychological problem and a great deal of time spent in activities necessary to obtain the substance.

From a psychiatric perspective, impulse control disorders are characterized by an increasing sense of tension or arousal before committing an impulsive act; pleasure, gratification, or relief at the time of committing the act; and regret, self-reproach, or guilt following the act. In contrast, compulsive disorders are characterized by anxiety and stress before committing a compulsive repetitive behavior and relief from the stress by performing the compulsive behavior. As an individual moves from an impulsive disorder to a compulsive disorder a shift occurs from positive reinforcement driving the motivated behavior to negative reinforcement driving the motivated behavior.

Binge, Withdrawal, Preoccupation/Anticipation

Collapsing the cycles of impulsivity and compulsivity yields a composite addiction cycle composed of three stages – preoccupation/anticipation, binge/intoxication, and withdrawal/negative affect – in which impulsivity often dominates at the early stages and compulsivity dominates at terminal stages. Negative reinforcement can be defined as the process by which removal of an aversive stimulus (e.g., negative emotional state of drug withdrawal) increases the probability of a response (e.g., dependence-induced drug intake). These three stages are conceptualized as interacting with each other, becoming more intense, and ultimately leading to the pathological state known as addiction, but a key component of this addiction process remains what is defined here as a motivational withdrawal state. This article focuses on the role of withdrawal in addiction, with a focus on the negative emotional state comprising the motivational withdrawal state of the withdrawal/negative affect stage of the addiction cycle.

Somatic versus Motivational Withdrawal

Somatic Withdrawal Measures in Humans and Animals with Different Drugs of Abuse

Two drugs, opioids and alcohol, provide the most dramatic examples of the somatic signs of withdrawal and have served as models for measures of withdrawal *per se*. These somatic signs are called "somatic" because they are expressed as responses of the body (e.g., gasps and writhes) and are both centrally and peripherally mediated. For opioids, somatic withdrawal signs in humans are dramatic, dose- and duration-of-abstinence-dependent, and include a number of overt measurable signs such as yawning, lacrimation, rhinorrhea, perspiration, gooseflesh, tremor, dilated pupils, anorexia, nausea, emesis, diarrhea, weight loss, and elevations in temperature and blood pressure (Table 1). In animals (rodents), opioid withdrawal signs are well characterized when precipitated by administration of a competitive opioid antagonist such as naloxone. A weighted scale was

Table 1 Human symptoms of physical/somatic withdrawal

<i>Drug</i>	<i>Withdrawal signs</i>	
<i>Opiates</i>	Craving Anxiety Yawning Perspiration Runny nose Lacrimation Mydriasis Gooseflesh Piloerection Tremors Muscle twitches Hot/cold flashes Aching bones/muscles Anorexia Insomnia	Increased blood pressure Increased temperature Increases respiration Increased pulse Restlessness Nausea Febrile facies Vomiting Diarrhea Weight loss Spontaneous ejaculation/orgasm Hemoconcentration leucocytosis Eosinopenia Increased blood sugar
<i>Alcohol</i>	Anorexia Insomnia Tremors Mild disorientation Convulsions	Increased blood pressure Increased temperature Increased heart rate Delirium tremens
<i>Nicotine</i>	Restlessness Insomnia	Hunger Weight gain
<i>Marijuana</i>	Decreased appetite Weight loss Sleep difficulties Strange dreams	Stomach ache Chills Shakiness Sweating

developed and widely adopted that included both graded signs of weight loss, diarrhea, escape attempts, wet dog shakes, abdominal constrictions, facial fasciculations/teeth chattering, salivation, ptosis, abnormal posture, penile

grooming/erection/ejaculation, and irritability (**Table 2**). When the somatic signs of withdrawal from opioids are directly compared with more motivational measures, the motivational measures are more sensitive

Table 2 Rodent symptoms of physical/somatic withdrawal

<i>Drug</i>	<i>Withdrawal signs</i>	
<i>Opiates</i>	Weight loss Increased escape attempts Abdominal constrictions Wet dog shakes Diarrhea Facial fasciculations Teeth chattering	Swallowing movements Profuse salivation Chromodacryorrhea Ptosis Abnormal posture Erection/ejaculation Irritability
<i>Alcohol</i>	Irritability Hyperresponsiveness Abnormal motor responses	Anxiety-like behavior Decreased reward sensitivity Seizures
<i>Nicotine</i>	Teeth chattering Chews Gasps Writhes Ptosis	Shakes Tremors Yawning Dyspnea Seminal ejaculation
<i>Marijuana</i>	Wet dog shakes Facial rubbing Ataxia Hunched posture Tremor	Ptosis Piloerection Locomotor depression Mastication

and show more efficacy in defining the withdrawal state. Spontaneous withdrawal shows many of the same signs, but they are significantly less intense.

For alcohol, the somatic signs of withdrawal in humans are equally dramatic but also life threatening and are characterized by tremor, increases in heart rate, increases in blood pressure, increases in body temperature, anorexia, and convulsions. In its severest form, alcohol withdrawal can result in pronounced hyperthermia that can evolve into delirium tremens, a state of marked sympathetic hyperactivity, hyperthermia (which can be fatal), and hallucinations (**Table 1**). In animals (rodents), alcohol withdrawal signs are characterized by hyperactivity, tail tremors, tail stiffness, head tremors, general tremors, ventromedio distal flexion, wet shakes, teeth chattering, akinesia, spastic rigidity, and induced and spontaneous convulsions (**Table 2**). With alcohol, the withdrawal is only spontaneous because no known competitive antagonist can precipitate withdrawal. Similar to opioids, withdrawal from alcohol is dose- and duration-of-abstinence-dependent, with peak withdrawal occurring between 10 to 16 h post-alcohol administration, with high-dose blood alcohol levels at the time of withdrawal (300–400 mg dl⁻¹).

For nicotine, a nicotine abstinence syndrome in humans after chronic nicotine exposure has been characterized and has both somatic and affective components. Acute nicotine withdrawal is characterized by somatic symptoms, such as bradycardia, gastrointestinal discomfort, and increased appetite leading to weight gain, as well as motivational symptoms including depressed mood, dysphoria, irritability, anxiety, frustration, increased reactivity to environmental stimuli, and difficulty concentrating.

In rats, a somatic syndrome associated with nicotine withdrawal also has been characterized (see **Table 2**). The somatic signs of nicotine withdrawal resemble those seen in opiate withdrawal, including the symptoms of abdominal constrictions, facial fasciculation, and ptosis. These somatic signs are called somatic because they are expressed as responses of the body (e.g., gasps and writhes) and are both centrally and peripherally mediated. The nicotine withdrawal syndrome has been observed after spontaneous nicotine withdrawal, as well as withdrawal precipitated by nicotinic acetylcholine receptor antagonists.

For marijuana, cannabis withdrawal in humans was not included in the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, but an accumulation of data from both inpatient and outpatient studies has led to a proposal for criteria for cannabis withdrawal in humans (see **Table 1**). The most common symptoms associated with cannabis withdrawal are decreased appetite/weight loss, irritability, nervousness, anxiety, anger, aggression, restlessness, and sleep disturbances. Onset of withdrawal

typically occurs between days 1 and 3. Peak effects are experienced between days 2 and 6, and most symptoms last 4 and 14 days. The long onset of cannabis withdrawal appears to be directly related to the long half-life and slow decline of blood levels of Δ^9 -tetrahydrocannabinol (THC).

Some clinicians have argued for the existence of a protracted abstinence state of cannabis withdrawal that can be quite prolonged and may last up to 15–18 months. Mild flu-like symptoms may occur a week or more later, and other subjective effects of protracted abstinence include cognitive deficits and sleep disturbances.

Cannabis withdrawal syndromes have been described in both rats and mice that involve precipitation of withdrawal with a cannabinoid-1 receptor antagonist. Spontaneous somatic withdrawal from THC has not been observed, but a mild somatic withdrawal syndrome has been observed during abstinence from a synthetic cannabinoid. Rats show a variety of somatic withdrawal signs, including wet dog shakes, scratching, facial rubbing, ptosis, mastication, hunched posture, and ataxia (**Table 2**). In mice exposed to chronic administration of THC, administration of the cannabinoid-1 receptor antagonist SR14716A produced a robust withdrawal syndrome. Signs, in order of prevalence, included wet dog shakes, facial rubbing, ptosis, hunched posture, frontpaw tremor, piloerection, and ataxia. The signs of wet dog shakes, ptosis, and hunched posture are similar to the signs observed in rats. These signs are also common with opioid withdrawal.

No generally established spontaneous or precipitated somatic withdrawal signs have been observed for psycho-stimulant drugs. In humans, some have argued that increased appetite, hypersomnia, and fatigue constitute somatic signs, but for the conceptual framework of this article, these symptoms still fall into the motivational withdrawal category.

Motivational Withdrawal Measures in Humans and Animals: Anxiety-Like Symptoms, Aversive-Like Symptoms, Elevations in Reward Thresholds

A common response to acute withdrawal and protracted abstinence from all major drugs of abuse is the manifestation of anxiety-like responses. Animal models of the withdrawal/negative affect stage include measures of anxiety-like responses, measures of conditioned place aversion (rather than preference), and measures of increases in reward thresholds using brain stimulation reward to precipitated withdrawal or spontaneous withdrawal from chronic administration of a drug (**Table 3**).

Animal models have revealed anxiety-like responses to all major drugs of abuse during acute withdrawal, with the dependent variable often a passive response to a novel

Table 3 Symptoms of motivational withdrawal

↑ Anxiety-like behavior
↑ Conditioned place aversions
↑ Drug-induced elevations in reward thresholds

and/or aversive stimulus, such as the open field or elevated plus maze, or an active response to an aversive stimulus, such as defensive burying of an electrified metal probe. Withdrawal from repeated administration of cocaine produces an anxiogenic-like response in the elevated plus maze and defensive burying test. Precipitated withdrawal in opioid dependence and nicotine dependence also produces anxiety-like effects. Spontaneous ethanol withdrawal produces anxiety-like behavior.

Place aversion has been used to measure the aversive stimulus effects of withdrawal, mostly in the context of opioids. In contrast to conditioned place preference, rats exposed to a particular environment while undergoing precipitated withdrawal from opioids spend less time in the withdrawal-paired environment when subsequently presented with a choice between that environment and an unpaired environment. Such an association continues to be manifested weeks after animals are detoxified (e.g., after the morphine pellets are removed) and can be measured from 24 h to 16 weeks later. Additionally, a place aversion in opioid-dependent rats can be observed with doses of naloxone below which somatic signs of withdrawal are observed and following a single injection of morphine. Similar acute withdrawal-like effects have been observed using anxiety-like responses following bolus injections of ethanol.

Electrical brain stimulation reward or intracranial self-stimulation has a long history as a measure of activity of the brain reward system and of the acute reinforcing effects of drugs of abuse. All drugs of abuse, when administered acutely, decrease brain reward thresholds. Brain stimulation reward involves widespread neurocircuitry in the brain, but the most sensitive sites defined by the lowest thresholds involve the trajectory of the medial forebrain bundle that connects the ventral tegmental area with the basal forebrain. Although much emphasis was focused initially on the role of the ascending monoamine systems in the medial forebrain bundle, other nondopaminergic, descending systems in the medial forebrain bundle clearly have a key role.

Acute intravenous cocaine self-administration in animals reduces reward thresholds, consistent with the well-documented effects of drugs of abuse in lowering brain reward thresholds. However, with more prolonged access to the drug, the decreases in reward thresholds (i.e., rewarding effects) are replaced with elevations in reward thresholds (i.e., antirewarding effects) after the initial decrease in reward thresholds, presumably reflecting an

acute withdrawal or opponent process-like effect. Such elevations in reward thresholds begin rapidly, can be observed within a single session of self-administration, and are greater with greater exposure to cocaine, bearing a striking resemblance to human subjective reports. Chronic administration or self-administration of all drugs of abuse produces elevations in reward thresholds during spontaneous or precipitated acute withdrawal (**Table 3**). These elevations in threshold can be short (minutes to hours) or can last for days, depending on dose, drug, time of exposure, and precipitant.

Neurobiological Bases of Withdrawal

Neurobiological Mechanisms of Somatic Signs of Withdrawal

No single structure appears responsible for expression of the somatic opioid withdrawal syndrome, suggesting a widespread distribution of the neuroanatomical substrate for the somatic signs of withdrawal. Key areas hypothesized to be involved in the somatic signs of opioid withdrawal include the locus coeruleus, amygdala, medial thalamus, globus pallidum, periaqueductal gray, locus coeruleus, nucleus paragigantocellularis, nucleus raphe magnus, and spinal cord. Numerous neurochemical systems have been implicated in the somatic signs of opioid withdrawal, and include α_1 noradrenergic antagonists, β noradrenergic antagonists, α_2 noradrenergic agonists, glutamatergic antagonists, γ -aminobutyric acid (GABA) agonists, nociceptin agonists, neuropeptide Y, cholecystokinin agonists, neurokinin-1 antagonists, neuropeptide FF antagonists, and nitric oxide synthase inhibitors. The somatic signs of withdrawal from other drugs of abuse have been less extensively studied but presumably involve similar structures and similar neurochemical systems for similar signs. For example, α_2 adrenergic agonists, GABA agonists, nitric oxide synthase inhibitors, neuropeptide Y, and serotonin reuptake blockers block the irritability, tremor, and rigidity signs of ethanol withdrawal.

Motivational Withdrawal: Decreased Reward Neurotransmission

The acute reinforcing effects of drugs of abuse are mediated by the activation of dopamine, serotonin, opioid peptides, and GABA systems either by direct actions in the basal forebrain (notably the nucleus accumbens and central nucleus of the amygdala) or by indirect actions in the ventral tegmental area. Thus, a logical explanation for motivational withdrawal would be compromised reward system neurotransmission. Within-system neuroadaptations to chronic drug exposure include decreases in function of the same neurotransmitter systems in the

same neurocircuits implicated in the acute reinforcing effects of drugs of abuse. Decreases in activity of the mesolimbic dopamine system and decreases in serotonergic neurotransmission in the nucleus accumbens occur during drug withdrawal in animal studies. Imaging studies in drug-addicted humans have consistently shown long-lasting decreases in the numbers of dopamine D₂ receptors in drug abusers compared with controls. Additionally, cocaine abusers have reduced dopamine release in response to a pharmacological challenge with a stimulant drug. Decreases in the number of dopamine D₂ receptors, coupled with the decrease in dopaminergic activity, in cocaine, nicotine, and alcohol abusers have been hypothesized to result in decreased sensitivity of reward circuits to stimulation by natural reinforcers. Support for such a hypothesis in animal studies are data showing increased sensitivity to dopamine receptor antagonists in decreasing drug self-administration in dependent animals (i.e., dependence defined as signs of withdrawal following withdrawal from extended access). These findings suggest an overall reduction in the sensitivity of the dopamine component of reward circuitry that may at least partially explain the dysphoria associated with motivational withdrawal.

Alcohol dependence has long been associated with decreased GABAergic neurotransmission during alcohol withdrawal. Chronic ethanol decreases GABA_A receptor function, and chronic ethanol decreases GABA release in interneurons in the central nucleus of the amygdala. The observation that very low doses of the GABA_A agonist muscimol, when injected into the central nucleus of the amygdala, blocks the increased ethanol intake associated with acute withdrawal suggests that the changes in GABAergic function in the central nucleus of the amygdala may have some motivational significance in ethanol dependence.

Decreases in reward neurotransmission have been hypothesized to reflect a within-system neuroadaptation and contribute significantly to the negative motivational state associated with acute drug abstinence. Decreased reward system function may also persist in the form of long-term biochemical changes that contribute to the clinical syndrome of protracted abstinence and vulnerability to relapse. For example, although the activation of cyclic adenosine monophosphate response-element binding protein and c-fos triggered by activation of dopamine systems is relatively short-lived, more long-term changes in other transcription factors such as ΔFosB may persist for weeks.

Brain neurochemical systems involved in arousal-stress modulation may also be engaged within the neurocircuitry of the brain stress systems in an attempt to overcome the chronic presence of the perturbing drug and to restore normal function despite the presence of drug. Both the hypothalamic–pituitary–adrenal axis and

the brain stress system mediated by corticotropin-releasing factor (CRF) are dysregulated by chronic administration of all major drugs with dependence or abuse potential, with a common response of elevated adrenocorticotropic hormone, corticosterone, and amygdala CRF during acute withdrawal. Acute withdrawal from all drugs of abuse produces an anxiety-like state that can be reversed by CRF antagonists, and CRF antagonists also block the increased drug intake associated with dependence (Table 4).

A particularly dramatic example of the motivational effects of CRF in dependence can be observed in animal models of ethanol self-administration in dependent animals. During ethanol withdrawal, extrahypothalamic CRF systems become hyperactive, with an increase in extracellular CRF within the central nucleus of the amygdala and bed nucleus of the stria terminalis of dependent rats (Table 4). The dysregulation of brain CRF systems is hypothesized to underlie both the enhanced anxiety-like behaviors and enhanced ethanol self-administration associated with ethanol withdrawal. Supporting this hypothesis, the subtype nonselective CRF receptor antagonists α-helical CRF_{9–41} and D-Phe CRF_{12–41} (intracerebroventricular administration) reduce both ethanol withdrawal-induced anxiety-like behavior and ethanol self-administration in dependent animals. Exposure to repeated cycles of chronic ethanol vapor produces substantial increases in ethanol intake in rats both during acute withdrawal and during protracted abstinence (2 weeks post-acute withdrawal). Intracerebroventricular administration of a CRF₁/CRF₂ antagonist blocks the dependence-induced increase in ethanol self-administration during both acute withdrawal and protracted abstinence. When administered directly into the central nucleus of the amygdala, CRF antagonists also attenuate anxiety-like behavior and ethanol self-administration in ethanol-dependent rats. These data suggest an important role for CRF, primarily within the central nucleus of the amygdala, in mediating the increased self-administration associated with dependence.

Systemic injections of small-molecule CRF₁ antagonists also block both anxiety-like responses and the increased ethanol intake associated with acute withdrawal. CRF antagonists injected intracerebroventricularly or systemically also block the potentiated anxiety-like responses to stressors observed during protracted abstinence and the increased ethanol self-administration associated with protracted abstinence. Similar interactions with CRF have been observed with the dependence associated with extended access to intravenous self-administration of cocaine, nicotine, and heroin (see Table 4).

Functional norepinephrine antagonists block the anxiogenic-like and aversive effects of opiate withdrawal and block excessive drug intake associated with dependence on ethanol, cocaine, and opioids (see Table 4). A

Table 4 CRF, κ -opioid, and norepinephrine antagonists vs. motivational withdrawal

Drug	CRF Antagonist Effects			
	Withdrawal-induced changes in extracellular CRF in CeA	Withdrawal-induced anxiety-like or aversive responses	Baseline self-administration or place preference	Dependence-induced increases in self-administration
Cocaine	↑	↓	—	↓
Opioids	↑	↓	—	↓
Ethanol	↑	↓	—	↓
Nicotine	↑	↓	—	↓
Δ^9 -THC	↑	↓	—	—

Drug	Noradrenergic Antagonist Effects			
	Withdrawal-induced changes in extracellular norepinephrine in CeA	Withdrawal-induced anxiety-like or aversive responses	Baseline self-administration or place preference	Dependence-induced increases in self-administration
Cocaine	↓	—	—	↓
Opioids	↑	↓	—	↓
Ethanol	—	↓	↓	↓
Nicotine	—	—	—	—

Drug	κ -Opioid Antagonist Effects			
	Withdrawal-induced changes in dynorphin peptide and prodynorphin mRNA in NAc	Withdrawal-induced anxiety-like or aversive responses	Baseline self-administration or place preference	Dependence-induced increases in self-administration
Cocaine	↑	—	—	—
Opioids	↑	—	—	↓
Ethanol	↑	↑	—	↓
Δ^9 -THC	—	—	—	—

—, no effect; blank entries indicate not tested. CeA, central nucleus of the amygdala.

focal point for many of these effects is the extended amygdala but at the level of the bed nucleus of the stria terminalis. The dynamic nature of the brain stress system response to challenge is illustrated by the pronounced interaction of central nervous system CRF systems and central nervous system norepinephrine systems.

Conceptualized as a feed-forward system at multiple levels (e.g., in the pons and basal forebrain), CRF activates norepinephrine, and norepinephrine in turn activates CRF. Norepinephrine stimulates CRF release in the paraventricular nucleus of the hypothalamus, the bed nucleus of the stria terminalis, and the central nucleus of the amygdala. Such feed-forward systems were further hypothesized to have powerful functional significance for mobilizing an organism's response to environmental challenge, but such a mechanism may be particularly vulnerable to pathology.

Much evidence shows that dynorphin is increased in the nucleus accumbens in response to dopaminergic activation and, in turn, that overactivity of the dynorphin systems can decrease dopaminergic function. κ Opioid agonists are aversive, and withdrawal from cocaine, opioids, and ethanol is associated with increased dynorphin in the nucleus accumbens and/or amygdala. Evidence demonstrates that CRF-induced place aversions are blocked by κ antagonists (see **Table 4**).

Implications for Addiction

Motivational Properties of Acute Withdrawal

The development of the aversive emotional state that drives the negative reinforcement of addiction has been defined as the dark side of addiction and is hypothesized to be the motivational withdrawal component of the hedonic dynamic known as opponent process when the initial drug effect is euphoria. The negative emotional state that comprises the withdrawal/negative affect stage defined above consists of key motivational elements, such as chronic irritability, emotional pain, malaise, dysphoria, alexithymia, and loss of motivation for natural rewards, and is characterized in animals by increases in anxiety-like behavior, dysphoric-like responses, and reward thresholds during withdrawal from all major drugs of abuse. Two processes are hypothesized to form the neurobiological basis for motivational withdrawal: loss of function in the reward systems (within-system neuroadaptation) and recruitment of the brain stress or anti-reward systems (between-system neuroadaptation). Antireward is a construct based on the hypothesis that brain systems are in place to limit reward. As dependence and withdrawal develop, brain stress systems such as CRF, norepinephrine, and dynorphin are recruited, producing aversive or stress-like states. Concurrently, within

the motivational circuits of the ventral striatum-extended amygdala, reward function decreases. The combination of decreases in reward neurotransmitter function and recruitment of antireward systems provides a powerful source of negative reinforcement that contributes to compulsive drug-seeking behavior and addiction.

Motivational Properties of Protracted Abstinence

The overall conceptual theme argued here is that drug addiction represents a break with homeostatic brain regulatory mechanisms that regulate the emotional state of the animal. However, the view that drug addiction represents a simple break with homeostasis is not sufficient to explain a number of key elements of addiction. Drug addiction, similar to other chronic physiological disorders such as high blood pressure, worsens over time, is subject to significant environmental influences, and leaves a residual neuroadaptive trace that allows rapid re-addiction even months and years after detoxification and abstinence. Relapse, or the return to drug abuse following periods of abstinence, is one of the principal characteristics of substance dependence. The development of dependence has been suggested to play an important role in the maintenance of compulsive use and relapse following periods of abstinence.

In human alcoholics, numerous symptoms that can be characterized by negative emotional states persist long after acute physical withdrawal from ethanol. Fatigue and tension have been reported to persist up to weeks post-withdrawal. Anxiety has been shown to persist up to months, and anxiety and depression have been shown to persist in up to 20–25% of alcoholics for up to 2 years post-withdrawal. These symptoms, post-acute withdrawal, tend to be affective in nature and subacute and often precede relapse. Negative emotion, including elements of anger, frustration, sadness, anxiety, and guilt, is a key factor in relapse, and the leading precipitant of relapse is negative affect. This state has been termed protracted abstinence and has been defined in humans with a Hamilton Depression rating ≥ 8 , with the following three items consistently reported by subjects: depressed mood, anxiety, and guilt.

Animal work has shown that prior dependence lowers the dependence threshold, such that previously dependent animals made dependent again display more severe physical withdrawal symptoms than groups receiving alcohol for the first time. This supports the hypothesis that alcohol experience and the development of dependence in particular can lead to relatively permanent alterations in responsiveness to alcohol. However, relapse often occurs even after physical withdrawal signs have ceased, suggesting that the neuropharmacological changes

that occur during the development of dependence can persist beyond the final overt signs of withdrawal (“protracted motivational withdrawal syndrome”). Such protracted withdrawal has motivational significance. A history of dependence in rats and mice can produce a prolonged elevation in ethanol self-administration in daily 30 min sessions long after acute withdrawal and detoxification. The increase in self-administration is also accompanied by increased behavioral responsiveness to stressors and increased responsiveness to antagonists of the brain CRF systems. These persistent alterations in ethanol self-administration and residual sensitivity to stressors can be arbitrarily defined as a state of protracted abstinence. Protracted abstinence defined as such in the rat spans a period after acute physical withdrawal has disappeared when elevations in ethanol intake over baseline and increased behavioral responsiveness to stress persist (2–8 weeks post-withdrawal from chronic ethanol). The persistent increase in drug self-administration during protracted abstinence has been hypothesized to involve an allostatic-like adjustment such that the set point for drug reward is elevated (hedonic tolerance). These characteristics of drug addiction imply more than simply a homeostatic dysregulation of hedonic function and executive function, but rather a dynamic break with homeostasis of these systems that has been termed allostasis.

Allostatic View of Addiction

Allostasis, originally conceptualized to explain persistent morbidity of arousal and autonomic function, is defined as stability through change. Allostasis involves a feed-forward mechanism rather than the negative feedback mechanisms of homeostasis, with continuous re-evaluation of need and continuous readjustment of all parameters toward new set points. Thus, the very physiological mechanism that allows rapid responses to environmental challenge becomes the driving force of pathology if adequate time or resources are not available to shut off the response (e.g., the interaction between CRF, norepinephrine, and dynorphin in the basal forebrain that could lead to pathological anxiety and dysphoria). Allostatic mechanisms have also been hypothesized to be involved in maintaining a functioning brain emotional system that has relevance for the pathology of addiction. The plasticity is hypothesized to account for the negative emotional state associated with addiction and involves decreased function of brain reward transmitters and circuits and recruitment of the brain antireward or brain stress systems (**Figure 1**). Repeated challenges, such as the case with drugs of abuse, lead to attempts of the brain via molecular, cellular, and neurocircuitry changes to maintain stability but at a cost, which

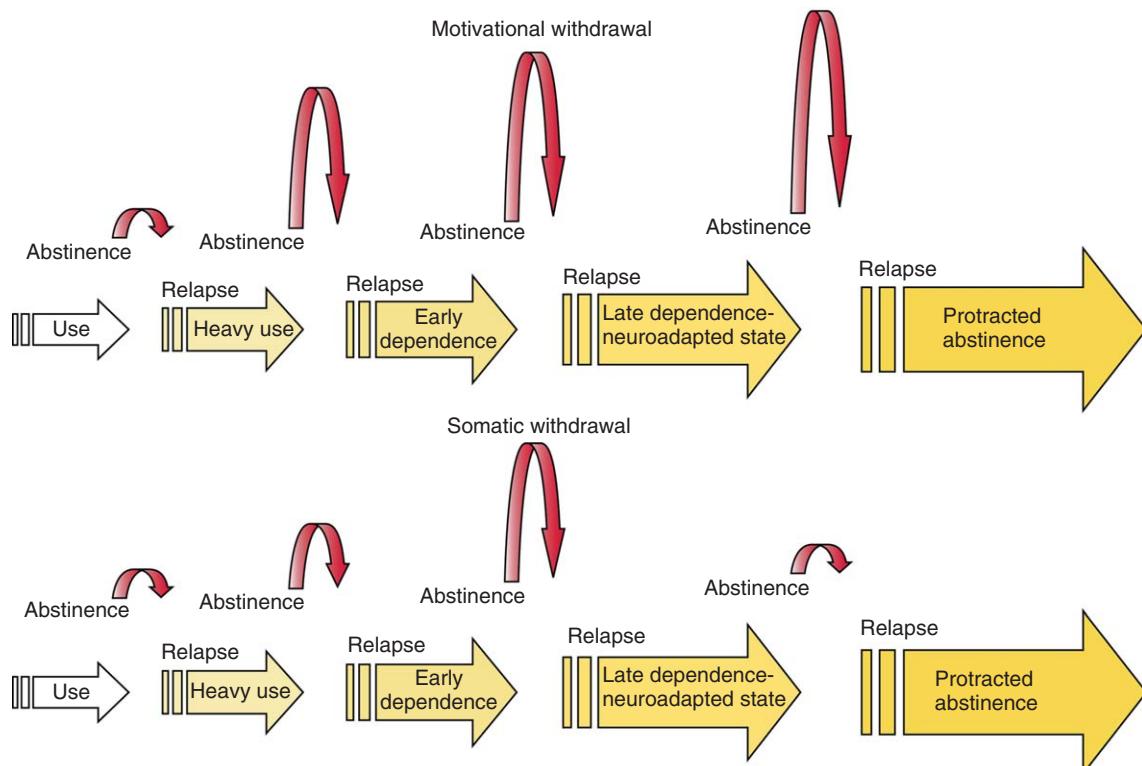


Figure 1 The hypothetical intensity of somatic and motivational withdrawal over the time-course of the development of addiction. Notice that motivational withdrawal is hypothesized to develop earlier, reach a higher intensity, and continue to sensitize with continued movement through the addiction trajectory. Reproduced with permission from Heilig M and Koob GF (2007) A key role for corticotropin-releasing factor in alcohol dependence. *Trends in Neurosciences* 30: 399–406.

includes a worsening of the negative emotional state during acute and protracted withdrawal. Such a cost fits the definition of allostatic load. For the drug addiction framework elaborated here, the residual negative emotional state is considered an allostatic state. How other known brain neurochemical systems hypothesized to be involved in emotional homeostasis and localized to the extended amygdala (e.g., vasopressin, orexin, and nociceptin) participate, where the extended amygdala projects to convey the emotional valence, and how individuals differ at the molecular-genetic levels of analysis to convey loading on these circuits remain challenges for future research.

Implications for Diagnosis of Addiction

The models of dependence with a ‘little d’ and other symptoms associated with Dependence with a ‘big D’ outlined above have both face and construct validity. Face validity is validation of the model by the similarity of the response (i.e., tremor responses in humans and in animals during the peak of alcohol withdrawal). Construct validity is validation in which the model has explanatory power for the human condition or functional equivalence for the human condition. For example, ample evidence indicates impaired reward function in animals showing escalation in drug intake with extended access to intravenous drugs of abuse and in animals with withdrawal-induced excessive drinking. Similarly, evidence exists of impaired stress responsivity during drug withdrawal that is paralleled in the human condition. The robust decrease in dopaminergic function in the mesolimbic dopamine system in rats during acute withdrawal suggests that a similar effect occurs in humans.

Such construct validity provides a powerful argument that a negative emotional state produced by excessive use of drugs is a key diagnostic for the syndrome of addiction. As such, two important issues are manifest. First, such a negative emotional state may not be as severe as those associated with anxiety disorders or depressive disorders, but clearly they may overlap in symptomatology. Second, the physical (somatic) signs of withdrawal are not the key elements of withdrawal that should be measured. Thus, a compelling argument can be made that the diagnostic criteria for addiction should indeed include an additional withdrawal measure, one for motivational withdrawal in addition to classic physical withdrawal. Defining the actual symptoms of such motivational withdrawal may ultimately require some biological measures in the domains of imaging, response to a drug challenge, or genetic markers.

See also: Alcoholism; Animal Models of Behavior: Alcohol Addiction; Cellular Plasticity in Cocaine and Alcohol Addiction; Drug Addiction; Pain and Addiction.

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Ethanol and Nicotine Interactions

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Glossary

Agonist – A substance that binds to and activates a receptor protein.

Antagonist – A substance that binds to a receptor protein without activating it but when bound to the receptor prevents an agonist from activating it.

Behavioral disinhibition – Alleviation of a constrained behavior.

Behavioral sensitization – Enhancement of the locomotor-activating effect of a drug upon repeated exposure (the opposite to tolerance development).

Conditioning – A neutral cue repeatedly presented together with a stimulus acquires the ability to elicit the reaction produced by the stimulus itself.

Drug discrimination – The ability to detect the presence of a substance producing an internal state similar to that produced by a well-experienced drug.

Mesolimbic dopamine system – A group of dopamine-carrying neurons originating in the ventral tegmental area in the midbrain and projecting to the nucleus accumbens in the ventral striatum.

Smoking and drinking often occur together and the use of either substance can trigger the use of the other in drug-experienced individuals. These associations have been established in epidemiological and experimental studies. Thus, 70–90% of alcoholics smoke, as compared to 20–30% in nonalcoholic comparable populations. Smokers also consume twice as much alcohol as do nonsmokers, and the risk for being a heavy smoker increases with the number of DSM-IV criteria fulfilled for alcoholism. Alcoholism is 10–14 times more common among smokers than nonsmokers, and collecting information about smoking habits has been advanced as a possible screening tool for heavy alcohol consumption. Smoking cessation is more difficult to attain in former or current alcohol problematics and successful smoking or alcohol cessation may improve the likelihood of alcohol reduction or smoking cessation, respectively, although evidence to the contrary is also available. Interestingly, there is a correlation between onset of tobacco addiction at early age and addiction to alcohol or other drug abuse later in life.

The reasons for the extensive co-abuse of nicotine and ethanol are multifactorial, including cultural, psychosocial, psychiatric, genetic, and pharmacologic/toxicologic factors. Here psychiatric, genetic, and pharmacologic/toxicologic factors will be considered.

Ethanol Nicotine and Society

Alcohol and nicotine are the two drugs of abuse that produce the most severe health problems and the highest costs to society. In the United States, in 1998, the total costs of alcohol abuse and dependence was estimated to be 184 636 million dollars. In Sweden (9 million inhabitants and the home country of the author), the number of annual deaths related to excessive alcohol intake is approximately 5000 and that to smoking (nicotine) approximately 8000. The co-abuse of these drugs may potentiate each other's detrimental effects and more alcoholics die from smoking-related diseases than from alcohol-related ones, reflecting the fact that a large proportion of alcoholics are smokers. The main reasons for the individual and society having difficulties in reducing the use of ethanol and nicotine are the well-established addictive properties of both drugs, and that both drugs are legal and are culturally accepted.

Psychiatric Disorders and Abuse of Ethanol and/or Nicotine

Several psychiatric disorders are associated with enhanced rates both of nicotine and ethanol dependence, and it is possible that the genetic and neurochemical underpinnings of such disorders at least partly underlie the extensive abuse of nicotine and/or ethanol that is observed. Of special interest in this respect are schizophrenia, bipolar disorder, and attention-deficit hyperactivity disorder (ADHD) in which the rates of nicotine and ethanol dependence are especially high and which all have been connected to dysregulations of brain dopamine function. Dopamine systems have been heavily implicated also in ethanol and nicotine dependence, as well as in other substance dependencies. There are also associations with underlying personality traits, which to a large extent are genetically determined. Heightened impulsivity, novelty seeking, reward dependence, and so

on are traits associated particularly with Cloninger's type II alcoholism and neuroticism with type I. Some of these personality traits are also seen in connection with ADHD, especially when presented in combination with conduct disorder (CD), which indicate that they may be manifestations of neurochemical alterations associated with these neuropsychiatric conditions and their genetic background. That this may be the case is suggested by the fact that many of these personality traits are abolished upon pharmacological treatment of ADHD. Individuals with ADHD-CD generally debut very early with cigarette smoking and alcohol use and often display a rapid foray into the antisocial type of alcoholism (type II).

The neurochemistries underlying ADHD, schizophrenia, and bipolar disorder thus may be directly involved in mediating the increased risk for both ethanol and nicotine abuse. However, the extensive drug use in these groups of patients could also represent self-medication. The cognitive enhancing effects of nicotine have been suggested to explain the extensive smoking in schizophrenia and could be related to nicotine's stimulatory effect on cholinergic and mesocortical dopamine systems. The same mechanisms could underlie extensive nicotine use in ADHD. Indeed, cognitive symptoms observed in schizophrenia and in ADHD, for example, attention and working memory deficits and disturbed executive function, are overlapping and, in both conditions, reduced catecholamine transmission in the frontal cortex may be involved. When interpreted in terms of self-medication, the use of alcohol is probably best explained by the drug's anxiolytic and calming effects. Anxiety and restlessness are prominent features of schizophrenia, bipolar disorder, and ADHD.

Heredity

It has become increasingly recognized that hereditary factors influence the risk for developing drug addiction. Adoption studies and numerous twin studies performed in various populations have demonstrated that 50% of the variance with respect to alcoholism is explained by genes. The collaborative study of the genetics of alcoholism (COGA) has further underlined the importance of genes, as have a number of candidate gene studies. Further, for habitual smoking, the genetic contribution is high, as much as 60–70%, and the genetic risk for alcoholism correlates with that for habitual smoking. Whether the genetics underlying alcoholism and nicotine dependence are shared with those of other drug addictions has not been clearly established. Twin studies indicate that the debut of drug use is determined by sociocultural factors, whereas continued use and dependence is determined by genes and nonshared environmental factors.

The common genetic background to nicotinism and alcoholism could involve a number of neurotransmitter systems in the brain, since both drugs have a rich pharmacology. Systems related to the intoxicating and feel-drug effects of alcohol have been implicated, since these effects are diminished in young men with alcoholic fathers compared to young men with nonalcoholic fathers, and this low alcohol response predicts future alcohol dependence. Further, nicotine users display a decreased sensitivity to the intoxicating effect of ethanol. Animal studies provide further support for common genetic factors underlying the sensitivity to both ethanol and nicotine. One of these factors could be a polymorphism in the α_4 subunit of the nicotinic acetylcholine receptor (nAChR).

Pharmacology

Interactions with Nicotinic Acetylcholine Receptors

The nAChR is a member of the family of cysteine-loop ligand-gated ion-channels, including also GABA_A, 5-HT₃, and glycine receptors. The nAChR is composed of five subunit proteins forming a cation channel passing through the neuronal cell membrane. Acetylcholine and nicotine bind to the receptor and thereby regulate the permeability of the ion-channel, neuronal activity, and transmitter release. The different subunits ($\alpha_2-\alpha_{10}$, $\beta_2-\beta_4$) are differentially expressed in the brain. Nicotine produces its pharmacological effects via nAChRs, especially via $\alpha_4\beta_2$ and α_7 homomeric receptors (see further below), that also are the most abundant nAChRs in the brain. *In vitro* and *in vivo*, the nAChR may undergo desensitization upon agonist exposure and some of the pharmacodynamic effects of nicotine may derive from activation of nAChR and others from inactivation (desensitization). Nicotinic acetylcholine receptors are present also in the peripheral nervous system, both in ganglia and at the motor endplate.

Relevant concentrations of ethanol interact with various cysteine-loop receptors. As early as in 1967, results indicated that ethanol interacts with nAChR in the peripheral nervous system of the frog, and based on similar electrophysiological studies, it was suggested in 1980 that ethanol's interaction with nAChRs could underlie the addictive properties of the drug. A number of studies have later been performed on nAChRs from *Torpedo*, showing how ethanol affects nAChRs with respect to both activation and deactivation. Several studies have shown that ethanol directly interacts also with central nAChR *in vivo*, in neuronal cell cultures and in different cells expressing nAChRs from rat or man. Ethanol's interaction with nAChRs is determined by the type of receptor

expressed – response potentiation is observed in some subtypes (e.g., $\alpha_2\beta_4$, $\alpha_4\beta_4$, $\alpha_2\beta_2$, $\alpha_4\beta_2$ (human); $\alpha_3\beta_4,\alpha_2\beta_4$ (rat)), whereas antagonism (e.g., α_7 oligomers (human and rat) or no effect is observed in others. Ethanol is a nAChR co-agonist rather than an agonist in its own right, that is, it potentiates the effect of acetylcholine but does not activate the receptor by itself. Chronic ethanol administration influences radioligand binding of nicotine to nAChRs, but the effects vary across studies and depend on what brain region is investigated.

Interactions with the Brain Reward System

Drugs of abuse activate brain catecholamine systems and this activation is associated with locomotor stimulation. It was suggested in the late 1970s that these effects are of importance also for the rewarding properties of addictive drugs. All drugs of abuse have subsequently been demonstrated to increase dopamine release, and this effect is most pronounced in the nucleus accumbens (NAcc), a major target of the mesolimbic dopamine system and a central component of the brain reward system. The mesolimbic dopamine system has its cell-bodies in the ventral tegmental area (VTA) of the mid-brain. The function of the mesolimbic dopamine system is severely impaired upon cessation of subchronic exposure to drugs of abuse, including ethanol and nicotine.

The mechanisms underlying the mesolimbic dopamine activations have been disclosed for both nicotine and ethanol. Nicotine directly stimulates $\alpha_4\beta_2$ nAChRs probably located on the cell-bodies of mesolimbic dopamine neurons in the VTA. Thereby, the regular firing rate of the neurons is increased. Nicotine increases also burst firing of dopamine neurons and this effect is mediated via activation of α_7 nAChRs located on afferent glutamatergic neuronal terminals. Stimulation of these α_7 receptors releases glutamate that, in turn, excites the dopamine neurons into burst firing and causes pronounced dopamine release in the NAcc.

The dopamine synthesis and locomotor stimulating effects of ethanol in mice are at least partly mediated by central nAChRs. In addition, the dopamine releasing and dopamine synthesis enhancing effects of ethanol in the limbic system of the rat involve nAChRs in the mesolimbic cell-body region. Thus, ethanol-induced mesolimbic dopamine activation involves nAChRs in the VTA, and the effect appears localized to the anterior VTA. However, local perfusion of ethanol in the VTA does not increase dopamine release in the NAcc, whereas local perfusion in the NAcc does, and this effect is prevented by nAChR blockade in the anterior VTA. These results indicate that ethanol produces an effect in the NAcc that secondarily increases endogenous ACh release and nAChR activation in the anterior VTA. These *in vivo* findings contrast to results obtained *in vitro* showing that

ethanol directly stimulates neuronal activity in isolated dopamine neurons. Whether or not this latter effect involves nAChRs is not known. The idea of an indirect mechanism of action put forward above is reinforced by findings that ACh depletion prevents ethanol-induced dopamine release and that ethanol consumption enhances ACh release in the VTA, in parallel with dopamine release in the NAcc. The event produced by ethanol in the NAcc that eventually leads to nAChR activation in the VTA is an interaction with another cysteine-loop receptor – the glycine receptor. Ethanol may still interact directly with nAChRs in the VTA, provided that it is simultaneously present in the NAcc. This could be explained by the fact that ethanol is a co-agonist rather than an agonist at nAChR, and when ethanol is simultaneously applied in the NAcc, acetylcholine is released in the VTA, enabling ethanol to influence ventral tegmental nAChRs.

Contrary to the case of nicotine, $\alpha_4\beta_2$ nAChRs are not involved in ethanol's effects outlined above. Instead, $\alpha_3\beta_2$ and/or β_3 containing receptors have been implicated. As regards α_7 receptors, ethanol is an antagonist rather than agonist at these receptors. Consequently, the dopamine-related effects of ethanol are not altered by α_7 receptor blockade. Such blockade also fails to mimic the ethanol effect. In conclusion, the available evidence indicates that ethanol increases mesolimbic dopamine activity via indirect and/or direct activation of $\alpha_3\beta_2$ and/or β_3 containing nAChRs in the anterior VTA.

Co-administration of ethanol and nicotine produces complex results with respect to ethanol-induced locomotor stimulation in mice, where nicotine either potentiates or counteracts the stimulatory effect of ethanol depending on the dose of ethanol. In rats, nicotine and ethanol produce additive effects on dopamine release in NAcc, both when the drugs are administered systemically and when ethanol is given systemically and nicotine locally in the VTA.

Alcohol Consumption/Self-Administration Studies

Systemic administration of a nicotinic antagonist reduces ethanol consumption in a two-bottle choice test and both ethanol and nicotine self-administration in operant procedures. Furthermore, local nAChR antagonism in the VTA reduces lever-pressing for nicotine self-administration as well as ethanol intake and preference in the two-bottle test. Dopamine levels in the NAcc have been concomitantly measured and the results parallel the ethanol consumption findings. In analogy with the pharmacological studies, $\alpha_4\beta_2$ receptors mediate nicotine self-administration but not alcohol consumption that instead involves α_3 and α_6 containing nAChRs. The partial nAChR antagonist varenicline, an established

smoking cessation agent, also reduces ethanol consumption in rats. However, there are also studies that have failed to demonstrate an ethanol consumption-reducing effect of nAChR antagonists.

It has been proposed that nAChRs are involved not only in the pharmacological effects of ethanol but also in mediating ethanol-conditioned dopamine release. Receptor nAChR blockade, thus, may prevent dopamine-induced orientation toward the ethanol bottle. This hypothesis received support when it was shown that a stimulus previously associated with ethanol intake later by itself increased dopamine output in the NAcc, an effect prevented by nAChR blockade. Further, responding with conditioned reinforcement for alcohol-associated stimuli involves nAChRs, and α_3 and α_6 containing receptors, rather than $\alpha_4\beta_2$, may be involved also in these effects. Brain acetylcholine systems, probably via nAChR activation, have been implicated in mediating conditioning also to various other rewards.

Taken together, a certain nAChR population (containing $\alpha_3\beta_2$ and/or β_3 subunits) in the cell-body region of the mesolimbic dopamine system is involved both in ethanol-conditioned activation of the system and in the pharmacological activation produced by ethanol. This coincidence is interesting, since ethanol intake in alcoholics often triggers further ethanol consumption, even though the individual already may be heavily intoxicated. This phenomenon could be explained if ethanol pharmacologically activates the same neuronal mechanisms as those involved in conditioned initiation of ethanol consumption – establishing a *circulus viciosus*. If the same neurocircuitry mediates conditioned dopamine release to stimuli associated also with other drugs, ethanol's pharmacological activation of it could contribute to ethanol-induced relapse to other addictive drugs, as often observed among drug-dependent individuals.

In addition, in humans, nAChR blockade decreases the stimulatory and euphoric effects of ethanol. Some evidence further indicates, that ethanol consumption as such is decreased by nAChR blockade in alcohol-dependent individuals.

Drug Discrimination Studies

Generally, ethanol does not substitute for nicotine in rats trained to discriminate nicotine. This is not surprising, since ethanol produces a range of effects of which those related to activation and blockade of certain γ -aminobutyric acid (GABA) and glutamate receptors, respectively, may be the most important for producing the ethanol discriminative cue. These findings do not exclude the fact that ethanol interacts with nAChRs but indicate that this interaction does not contribute significantly to its discriminative cue in rats.

Subchronic/Chronic Interactions

According to epidemiologic studies, an early smoking debut is a risk factor for future alcohol dependence. Animal studies show that subchronic nicotine administration changes the sensitivity of the mesolimbic dopamine system not only to nicotine but also to ethanol, indicating that the increased risk for alcohol dependence among nicotine users is due to pharmacological consequences of the drug. Indeed, studies have shown that chronic nicotine administration increases ethanol consumption in rats, even though contradictory results also are available. The presence of nicotine during ethanol consumption is not required to produce the effect. Instead, nicotine appears to produce a sustained neurochemical effect that promotes ethanol intake. Further, the findings that the exposure of mice to nicotine during a limited period very early in life enhances ethanol consumption in adult life support this idea.

That the ethanol intake-promoting effect of smoking is related to nicotine rather than to other components of cigarette smoke is supported not only by animal experiments, but also by clinical studies showing that ethanol consumption is also increased in nicotine gum users and in snuffers. Ethanol consumption is most pronounced in individuals using both cigarettes and snuff, indicating dose-dependency and/or complementary mechanisms (see below).

Relation to Behavioral Disinhibition/Impulsivity

The mechanisms underlying the chronic nicotine effect is unknown but could involve an increased responsiveness of mesolimbic dopamine neurons, perhaps on the basis of a paradoxical upregulation of the number of central nAChRs in response to chronic nicotine exposure. Such upregulation has been demonstrated both in rats and in humans. A puzzling finding is that the ethanol intake enhancing effect of nicotine in rats is resistant to concomitant chronic antagonism pretreatment and that both a blood-brain barrier penetrating and a nonpenetrating nAChR antagonist increase ethanol intake by themselves after chronic pretreatment. To account for these findings, it has been proposed that the ethanol-consumption-promoting effect of nicotinic drugs is related to intermittent blockade of ganglionic nAChRs rather than to stimulation of central nAChRs. Furthermore, the effect on ethanol consumption pharmacologically and statistically correlates with alterations of nicotine-induced behavioral disinhibition rather than with sensitization to the locomotor-stimulatory effect of nicotine, as originally hypothesized. The expression of nicotine-induced behavioral disinhibition is prevented by co-treatment with serotonin-facilitating drugs. Decreased serotonin

transmission has since long been linked to impulsive features and to increased ethanol consumption.

Chronic studies show that the combined treatment with a blood–brain barrier nonpenetrating nAChR antagonist and nicotine produces the largest effects both on ethanol consumption and on nicotine-induced behavioral disinhibition. Thus, intermittent blockade of peripheral nAChRs in combination with intermittent stimulation of central receptors appears to be the most powerful treatment to promote ethanol consumption. This result may parallel the findings in human snuffers who also smoke – snuffers more or less constantly expose themselves to nicotine, which should desensitize peripheral nAChRs, resembling peripheral blockade. Recent results have shown that subchronic pretreatment with a peripheral nAChR blocker decreases GABA_A-mediated depression of dopamine release in NAcc, resulting in prolonged dopamine release after systemic as well as local ethanol administration.

In conclusion, chronic nicotine administration enhances behavioral disinhibition, reflected i.a. in increased ethanol intake, which may derive from long-term consequences of intermittent desensitization of peripheral nAChRs. Human studies parallel these findings, showing that smokers are more impulsive than nonsmokers and ex-smokers, both as estimated with personality inventories and in experimental models. These results indicate that nicotine-induced impulsivity is reversible or that those who manage to quit smoking do so because they are less impulsive. Prospective studies are needed to resolve this issue. In the context of behavioral disinhibition, it is interesting to note that nicotine exposure during the last trimester has been associated with enhanced ethanol consumption in adult life in mice and with dose-dependent risks for ADHD symptoms and for being subjected to criminal arrests or being hospitalized for substance abuse in humans.

See also: Alcoholism; Animal Models of Behavior: Alcohol Addiction; Animal Tests for Anxiety; Antisocial Substance Dependence; Brain Imaging and Addiction; Brain Stimulation and Addiction; Cellular Plasticity in Cocaine and Alcohol Addiction; Drug Addiction; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Drug Priming; Drug Sensitization and Drug Abuse; Drug Withdrawal – Motivational View; Incentive Motivation and Incentive Salience; Molecular Neurobiology of Addiction; Motivation; Neural Basis of Attention-Deficit/Hyperactivity Disorder; Neural Systems

of Motivation; Neurophysiology of Drug Reward; Nicotine; Psychostimulants; Rewarding Brain Stimulation; Stress and Drug Craving; Stress and Reward; Transition to Addiction; Vulnerability Factors in Addiction Disorders.

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Hallucinations in Neuropsychiatry and Drug Abuse: From Phenomenology to Pathophysiology

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Glossary

Delusions – A false belief based on incorrect inference about external reality that is firmly sustained despite what almost everyone else believes and despite what constitutes incontrovertible and obvious proof of evidence to the contrary.

Hallucinations – Perceptions that occur without external stimulation of the relevant sensory organ.

Hallucinosis – This poorly defined term has been applied to a variety of clinical situations, including hallucinatory syndromes without associated delusions, or hallucinations in the setting of neurological conditions or sensory impairment, usually with preserved insight.

Illusions – A misperception or a misinterpretation of a real external stimulus.

Insight – In neuropsychiatry, insight refers to people's understanding of their illness, and to understanding how the illness affects individuals' interactions with the world. Applied to hallucinations, insight refers to the awareness of the hallucinatory nature of the experience.

received various definitions (e.g., hallucinations with preserved insight or vivid internal images), none of which is universally accepted. The same holds true for the term 'hallucinosis' which has been applied to a variety of clinical situations. Both terms are confusing and unnecessary. Finally, hallucinations are also distinct from vivid mental imagery and from dreams, although, in the latter case, transitional forms exist.

Frequency

The prevalence of hallucinations in the general population is not negligible. In one prevalence study, hallucinations were occasionally present during the daytime in the quarter of a large, noninstitutionalized sample aged 15 years or over. In another study, the general incidence was 10–30 cases per 1000 persons per year, with variations related to age and gender. Although hallucinations occur in a range of organic and psychiatric conditions, and under the influence of drugs or alcohol, they also occur in normal individuals. The rest of this article focuses on hallucinations occurring in the course of neurological or sensory diseases, in psychiatric conditions, or under the influence of hallucinogenic substances.

Introduction: General Aspects

Definitions

The term hallucination in its modern sense was first introduced by Jean Etienne Esquirol in his textbook *Des maladies mentales* (1837). Hallucinations are generally defined as perceptions that occur without external stimulation of the relevant sensory organ. Hallucinations are therefore linked to a sensory modality and may be auditory, visual, somatosensory (tactile or somatic), olfactory, gustatory, or multimodal, occurring in more than one modality. Visual hallucinations may be elementary or simple (lines, dots, geometrical patterns) or complex (objects, animals, people, landscapes, etc.). Auditory hallucinations may also be simple (knocks and rings) or complex (music and voices). The person may or may not have insight into the fact he or she is having a hallucination.

Hallucinations should be distinguished from illusions, which are misperceptions or misinterpretations of a real external stimulus. The term 'pseudohallucinations' has

Evaluation

To identify and rate hallucinations, the examiner relies on the patient's and/or the caregiver's accounts. Many patients do not spontaneously report their hallucinations, so that the information has to be sought by using specific questions or scales. To identify hallucinations, single items from scales have been used, as well as self-developed questionnaires or inventories. The latter are useful to record the variety of psychotic symptoms, but they do not allow examiners to rate the symptoms. Scales for rating hallucinations and other psychotic symptoms have been developed mainly in the field of psychiatry (e.g., the Positive and Negative Syndrome Scale, the Scale for Assessment of Positive Symptoms, or the Brief Psychiatric Rating Scale) and in the field of dementia (e.g., the Neuropsychiatric Inventory).

Hallucinations Associated with Neurological or Sensory Disease

Cortical Activation as a Common End Pathway for Hallucinations

Pioneering works following World War I established that occipital stimulation generated visual hallucinations, and that hallucinations became more complex when stimulation shifted from primary visual cortex to association visual cortices. It was also shown that seizures secondary to occipital lesions were often preceded by a visual aura. The role of the cortex in generating hallucinations was confirmed by Penfield and colleagues who obtained auditory and visual hallucinations in patients undergoing surgery for epilepsy by stimulating the auditory and visual cortices. The role of specific areas of cortex has been further demonstrated by using functional imagery. Using functional magnetic resonance imaging (fMRI) in patients with Charles Bonnet syndrome (see below), it was shown that various types of visual hallucinations correlate with cerebral activity in ventral extrastriate visual cortex, that the content of the hallucinations reflects the functional specializations of the region, and that the patients who hallucinate have increased ventral extrastriate activity, which persist between hallucinations. To summarize, hallucinations in a sensory modality result from the activation of cortical areas normally associated with the processing of sensory stimuli in the same modality. Simple (elementary) hallucinations result from primary sensory cortex activation, and complex hallucinations result from the activation of association sensory areas. In the context of neurological or sensory diseases, visual hallucinations are the more prevalent type, and cortical activation underlying hallucinations may result from various mechanisms which are now reviewed.

Cortical Irritation

In this model, hallucinations result from intrinsic overactivity in the corresponding sensory cortical area. This mechanism is generally considered to occur in migraine and in epilepsy auras. In migraine, visual symptoms precede the headache in 20% of the cases. Typically, the aura is a flickering uncolored unilateral zigzag line in the center of the visual field, which progresses toward the periphery, leaving a scotoma. The phenomenon is reversible in less than 30 min. Clinical and imaging studies indirectly suggest a relationship between migraine aura and cortical spreading depression, a wave of neuronal and glial depolarization, followed by long-lasting suppression of neural activity. In partial epilepsy, hallucinations were part of the aura in 13% of the cases in one large series. They are more often simple than complex, and involve the following sensory modalities in

decreasing frequency order: visual, somato-sensory, auditory, olfactory, and gustatory. The simple hallucinations probably reflect epileptic discharges in a primary sensory cortex. However, other mechanisms have been advocated for epileptic complex hallucinations, relying on the Jacksonian concept of dissolution, or on the concept of activation of specific circuits by a focal discharge.

Deafferentation and Release Phenomena

General disinhibition theories for hallucinations were first forwarded by Jackson. In the perceptual release theory of West, hallucinations arise when the constant flow of sensory inputs is impaired, allowing the emergence of earlier perceptions or traces into consciousness. More specifically, the release or deafferentation theory has been applied to visual hallucinations associated with impairment of visual input. The core hypothesis is that stimulus-driven, bottom-up visual processing inhibits the spontaneous activity of the visual cortical areas and facilitates the release of stored images. More recent conceptualizations suggest that the lack of input leads to chronic hyperexcitability. Visual hallucinations associated with eye disease in nondemented persons have received the name of Charles Bonnet syndrome. The prevalence rate of visual hallucinations in visually impaired populations is around 10%, and their main risk factors are a lower visual acuity, a lower contrast sensitivity, and an older age. Hallucinations may be simple or, more often, complex. Insight is preserved. The neurophysiological link from deafferentation to hallucinations remains unclear. Using induced hallucinations in normal-sighted individuals, ffytche has proposed that hallucinations in eye disease result from the shift from tonic to burst firing in thalamocortical circuitry. Lesions of the retrochiasmal visual pathways, typically ischemic, are associated in up to 40% of the cases with hallucinations in the hemianopic field. Hallucinations, simple or complex, are usually transient. Hallucinations are associated with circumscribed, occipital ischemic lesions, while larger lesions, affecting the temporal–visual associative areas, preclude the development of hallucinations. This finding supports the hypothesis that hallucinations result from release from inhibitory input of visual areas bordering the damaged occipital lesion.

The concept of cortical release has been applied to hallucinations in nonvisual sensory modalities associated with the impairment of the corresponding sensory pathways. For instance, hearing loss may be associated with auditory hallucinations, typically of a musical nature. In a series of 125 elderly subjects with hearing impairment, one-third had hallucinations, mostly elementary (including tinnitus), and 5% heard voices or music. In patients with musical hallucinations and deafness, a positron emission tomography (PET) study showed that brain activity

increased as a function of the severity of the hallucination in a distributed network distinct from the primary auditory cortex, which included the posterior temporal lobes, the right basal ganglia, the cerebellum, and the inferior frontal cortices. Phantom sensations, that is, the vivid impression that an amputated limb is still present, and, in some cases, is painful, are present in almost all patients with limb amputation. In this case, the hallucinatory phenomenon seems secondary to the reorganization of the somato-sensory cortical maps following the deafferentation.

Dream Intrusions and Status Dissociatus

Relations between dreams and hallucinations have been debated for a long time. It has been postulated that hallucinations may result from a dissociation between dream and sleep mechanisms. Jean Lhermitte, in the 1920s, first suggested this dissociation could occur in patients with peduncular hallucinosis (hallucinations secondary to a lesion of the upper brainstem). Hypnagogic and hypnopompic hallucinations (perceptions that occur while going to sleep and on waking) represent transitional forms of misperceptions, between dream and hallucination. They are not considered as hallucinations proper in some classifications. Hypnagogic and hypnopompic hallucinations occur in normal persons, but they are more frequent and severe in patients with narcolepsy, a chronic sleep disorder characterized by excessive daytime sleeping and cataplexy. In this condition, hypnagogic hallucinations occur when patients fall asleep directly into rapid eye movement (REM) sleep, suggesting that the hallucinations share mechanisms with dreams. Polysomnographic studies have suggested that narcoleptic-like mechanisms could explain some hallucinations in other conditions, such as Parkinson's disease. Prolonged dream-like vivid hallucinations are also present in status dissociatus, a parasomnia due to extreme dissociation between wakefulness, REM sleep, and nonrapid eyemovement (NREM) sleep. Status dissociatus is observed in various medical diseases, such as treated narcolepsy–cataplexy, dementia, multiple system atrophy, status-post-cardiac surgery, Morvan's chorea, protracted alcohol withdrawal, fatal familial insomnia, and the Guillain–Barré syndrome.

Multifactorial Models

Hallucinations may occur in the course of neurodegenerative diseases. Their lifetime prevalence is approximately 50% in patients with Parkinson's disease, and the prevalence is even higher in patients with dementia with Lewy bodies. In most cases, hallucinations occur with a clear sensorium and a chronic course. Complex visual hallucinations are the most common type, but other sensory modalities may be involved. Hallucinatory

images are superimposed on the normal background scene, they may be relatively stereotyped in a given patient and, in most instances, the patient is an observer rather than an actor in the hallucinated scene. Insight may be preserved, fluctuating, or lost. In the two latter cases, cognitive impairment is usually present. Hallucinations often combine with other minor phenomena such as visual illusions and sense of presence, and, mostly in demented patients, with delusions. Although a number of clinical and biological risk factors have been identified, the pathophysiology of Parkinson's disease-associated psychosis remains unclear. Hallucinations could result from various and probably concomitant mechanisms, including: (1) dopaminergic overactivity and/or imbalance in monoaminergic (relatively preserved) and cholinergic (altered) neurotransmission; (2) alteration of brainstem sleep/wake and dream regulation; (3) dysfunction of the visual pathways, nonspecific (coincidental ocular disease) and/or specific, such as PD-associated retinal dysfunction and functional alterations in the ventral stream of visual cortical pathways; (4) dysfunction of top-down mechanisms of vision, such as impaired attentional focus; and finally, (5) antiparkinsonian drugs and other pharmacological agents may interfere with the preceding mechanisms at many levels. No simple model can account for the full diversity and heterogeneity of factors associated with hallucinations in PD. Diederich *et al.* forwarded an integrative model based on Hobson's work on factors regulating consciousness. This model emphasizes dysregulation of the gating and filtering of external perception and internal image production. A more general model for recurrent complex visual hallucinations occurring in the course of a variety of conditions has been proposed by Collerton *et al.*, based on cognitive models of scene perception. In this Perception and Attention Deficit model, a combination of impaired attentional binding and poor sensory activation of a correct proto-object (or template), in conjunction with a relatively intact scene representation, bias perception to allow the intrusion of a hallucinatory proto-object into a scene perception.

Hallucinations in Psychiatry

Hallucinations and Schizophrenia

Prevalence and phenomenology

Hallucinations are a core clinical feature of major psychiatric disorders. Besides disorganization and negative symptoms, hallucinations constitute a core dimension of schizophrenia with delusions (Schneider's first rank symptom), and are associated with poor social functioning. Hallucinations in schizophrenia may occur in any sensory modality, but auditory verbal hallucinations are the most common and characteristic. Voices are perceived

as distinct from the person's own thought and as coming from the extrapersonal space. The content is often pejorative or intrusive. The classical view is that schizophrenic patients lack insight into their illness and psychotic symptoms. However, poor awareness of psychotic experience seems to be a trait of the acute rather than the chronic psychopathology of schizophrenia. Lifetime prevalence rates of auditory vocal hallucinations in schizophrenia range from 50% to 70%. The phenomenology and the prevalence of hallucinations in schizophrenic patients vary according to the marital status, the educational level, and the cultural background, suggesting that environmental factors influence the expression of the hallucinations. The phenomenology may vary according to the age of onset. The French concept of 'chronic hallucinatory psychosis' refers to a chronic hallucinatory and delusional disorder affecting mainly women and that differs from paranoid schizophrenia by a late onset, the absence of formal thought disorder and intellectual impairment, a better response to treatment, and a better outcome. Accusatory or abusive auditory verbal hallucinations are more frequent in these late-onset cases. This entity is included into late-onset schizophrenia in current classifications such as the *Diagnostic and Statistical Manual of Mental Disorders* (DSM).

Brain structure and functional neuroimaging studies

Recent works using sophisticated analysis techniques such as voxel-based morphometry found that verbal hallucinations are associated with reduced gray matter volumes in the temporal lobe, more specifically in the left superior temporal gyrus, including the primary auditory gyrus, and in nonsensory regions such as the right dorsolateral frontal cortex. Studies aiming at capturing the cerebral activity associated with verbal hallucinations have shown temporal lobe activation, on the left side in most studies. This activation may include, or not, the primary auditory cortex (Heschl's gyrus). Nonsensory cortical and subcortical areas are also involved in some studies, including language production area (Brodmann's area), anterior cingulate area, and cerebellar regions, suggesting that disturbances in a distributed network may be associated with verbal hallucinations. Finally, some studies have addressed the question of cerebral asymmetry and connectivity in patients with verbal hallucinations. Evidence for altered asymmetry is inconclusive, but there is evidence, from works using diffusion tensor imaging or fMRI, for disrupted connectivity between the temporal, prefrontal, and anterior cingulate cortex.

Cognitive models

The prevailing cognitive model was proposed by Frith in the 1990s and postulates that verbal hallucinations derive from inner speech that has been misidentified as coming

outside the self, because of defective self-monitoring. In this model, frontal regions involved in verbal generation fail to modulate activation in areas involved in speech perception. This view has been later criticized or refined. Although most authors postulate an impaired processing of inner speech, the way to integrate the somewhat heterogeneous functional neuroimaging data may vary. Some models insist on the interactions between top-down and bottom-up processes. Furthermore, the role of emotion and attention is also supported by some activation studies.

Neurotransmitters

At a molecular level, studies in schizophrenic patients have reported dopaminergic abnormalities in a number of cortical and subcortical regions that have been related to positive symptoms such as hallucinations. Direct or indirect (preclinical or pharmacological) evidence suggest that schizophrenia is associated with excessive stimulation of striatal dopamine D2 receptors, which has been positively correlated with positive symptoms and more specifically hallucinations, and deficient stimulation of prefrontal dopamine D1 receptors, which could be a factor in the cognitive impairments of schizophrenia. However, other neurotransmitters, including serotonin, glutamate, γ -amino butyric acid (GABA), and acetylcholine have been implicated, either directly, or through interactions with dopaminergic systems. How multiple neurotransmitters and risk genes interact to produce the positive symptoms of schizophrenia is poorly understood.

Hallucinations and smoking

Recent research has focused on the fact that schizophrenic subjects have an auditory sensory-gating deficit which could be corrected by tobacco use. The prevalence of smoking among schizophrenic subjects is higher than in the general population. Schizophrenic smokers have higher psychotic symptoms scores (including hallucinations) than nonsmokers, and the intensity of symptoms is positively correlated to tobacco consumption. These findings suggest a form of self-medication, considering that tobacco could counteract the sensory deficit. Eighty percent of schizophrenic patients have an impaired auditory sensory transmission of repeated auditory stimuli (P50 wave inhibition deficit), which is transiently improved by nicotine. Current theories imply that the P50 wave inhibition prevents schizophrenic patients from filtering unimportant auditory stimulation, thus facilitating the emergence of auditory hallucinations and delusional interpretations.

Hallucinations and Bipolar Disorders

Few studies are devoted to hallucinations in the course of bipolar disorders. Compared with those of schizophrenia,

hallucinations in bipolar disorders are less severe, more often visual, and less often auditory. Characteristics of hallucinations seem to be similar among manic and both bipolar- and unipolar-depressed subjects. Among patients with major affective disorders, those with hallucinations are less well-educated, have higher anxiety scores, less insight into the illness, and longer hospitalizations. Recent researches have shown that childhood sexual abuse and other early traumas are associated with serious mental illness and more specifically positive symptoms, particularly hallucinations. In one study, about half of the bipolar patients had experienced hallucinations (mostly auditory followed by visual) and 16% revealed sexual abuse. These findings suggest that childhood sexual abuse could increase the vulnerability of bipolar patients to later experience auditory hallucinations.

Posttraumatic Stress Disorders and Borderline Personality Disorders

Several studies suggest that patients with posttraumatic stress disorders (PTSDs) have perceptual disturbances, and are predisposed to hallucinations and paranoia. Psychotic symptoms, especially hallucinations, are frequently experienced by survivors of early (e.g., sexual abuse) and later (e.g., exposure to military combat) trauma. The nature of delusional and hallucinatory symptoms in borderline personality disorder (known to have experienced trauma in childhood) is not well documented. Psychotic episodes are common among patients with borderline personality disorder, narrowly due to concomitant disorders. However, the nature of the relationship between trauma and hallucinations is poorly understood from a psychological perspective. As previously stated, hallucinations may result from the misattribution of mental events to an external source: this is likely to occur when experiencing mental events that are automatic and associated with low cognitive effort such as intrusive memories of trauma. The latter may be experienced as hallucinations by individuals whose source-monitoring abilities are compromised by severe mental illness. This mechanism may be reinforced during stressful periods, for instance, when an adult survivor of abuse suffers additional negative experiences. In line with this hypothesis, many patients experiencing hallucinations report that hallucinations began following a retraumatising experience.

Ekbom Syndrome: the Delusional Parasitosis

In this condition, the subject has the strong delusional belief of being infested with parasites. Patients give detailed descriptions of the activity of the parasites (crawling, biting, burrowing), translating tactile hallucinations. Delusional parasitosis is referred to as the

Ekbom's syndrome, after the Swedish neurologist, who published seminal cases in 1937, or as 'delusional disorder, somatic type' in the DSMIV criteria. Secondary functional delusional parasitosis can occur in psychiatric conditions such as schizophrenia or depression.

Hallucinogens and Alcohol

Hallucinogenic Substances

Hallucinogens include natural substances extracted from plants, such as mescaline and psilocybin, and synthetic substances such as lysergic acid diethylamide (LSD), 3,4-méthylène-dioxy-méthylamphétamine (MDMA, or ecstasy), and phencyclidine (PCP). The perceptual, cognitive, and psychological effects of these compounds are unpredictable, and are heavily dependent on the expectations of the user and the environment. The effects also depend on the dose, with differences in nature, and not only in intensity, with higher or lower doses. The hallucinations induced by these compounds are mainly auditory and visual, and are associated with, or preceded by, intensification of perceptions, synesthesia, illusions, and derealization. Visual hallucinations often consist of geometric patterns, sometimes persons or objects. Geometric patterns have been classified into four groups, called 'form constants.' It has been postulated that these hallucinations arise in the primary visual cortex (V1), and that the form of the retino-cortical map and the micro-architecture of V1 determine their geometry. In most cases, insight on the hallucinatory nature of the phenomenon is maintained. Perceptual symptoms may be re-experienced in the absence of any recent hallucinogen intoxication (hallucinogen persisting perception disorder, or flashbacks).

The drug category of hallucinogens encompasses many compounds, which exhibit a wide range of pharmacological properties. The three prototypical drugs – mescaline, psilocybin, and LSD – are agonists at 5-HT_{2A} (serotonergic) receptors. Recent research has confirmed that 5-HT_{2A} receptors are an important site of action for the hallucinogens, and have also directed attention on the modulatory roles of 5-HT_{2C} (for the phenethylamines) and 5HT_{1A} receptors (for the tryptamines).

Other drugs may act through different mechanisms such as cannabinoid agonism (tetrahydrocannabinol), N-methyl-D-aspartate (NMDA) antagonism (phencyclidine), muscarinic receptor antagonism (scopolamine), and mixed action monoamine release (MDMA).

Alcohol

Hallucinations occur in up to 10% of alcohol withdrawal patients. They are commonly visual, though they may be

auditory, tactile, and olfactory. They usually occur early, in the first 24 h following the last drink, and are associated with insomnia, agitation, and enacting-dream behaviors with partial or absent awareness of reality. Polysomnographic studies have shown the presence of an atypical transitional state between REM-sleep and wake (status dissociatus, see above).

Alcoholic hallucinosis is a much rarer condition of acute onset, occurring after one or more decades of heavy alcohol consumption. The clinical picture includes hallucinations, generally auditory, often accompanied by delusions of reference and persecution, persisting for variable periods of time, regardless of whether the patient is abstinent. The toxic effect of alcohol on frontal lobe functions is well known, suggesting that alcohol hallucinosis may share mechanisms with the positive symptoms of schizophrenia.

Therapeutic Aspects

Antipsychotic Drugs

Antipsychotic drugs are the first-line treatment for patients suffering from hallucinations occurring in the course of psychiatric conditions, especially schizophrenia. In line with the dopamine theories of schizophrenia mentioned above, antipsychotics inhibit the actions of dopamine by acting on D₂ and D₃ dopaminergic receptors. Although it has been assumed that antipsychotics are antagonists at the D₂/D₃ receptors in the brain, *in vitro* assays suggest that they are in fact inverse agonists at these receptors. Recently, a D₂/D₃ partial agonist drug (aripiprazole) has also shown antipsychotic properties. Action at serotonin receptors (5HT_{1A}, 5HT_{2A}) might also be important for the action of some second-generation (atypical) antipsychotics. However, the exact characteristics that make these new antipsychotics atypical are a matter of debate, and there may be significant differences between the drugs within this class. The atypical antipsychotic effect could, at the molecular level, be due to a fast dissociation of the drug from the D₂ receptor, rather than to a high 5-HT₂ occupancy. All these drugs treat primarily the positive symptoms of schizophrenia, including the hallucinations. A meta-analysis has shown that some (but not all) of the second-generation antipsychotic drugs are most efficacious than first-generation drugs for treatment of positive (and negative) symptoms. However, the effect on hallucinations, among other positive symptoms, was not specifically assessed. Due to the side effects associated with the use of conventional (first- or second-generation) antipsychotics, there is a need for alternative pharmacological treatment.

Preliminary studies suggest that metabotropic glutamate receptor agonists could represent a promising new class of antipsychotics.

Repetitive Transcranial Magnetic Stimulation

Recently, repetitive transcranial magnetic stimulation (rTMS) has emerged as a possible alternative treatment of hallucinations in schizophrenic patients resistant to antipsychotic drugs. Several studies and recent meta-analysis of controlled studies suggest that low-frequency rTMS delivered to the left temporo-parietal cortex induce a significant, although modest-to-moderate, reduction of overall positive symptoms in patients receiving active treatment. However, the effect size is higher and more robust when only auditory hallucinations are taken into account, thus confirming that the temporal association cortex plays a crucial role in the pathophysiology of auditory hallucinations.

See also: Hallucinogens; Parkinson's Disease; Schizophrenia; Sleeping, Waking, and Dreaming; Vision.

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Hallucinogens

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Glossary

Classical hallucinogen – A drug that is capable of producing a complex syndrome of effects typically involving alterations in perception, mood, and cognition. It specifically refers to substances with pharmacology and behavioral effects similar to lysergic acid diethylamide (LSD), mescaline, and psilocybin.

M100907 – A serotonin antagonist that binds selectively to 5-HT_{2A} receptors, with approximately 70-fold lower affinity for the rat 5-HT_{2C} receptor. Other names for this compound, known chemically as (+)- α -(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol, include MDL 100,907 and volinanserin.

SER-082 – An indolonaphthyridine derivative developed by Sandoz that acts as a mixed 5-HT_{2C/2B} receptor antagonist.

Serotonin – This substance, also known as 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter found in the central nervous system of vertebrate animals.

Stereoselectivity – In reference to a chiral substance, the phenomenon that one enantiomer is more pharmacologically active than the other stereoisomer. The more potent enantiomer is referred to as the eutomer, whereas the less potent enantiomer is the distomer.

Tolerance – Reduction or loss of the response to a drug after repeated exposure.

WAY-100635 – *N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinyl-cyclohexanecarboxamide, a selective 5-HT_{1A} receptor antagonist.

Humans have used hallucinogenic drugs for thousands of years, typically as crude botanical preparations. Modern scientific interest in these drugs was stimulated by the identification of mescaline as the active agent in the Peyote cactus by Arthur Heffter, in 1897, and the discovery of the hallucinogenic properties of (+)-lysergic acid diethylamide (LSD) by Albert Hofmann, in 1943. Hallucinogens produce alterations of cognition, perception, and affect at nontoxic dosages. Most commonly, the effects of these agents involve marked perceptual disturbances, changes in thought and mood, depersonalization,

visual hallucinations, and somatic symptoms. It is important to note that several different pharmacological classes of agents produce effects in humans that are hallucinogen-like. These classes include cannabinoids such as tetrahydrocannabinol (THC) and the glutamate *N*-methyl-D-aspartate (NMDA) antagonists phencyclidine (PCP) and ketamine, in addition to the classical phenylalkylamines, tryptamines, and indolealkylamines acting on serotonergic systems. There is clearly some overlap between the subjective effects produced by the former two classes and those produced by the prototypical hallucinogens LSD, mescaline, and psilocybin. Nonetheless, human and animal subjects readily distinguish the subjective effects of THC, PCP, and ketamine from those of the hallucinogens, whereas it is reported consistently that LSD, mescaline, and psilocybin produce similar effects. To differentiate hallucinogens from other classes of drugs that do not fit within this classification, the term ‘classical hallucinogens’ has been proposed to describe substances whose psychopharmacology is similar to that of LSD, mescaline, and psilocybin. For the purposes of this article, only those substances that meet the above definition will be referred to as hallucinogens. One indication that the classical hallucinogens represent a discrete drug class is the finding that these agents produce reciprocal cross-tolerance. This effect does not occur between hallucinogens and members of other drug classes, indicating that the classical hallucinogens share a common mechanism of action.

Structure and Pharmacology of Hallucinogens

Classical hallucinogenic drugs belong to two groups of chemicals: indoleamines and phenylalkylamines (Figure 1). The indoleamine class includes two subsets, ergolines such as LSD and tryptamines such as *N,N*-dimethyltryptamine (DMT), 5-methoxy-DMT (5-MeO-DMT), and psilocybin. The phenylalkylamine class of hallucinogens includes phenethylamines such as mescaline, as well as phenylisopropylamines such as 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM), and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). Radioligand-binding studies have demonstrated that the tryptamines are nonselective serotonin (5-HT) receptor ligands that display moderate to high affinity for 5-HT₁ sites (including 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1D} receptors) and 5-HT₂ sites (including 5-HT_{2A} and

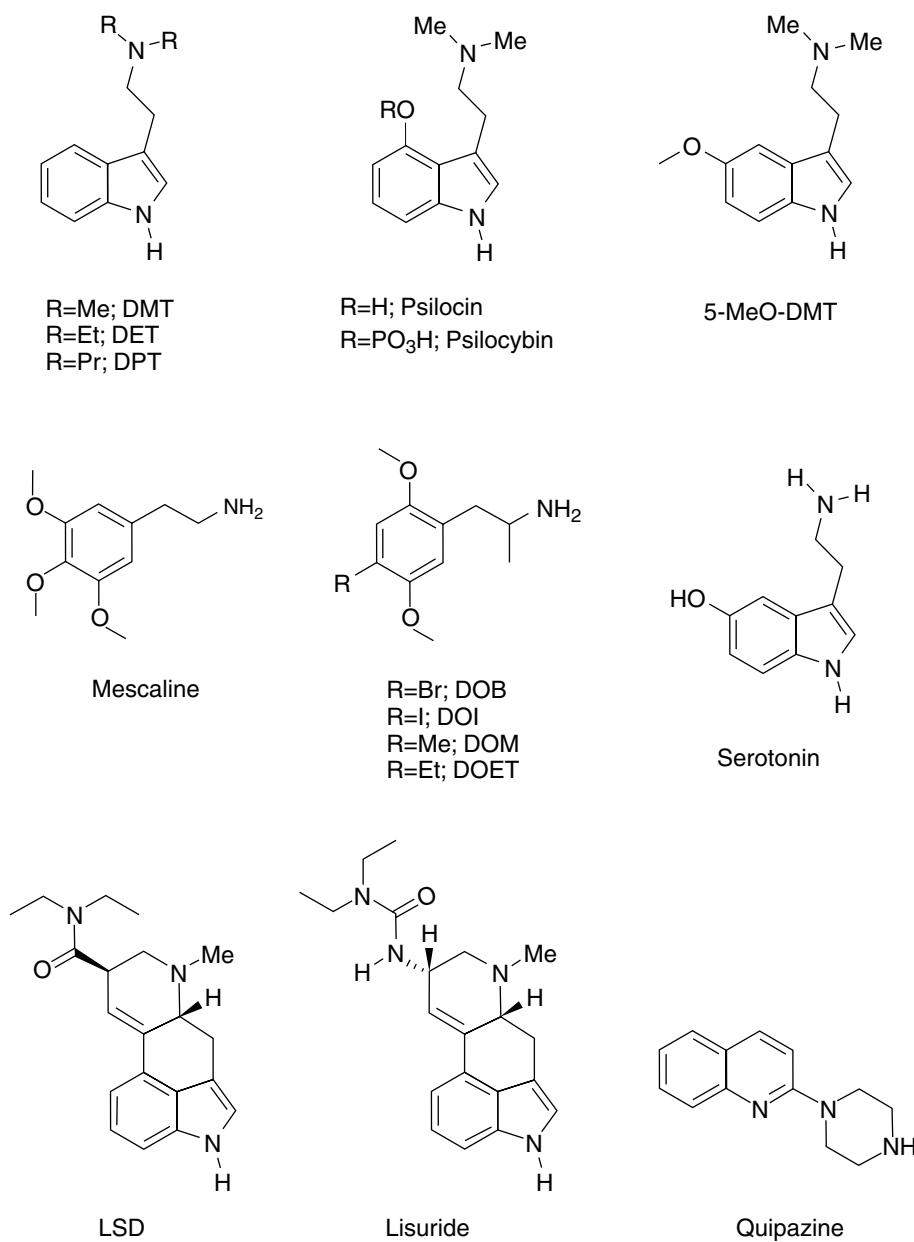


Figure 1 Chemical structures of indoleamine and phenylalkylamine hallucinogens and some related substances.

5-HT_{2C} receptors). LSD binds to a larger population of 5-HT receptors (5-HT_{1A/1B/1D}, 5-HT_{2A/2C}, 5-HT₅, 5-HT₆, and 5-HT₇), but also has high affinity for dopaminergic (DA) and adrenergic receptors. Conversely, phenylalkylamine hallucinogens such as DOM are relatively selective for 5-HT₂ sites, binding to 5-HT_{2A} and 5-HT_{2C} receptors with subnanomolar or nanomolar affinities but interacting with other receptors only at micromolar concentrations. Although the question of whether hallucinogens function as agonists or antagonists at 5-HT₂ receptors was a source of disagreement for many years, it is now widely accepted that these compounds display at least partial agonist efficacy at 5-HT_{2A} and 5-HT_{2C} receptors. Based on the results of behavioral studies in animals, the 5-HT_{2A} receptor is believed to be primarily

responsible for the psychoactive effects of hallucinogens. Importantly, this mechanism has now been confirmed by the finding, in human clinical trials, that the subjective effects of psilocybin are blocked by the administration of the 5-HT_{2A/2C} antagonist ketanserin and the 5-HT_{2A}/DA D₂ antagonist risperidone.

Use of Animal Behavioral Models to Study Hallucinogens

The effects of hallucinogens in humans are highly subjective and are normally assessed using verbal reports. Unfortunately, very few human clinical trials with

hallucinogens have been conducted during the last three decades due to regulatory and ethical considerations. Due to the constraints on human studies, animal behavioral models have become the primary methodology used to study the *in vivo* pharmacology of these drugs. Given the wide variability and the complexity of hallucinogen effects in humans, it has been difficult to define animal behavioral tests for hallucinogenic activity. Nonetheless, numerous animal models have been used over the last four decades to probe the pharmacology of these compounds, yielding a number of important insights.

Types of Animal Models

Animal models used to study the behavioral effects of hallucinogens can be divided into two groups: (1) models that test for behavior(s) that are analogous to hallucinogen effects in humans; and (2) models that test for a behavior that has no human counterpart. One advantage of the first type of model is the existence of a conceptual link to human phenomenology; hence, these models can be used to probe the neural and psychopharmacological mechanisms underlying the human response to hallucinogens. Animal models that belong to the second group are typically robust and have considerable predictive validity. However, with these animal models it is often difficult, if not impossible, to define how the effect(s) being detected relates to the subjective effects of hallucinogens in humans.

Susceptibility to False Positives

One problem associated with animal models used to assess compounds for hallucinogen-like activity is that they cannot always reliably differentiate the behavioral effects of hallucinogens from those of certain closely related compounds that are not hallucinogenic in humans (i.e., hallucinogen false positives). The two most common examples of compounds that generate false-positive results are lisuride and quipazine. Lisuride is an ergot derivative that has been used as a treatment for Parkinson's disease. Lisuride is structurally similar to LSD and is a nonselective agonist at serotonergic, dopaminergic, and adrenergic receptors. Quipazine is an arylpiperazine that acts as a nonselective 5-HT receptor agonist. Neither of these compounds reliably produces LSD-like hallucinogenic effects when tested in human subjects. Nevertheless, many of the behavioral paradigms used to study the effects of hallucinogens identify quipazine and lisuride as being hallucinogen-like. It is not clear why this discrepancy exists. Although the phenomenon of false positives does somewhat limit the predictive validity of these animal models, it is important to consider that the vast majority of agents tested are correctly classified by these paradigms.

Validation of Animal Models

The results obtained with animal models used to study hallucinogen effects should accurately reflect what is known about the pharmacology of these compounds in humans. Valid animal models should reflect the fact that, in humans: (1) tolerance rapidly develops to the behavioral effects of hallucinogens; (2) the action of phenylisopropylamine hallucinogens such as DOM is highly stereoselective; (3) indoleamine and phenylalkylamine hallucinogens produce similar effects; and (4) 5-HT_{2A} receptor antagonists block the subjective effects of hallucinogens.

Animal Models of Hallucinogen Effects

As noted earlier, numerous animal models have been used to study the effects of hallucinogens. The vast majority of these models have, however, fallen into disuse. Some of these models have been invalidated and others were never widely adopted. At present, there are five paradigms commonly used to assess the behavioral effects of hallucinogens. A brief description of each of these behavioral models is provided below. Included in the descriptions are some of the advantages and disadvantages of each model, information about their validation, the receptor mechanisms and neurochemical substrates for the behavioral effect, and the susceptibility of the model to false positives (see [Table 1](#)).

Drug Discrimination

The drug-discrimination paradigm has been widely used to study the pharmacology of hallucinogenic agents and has proven especially useful in structure-activity studies and in mechanistic analyses of these compounds. The paradigm of drug discrimination makes use of the fact that animals can be trained to discriminate the interoceptive stimulus evoked by a training drug from that of the nondrug state. Most commonly, animals are trained to discriminate drug versus vehicle using standard two-lever operant procedures. Once trained, animals can be challenged with novel agents to determine whether these test substances produce stimulus effects similar to those of the training drug (i.e., tests of stimulus generalization). Hirschorn and Winter first demonstrated in 1971 that rats could be trained to discriminate mescaline and LSD from saline. It was subsequently shown that a number of other hallucinogens, including DOM, DOI, DOB, DPT, psilocybin, and 5-MeO-DMT, are capable of serving as training drugs in the drug-discrimination paradigm. Importantly, cross-generalization occurs between these training drugs, suggesting that they evoke similar stimulus cues. The majority of drug-discrimination studies with

Table 1 Summary of behavioral models used to test for hallucinogen effects

	<i>DD</i>	<i>BPM</i>	<i>HTR</i>	<i>PPI</i>	<i>ESR</i>
Sensitivity to hallucinogen classes					
Indoleamines	Yes	Yes	Yes	Yes	No
Phenylalkylamines	Yes	Yes	Yes	Yes	Yes
Behavior is specific to hallucinogens	Yes	Yes	No	No	Yes
Lisuride false-positive	Yes	No	No	?	No
Quipazine false-positive	Yes	Yes	Yes	No	No
Human phenomenology	No	Yes	No	Yes	No
Hallucinogens act via 5-HT _{2A}	Yes	Yes ^a	Yes	Yes ^a	Yes
Sensitive to hallucinogen tolerance	Yes	Yes	Yes	?	Yes
Stereospecificity	Yes	?	Yes	?	Yes
Automated	Yes	Yes	No	Yes	No

^a = rare exceptions exist.

? = not yet tested.

Abbreviations: BPM, Behavioral Pattern Monitor; DD, drug discrimination; ESR, mouse ear-scratch response; HTR, head twitch response; PPI, prepulse inhibition of startle.

hallucinogens have used rats, although mice, pigeons, and monkeys have also been studied. The ability of agents to evoke hallucinogen-like responding in the drug-discrimination assay displays pronounced pharmacological specificity in that members of other drug classes (e.g., opioids, depressants, psychostimulants, glutamate NMDA antagonists, cannabinoids, and cholinergic agonists and antagonists) consistently fail to produce substitution in animals trained with classical hallucinogens. The effect of phenylisopropylamine hallucinogens in the drug-discrimination paradigm is stereoselective, with the *R*-(-)-enantiomer being the more potent stereo isomer. Although the drug discrimination paradigm is not an animal model of hallucinogenic activity *per se*, effective dose (ED₅₀) values for stimulus generalization of phenalkylamine and indoleamine hallucinogens are significantly correlated with the potencies of these compounds in humans.

The discriminative stimulus induced by hallucinogens is attenuated to varying degrees by nonselective 5-HT receptor antagonists such as methysergide, metergoline, mianserin, methiothepin, pizotifen (pizotyline or BC-105), and cyproheptadine. The observation that the selective 5-HT_{2A/2C} antagonists pirenperone and ketanserin are potent and highly effective antagonists of the discriminative stimulus properties of DOM and LSD – and of DOM-stimulus generalization to LSD, mescaline, and 5-MeO-DMT – led to the proposal that these drugs may be acting through 5-HT₂ receptors. Subsequent studies have provided evidence that the discriminative stimulus evoked by hallucinogens is mediated by agonist actions at the 5-HT_{2A} receptor subtype. A robust and significant linear correlation exists between the 5-HT_{2A} affinities of various phenylalkylamine hallucinogens and their ED₅₀ values obtained from stimulus-generalization studies using DOM as the training drug. Antagonist correlation analysis has demonstrated that the 5-HT_{2A} binding affinities of a number of structurally diverse 5-HT antagonists is strongly correlated with their potencies for blocking substitution of *R*-(-)-DOM in animals

trained with LSD. Furthermore, the highly selective 5-HT_{2A} antagonist M100907 can block stimulus control evoked by DOI and LSD. Although there has been some controversy over whether interactions with 5-HT_{2C} receptors contribute to the discriminative stimulus effects of hallucinogens, studies in rats demonstrate that the mixed 5-HT_{2C/2B} antagonists SB 200,646A and SB 206,553, and the selective 5-HT_{2C} antagonist SB 242,084, consistently fail to block hallucinogen-induced stimulus control. There is evidence that tolerance can develop to the hallucinogen cue; daily administration of 1.0 mg kg⁻¹ DOI for 8 days to rats trained to discriminate a lower dose of the drug produced a significant decrease in drug-appropriate responding. The decrease in DOI-lever responding was accompanied by a decrease in the number of 5-HT_{2A} receptors but not 5-HT_{2C} receptors. Taken together, these findings indicate that it is unlikely that 5-HT_{2C} receptor activation is essential for the induction of stimulus control by hallucinogens (at least in rats).

Although a preponderance of evidence demonstrates that interactions with 5-HT_{2A} receptors mediate hallucinogen-induced stimulus control, this evidence does not preclude the possibility that interactions with other receptors may contribute to or modify the discriminative stimulus effects of these drugs. Indeed, indoleamine hallucinogens are far less selective for 5-HT_{2A} receptors than the phenylalkylamines and it appears that the former class of agents have more complex stimulus properties than the phenylalkylamines. It is well established that the 5-HT₂ antagonist cinanserin antagonizes the effects of DOM and mescaline much more effectively than it suppresses LSD- and 5-MeO-DMT-appropriate responding. There is evidence that the 5-HT_{1A} subtype contributes to the discriminative effects of LSD. For example, the 5-HT_{1A} agonist ipsapirone produces partial drug-lever responding in LSD-trained animals, and the mixed 5-HT_{1A} agonist/α₂-adrenoceptor antagonist yohimbine has been reported, by several groups of investigators, to

produce LSD-like effects ranging from partial to full substitution. LSD also elicits partial substitution in rats trained with the 5-HT_{1A} agonist 8-OH-DPAT. LSD has high affinity for DA D₁, D₂, D₃, and D₄ receptors. Based on the finding that the 5-HT_{2A}/D₂ antagonist risperidone is much more effective than ritanserin as an antagonist of the LSD cue, it has been proposed that DA mechanisms may also play a role in LSD-induced stimulus control. There have also been reports that the discriminative stimulus effects of LSD occur in two temporal phases, with the first phase mediated by 5-HT_{2A} receptors and the second phase involving interactions with DA D₄ receptors. LSD evokes a compound stimulus; although the most salient component of the LSD stimulus is transduced through the 5-HT_{2A} receptor, ancillary interactions with 5-HT_{1A} and dopaminergic receptors are responsible for secondary nonessential components of the LSD stimulus complex. As with any drug possessing complex stimulus properties, the nature of the LSD stimulus will vary depending upon the training and testing conditions. In addition to LSD, other indoleamines produce stimulus effects that involve 5-HT_{1A}- and 5-HT_{2A}-mediated components. Indeed, the 5-MeO-DMT stimulus cue generalizes not only to DOM, but also to 8-OH-DPAT and ipsapirone. The 5-MeO-DMT discriminative stimulus seems to involve a substantial 5-HT_{1A} receptor-mediated component, as demonstrated by the finding that the 5-HT_{1A} receptor-selective antagonist WAY-100635 and the mixed 5-HT₁/β-adrenergic antagonist pindolol are more effective than pirenperone at blocking stimulus control by 5-MeO-DMT.

It has occasionally been reported that animals trained to discriminate a hallucinogen will generalize to a drug that is not hallucinogenic in humans (i.e., so-called 'false positives'). For example, some (but not all) investigations have found that lisuride can fully substitute for LSD and DOM. Nonetheless, rats can be trained to discriminate between LSD and lisuride using a three-lever drug-discrimination procedure. Thus, although LSD and lisuride have similar stimulus effects, the stimulus cues evoked by these two agents are apparently distinguishable. Quipazine has consistently been found to substitute for LSD and DOM. Substitution of quipazine in rodents trained with hallucinogens is blocked by ketanserin and pirenperone, suggesting it is mediated by 5-HT_{2A} receptors.

The discriminative stimulus effects of hallucinogens are centrally mediated. Systemic administration of xylymidine, a peripheral 5-HT₂ antagonist that does not cross the blood-brain barrier, has no effect on stimulus control induced by mescaline, DOM, or by moderate to high doses of LSD. Central administration of *R*(-)DOM and LSD via the intracerebroventricular route substitutes for the discriminative stimuli produced by systemic injection of those agents. The central neuroanatomical site(s) responsible for mediating the hallucinogen cue have not

been conclusively identified, although a few candidates have been proposed. Intracerebral microinjection studies have suggested that the nucleus accumbens and dorsal raphe nucleus may play a role in the effects of LSD, although very high doses of LSD had to be injected into the latter region in order to produce substitution. A more recent investigation has demonstrated that infusion of LSD directly into the anterior cingulate cortex (ACC) of rats produces full substitution in rats trained to discriminate LSD administered systemically. Importantly, administration of M100907 directly into ACC antagonized the stimulus effects of LSD administered either systemically or via intra-ACC infusion. These findings strongly indicate that the ACC may play a role in stimulus control induced by LSD.

Investigatory and Exploratory Behavior

Locomotor paradigms have frequently been used to assess the behavioral effects of psychoactive drugs. Typically, the amount of unconditioned locomotor activity in rodents is quantified to determine whether a compound acts as a stimulant or a depressant. However, these measures yield only a partial description of the behavioral state (i.e., they reveal nothing about qualitative behavioral changes) and do not address whether alterations in the response to sensory stimuli contribute to the behavioral effect. To address these shortcomings, the rat Behavioral Pattern Monitor (BPM) was developed. The BPM combines features of activity and holeboard chambers and was specifically designed to assess the quantity as well as the quality or patterns of activity by monitoring the temporal and spatial sequence of behavioral responses. In addition to measuring exploratory and investigatory behaviors (rearings and holepokes), the BPM can be used to assess the structure of motor activity. The BPM also enables assessment of the responsiveness of animals to environmental stimuli, including their sensitivity to novelty.

Classical hallucinogens produce a characteristic behavioral profile in the BPM. When tested in a novel environment, phenylalkylamine (mescaline, DOM, DOI, and DOET) and indoleamine (psilocin, DMT, and 5-MeO-DMT) hallucinogens produce the following behavioral effects: (1) reductions in locomotor activity; (2) decreases in investigatory behaviors (rearings and holepokes); and (3) decreased entries into and time spent in the center of the chamber. The effects of hallucinogens in the BPM are significantly reduced when animals are tested in a familiar environment. Based on these findings, it has been suggested that hallucinogens potentiate the neophobia normally exhibited by rats in response to a novel environment. The diminution of the behavioral effects of hallucinogens in a familiar test environment has been interpreted as representing an increased willingness of hallucinogen-treated animals to explore the

test chamber once the stimuli associated with the chamber become less threatening due to habituation. LSD produces a more complex behavioral profile in the BPM. As with other hallucinogens, LSD decreases investigatory behavior and increases avoidance of the center of the chamber, but it has biphasic effects on locomotor behavior, producing an initial reduction in activity followed by an increase as time progresses. The acute effects of LSD and DOI in the BPM are diminished after repeated treatment with the drugs, indicating that hallucinogen-induced tolerance is detectable in this paradigm.

The effects of DOI and DOM in the BPM paradigm are blocked by the nonselective 5-HT antagonist cyproheptadine and by the selective 5-HT₂ antagonists ketanserin and ritanserin. The effects of phenylalkylamine hallucinogens are also blocked by pretreatment with the selective 5-HT_{2A} antagonist M100907 but not with the 5-HT_{2C/2B}-selective antagonist SER-082, and are, therefore, likely solely mediated by 5-HT_{2A} receptors. The mechanistic basis for the effects of indoleamine hallucinogens in the BPM is more complex. For example, the effects of low doses of 5-MeO-DMT are antagonized by the selective 5-HT_{1A} antagonist WAY-100635 but not by M100907 or SER-082. For LSD, experiments have demonstrated that the initial suppression of locomotor activity induced by LSD is blocked by the mixed 5-HT₁/β-adrenergic antagonist propranolol but not by the 5-HT₂ antagonist ritanserin, whereas the delayed increase in locomotor activity is blocked by ritanserin but not by propranolol. More recent studies have demonstrated that the LSD-induced decrease in locomotion is attenuated by WAY-100635, whereas LSD-induced hyperactivity is blocked by M100907. The fact that both 5-HT_{1A} and 5-HT_{2A} receptors appear to contribute to the behavioral effects of LSD in the BPM is supported by the finding that chronic pretreatment with either 8-OH-DPAT or DOI produces cross-tolerance with LSD in this behavioral paradigm.

When tested in a novel environment, selective 5HT_{1A} agonists such as 8-OH-DPAT and ipsapirone produce dose-dependent decreases in investigatory and exploratory behavior, and increase avoidance of the center region. Thus, with regard to these behavioral measures, 5-HT_{1A} agonists induce hallucinogen-like effects. However, whereas the effects of hallucinogens in the BPM depend on the novelty of the testing environment, the effects of 5-HT_{1A} agonists persist in a familiar environment. Based on that finding, it has been concluded that the behaviorally suppressive effects of 5-HT_{1A} are reflective of a generalized sedative effect rather than an alteration in the animal's response to environmental stimuli.

The effects of hallucinogens on investigatory and exploratory behavior in the BPM are distinct from those produced by members of other drug classes (see **Table 2**). Furthermore, it is possible to distinguish the behavioral

Table 2 Drugs that do not reproduce the hallucinogen behavioral profile in the BPM

Drug class	Examples
5-HT _{1A} agonist	8-OH-DPAT Buspirone Gepirone Ipsapirone
5-HT _{1B} agonist	RU 24969
5-HT _{1B} /5-HT _{2C} agonist	mCPP TFMPP
5-HT releaser	Fenfluramine MDA MDMA MDEA MBDB α-Ethyltryptamine p-Chloroamphetamine
SERT inhibitor	Fluoxetine
D ₁ /D ₂ agonist	Apomorphine
DAT inhibitor	Cocaine GBR 12909 Nomifensine
DA/NE releaser	(+)-Amphetamine
α ₂ Agonist	Clonidine
β ₂ Agonist	Clenbuterol
β Antagonist	Nadolol
NET inhibitor	Chlorimipramine
Opioid agonist	Morphine
Adenosine agonist	Caffeine
NMDA antagonist	Phencyclidine MK-801
Muscarinic AChR antagonist	Scopolamine
Nicotinic AChR agonist	Nicotine
MAO inhibitor	Harmaline Clorgyline

Abbreviations: AChR, acetylcholine receptor; DA, dopamine; DAT, dopamine transporter; MAO, monoamine oxidase; NE, norepinephrine; NET, norepinephrine transporter; NMDA, N-methyl-D-aspartate; SERT, serotonin transporter.

effects of lisuride from those induced by LSD and other hallucinogens in the BPM. Low doses of lisuride produce a sedative effect with no evidence of exaggerated avoidance of the center of the chamber. Higher doses of lisuride induce stereotyped patterns of locomotor hyperactivity resembling those produced by the dopamine receptor agonist apomorphine. Conversely, quipazine produces hallucinogen-like effects in the BPM that are blocked by pretreatment with ketanserin and ritanserin.

In summary, the effects of hallucinogens on exploratory and investigatory behavior in the BPM are characteristic and can be used to reliably distinguish these drugs from members of other drug classes. Furthermore, because the BPM includes measures that assess the responsiveness of animals to environmental stimuli, it is possible to relate the effect of hallucinogens in the BPM to the subjective effects of these drugs in humans.

Head-Twitch Response

Corne and colleagues first reported, in 1963, that administration of the 5-HT precursor 5-hydroxytryptophan (5-HTP) to mice evokes the head-twitch response (HTR) – a paroxysmal rotational movement of the head resembling the pinna reflex. It was subsequently shown that hallucinogens such as LSD, DOM, DOI, psilocin, psilocybin, 5-MeO-DMT, and mescaline also induce head twitches in mice. Administration of 5-HTP or hallucinogens to rats induces an analogous behavior that is sometimes referred to as the ‘wet-dog shake’ because it frequently involves the head, neck, and trunk. The HTR induced by phenylisopropylamine hallucinogens is stereoselective, and there is substantial diminution of the response after repeated administration of DOM. The HTR has been widely adopted as a behavioral assay for detecting hallucinogen-like effects because the response is reliably induced by classical hallucinogenic drugs and does not require specialized equipment or training to assess.

The HTR to hallucinogens and to 5-HT precursor loading with 5-HTP can be blocked by administration of nonselective 5-HT receptor agonists such as cyproheptadine, metergoline, mianserin, pizotyline, and methysergide and is thus believed to reflect activation of 5-HT receptors. It was proposed in 1981 that the HTR induced by 5-HTP is mediated by 5-HT_{2A} receptors, based on evidence that the potency of 5-HT antagonists to block the behavior was significantly correlated with their 5-HT_{2A} affinity. Similar findings were later reported for the HTR induced by mescaline and DOI. Additional evidence for a linkage between 5-HT_{2A} receptors and the HTR emerged from studies demonstrating that the effects of 5-HTP and mescaline are blocked by pirenperone and ketanserin. Although those two antagonist ligands bind to both 5-HT_{2A} and 5-HT_{2C} receptors, a specific link between the 5-HT_{2A} receptor and head twitches in rodents is supported by the finding that the HTR to DOI and other hallucinogens is inhibited by the selective 5-HT_{2A} antagonist M100907 but not by the selective 5-HT_{2C} antagonist SB 242,084 or by the mixed 5-HT_{2C/2B} antagonists SB 200,646A and SER-082. The apparent involvement of 5-HT_{2A} receptors in the hallucinogen-induced HTR was confirmed by the recent finding that the ability of LSD and DOI to induce the HTR is abolished in 5-HT_{2A}^{-/-} knockout mice.

Quipazine induces the HTR, an effect that is blocked by ketanserin and pirenperone. Conversely, lisuride fails to provoke the HTR in mice and rats, and can actually antagonize LSD- and 5-HTP-induced head twitching. Although head twitching in rodents is used as a behavioral assay for hallucinogenic drugs, members of other drug classes are also capable of eliciting the HTR. In addition to 5-HTP, phencyclidine, certain benzodiazepines, the cannabinoid CB₁ antagonist SR 141716A, and the 5-HT_{1A} antagonists WAY-100635 and S-(–)-UH 301 have

also been shown to induce the HTR. Importantly, the HTR induced by these compounds is blocked by 5-HT₂ receptor antagonists, indicating that they may also induce the behavior via activation of 5-HT_{2A} receptors. A number of other agents, including [Met]enkephalin, the cholinergic agonist carbachol, and thyrotropin-releasing hormone, are also reported to produce head twitches; however, it is not clear whether the effects of these agents are blocked by 5-HT_{2A} antagonists. Nonetheless, the available evidence indicates that although the HTR cannot be used reliably to assess drugs for potential hallucinogenic activity, it does show utility as an assay for 5-HT_{2A} agonist activity. It is also important to note that the HTR induced by 5-HT_{2A} receptor activation can be modified by drugs acting at a variety of other receptor sites, including 5-HT_{1A}, DA D₁ and D₂, cannabinoid CB₁, μ -opioid, nicotinic cholinergic, α_2 -adrenergic, and ionotropic and metabotropic glutamate receptors. Hence, the HTR has been utilized as a tool to probe the neuronal and circuit interactions between 5-HT_{2A} receptors and other transmitter systems.

Pretreatment with xylamidine has no effect on the HTR induced by 5-HTP. Although this finding indicates that the HTR induced by systemic administration of 5-HT agonists is centrally mediated, there is disagreement in the literature with regard to the neuroanatomical locus that is responsible for mediating this behavioral effect. Bilateral infusion of DOI into the medial prefrontal cortex of rats has been reported to induce the HTR, an effect that was blocked by the systemic administration of ketanserin or M100907 but not SER-082. The conclusion that prefrontal cortical 5-HT_{2A} receptors are involved in the hallucinogen-induced HTR is supported by the finding that selective restoration of 5-HT_{2A} receptors in the cortex of 5-HT_{2A}^{-/-} knockout mice rescues the HTR to LSD. However, other evidence indicates that the frontal cortex is not required for the HTR induced by the systemic administration of 5-HT agonists. For example, removal of the frontal cortex in rats does not alter the HTR induced by quipazine or 5-HTP. Likewise, cutting the rat brain transversely at the level of the anterior commissure did not block the ability of 5-HTP to induce the HTR, whereas transection at the level of the posterior commissure abolished the response to 5-HTP. The latter finding suggests that structures in the caudal diencephalon and medial brainstem mediate the HTR to 5-HT agonists. It has also been reported that infusion of DOI into the region of the brainstem containing the raphe obscurus and inferior olive induces the HTR in rats. Taken together, the available evidence indicates that activation of 5-HT_{2A} receptors in multiple brain regions can elicit the HTR, although it is not clear which brain region(s) are responsible for the HTR induced by systemic administration of 5-HT agonists.

Prepulse Inhibition of Startle

Prepulse inhibition (PPI) of the startle reflex is defined as the response decrement that occurs when a startling acoustic stimulus is preceded immediately by a subthreshold stimulus. PPI serves as an operational measure of sensorimotor gating and has been found to be deficient in patients with schizophrenia. One advantage of this behavioral paradigm is that PPI is a cross-species phenomenon that can be assessed in humans and animals using similar testing procedures. Sipes and Geyer first reported, in 1994, that the hallucinogen DOI disrupts PPI in rats. Additional studies confirmed that other hallucinogens, including LSD, DOB, and 5-MeO-DMT, are capable of disrupting PPI. Ketanserin and risperidone reverse the effect of DOI on PPI. The ability of DOI and LSD to decrease PPI in rats is blocked by the selective 5-HT_{2A} antagonist M100907 but not by the 5-HT_{2C/2B} antagonist SER-082, indicating that the effect is mediated by 5-HT_{2A} receptors. The conclusion that these hallucinogens are not acting through the 5-HT_{2C} receptor to alter PPI is supported by the finding that the preferential 5-HT_{2C} agonist mCPP has no effect on PPI. Compared with DOI and LSD, the mechanism for the effect of 5-MeO-DMT on PPI is more complicated and apparently involves interactions with both 5-HT_{1A} and 5-HT_{2C} receptors.

With regard to the sensitivity of the PPI paradigm to drugs that often generate hallucinogen false-positive results, quipazine has no effect on PPI, whereas there are no published studies of the effect of lisuride on PPI. However, many other nonhallucinogenic agents disrupt PPI in rodents, limiting the predictive validity of the paradigm. Administration of DA agonists such as (+)-amphetamine and apomorphine decreases PPI. Nonhallucinogenic 5-HT-releasing agents such as 3,4-methylenedioxymethamphetamine (MDMA) and fenfluramine also decrease PPI via direct actions at 5-HT_{2A} receptors. Thus, PPI cannot be used to distinguish hallucinogenic from nonhallucinogenic drugs. Nonetheless, PPI continues to serve as a valuable animal paradigm to assess the behavioral effects of hallucinogens because it serves as a model of the effects of these drugs on sensorimotor gating in humans. Indeed, depending on the experimental parameters used, psilocybin has been shown to impair PPI when administered to human volunteers. However, it is not clear whether other hallucinogens such as LSD have similar effects on PPI in humans.

Mouse Ear-Scratch Response

Deegan and Cook reported, in 1958, that administration of mescaline to mice induces stereotypic scratching of the head and ears – an effect that was not observed in other species of animals. It was subsequently confirmed that DOM, DOI, and DOET could also induce the ear-scratch response (ESR). The ESR induced by these agents is stereoselective, with the R-(-)-enantiomers being

significantly more potent than the S-(+)-enantiomers. The ability of mescaline and DOI to induce the ESR is significantly reduced after repeated treatment. DOI-induced ESR is apparently mediated by 5-HT_{2A} receptors, since it is blocked by ketanserin and by spiperone, an antagonist of 5-HT_{1A/2A} and D₂ receptors. It should be noted that the ESR is not predictive of hallucinogenic activity since the behavior is not induced by indoleamine hallucinogens. In fact, LSD and 5-MeO-DMT actually block the ESR induced by mescaline and DOI, respectively. Based on the finding that the DOI-induced ESR is also inhibited by the 5-HT_{1B} agonist RU 24969, it appears that blockade of the ESR by the indoleamine hallucinogens may involve interactions with 5-HT_{1B} receptors. Neither quipazine nor lisuride induces the ESR – a finding that is consistent with the 5-HT_{1B} agonist activity of these compounds. Thus, the ESR has utility to test phenylalkylamines for 5-HT_{2A} agonist activity but cannot be used with indoleamines or other compounds that are nonselective 5-HT receptor agonists.

Conclusion

Animal behavioral models are the predominant methodology used to study the *in vivo* pharmacology of the classical hallucinogens. Although many animal models for hallucinogen effects have been developed, only a few of these paradigms are widely used. One commonality shared by the models described in this article is that they all demonstrate that the behavioral effects of hallucinogens involve interactions with the 5-HT_{2A} receptor. Therefore, although each of these models has certain disadvantages, they have all helped to elucidate the pharmacological interactions that are responsible for the behavioral effects of classical hallucinogens.

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See also: Drug Addiction; From Sensation to Perception; Habituation; Hallucinations in Neuropsychiatry and Drug Abuse: From Phenomenology to Pathophysiology; Knock-Outs: Learning and Memory; Mouse Genetic Approaches to Psychiatric Disorders; Neurotransmitters and Neuromodulators Regulating Sleep and Wakefulness; Novelty; Psychostimulants; Schizophrenia; Subjective Experience and the Expression of Emotion in Man; Δ9-THC.

Further Reading

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γ -Hydroxybutyric Acid (GHB)

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Glossary

Baclofen – Prototypic, direct agonist of the GABA_B receptor. Because of its myorelaxant effects, baclofen has been successfully used in the treatment of muscle rigidity from a variety of causes, including multiple sclerosis. More recently, baclofen has been proposed as an anti-addictive therapy. Baclofen and GHB share several psychopharmacological effects.

Conditioned place preference – Behavioral technique widely used to investigate the rewarding properties of psychoactive drugs. Laboratory animals are initially trained to associate the interoceptive cues produced by a drug with the external (neutral) stimuli of a specific environment and the absence of those effects with the stimuli of a different environment. After a number of conditioning sessions, animals are given a choice between the two environments: if the animal increases the time spent in the drug-paired context, it is inferred that the drug possesses rewarding properties.

Drug discrimination – In this behavioral technique, laboratory animals are trained to associate the interoceptive cues of a certain drug (training drug) with a measurable behavior, providing a sort of self-report whether they feel drugged or not. Studies of drug discrimination may provide information on the cellular sites of action of psychotropic drugs, as (1) drugs acting similarly at a given receptor have similar discriminative stimulus effects and (2) receptor antagonists block the discriminative stimulus effects of drugs acting at that specific receptor.

GABA (γ -aminobutyric acid) – Major inhibitory neurotransmitter in the central nervous system. It activates two classes of receptors: ionotropic GABA_A receptors and metabotropic GABA_B receptors. GABA is a precursor of GHB and, at least for a small portion, a product of GHB metabolism.

GABA_A-benzodiazepine receptor complex – Ionotropic receptor whose activation results in an increase of the chloride influx through the associate ion channel and, ultimately, fast inhibition of the excitability of the postsynaptic neuron. The activity of this receptor is potentiated by different positive modulators, including benzodiazepines, barbiturates, and endogenous

neurosteroids. There is evidence that the GABA_A receptor may partially contribute to the mediation of some effects of GHB.

GABA_B receptor – Heterodimer receptor comprised of two 7-transmembrane-spanning units. Its activation decreases adenylyl cyclase activity, increases potassium conductance, and decreases calcium conductance, with the ultimate result of a slow inhibitory action on excitability of the postsynaptic neuron. GHB is a weak agonist at the GABA_B receptor (at pharmacological concentrations).

GHB (γ -hydroxybutyric acid) – A natural constituent of the mammalian brain, likely acting as neurotransmitter or neuromodulator. When exogenously administered, GHB exerts a number of psychopharmacological effects, ranging from euphoria to anesthesia. GHB displays some therapeutic potential (narcolepsy and alcoholism) and abuse liability.

GHB-specific binding site – High- and low-affinity GHB-specific binding sites have been described in neuronal preparations. The high-affinity binding site is displaced only by GHB (at physiological concentrations) and GHB structural analogs, such as NCS-382.

Mesocorticolimbic dopamine system – The mesocorticolimbic dopamine system – composed of neurons originating in the ventral tegmental area and projecting in the nucleus accumbens, amygdala, and frontal and limbic cortex – constitutes the likely neural pathway mediating the reinforcing effects of psychostimulants and the motivational properties of many drugs of abuse (apparently including also GHB). Indeed, virtually all drugs of abuse activate this system, increasing the output of dopamine in the terminal areas.

Narcolepsy – Rare sleep disorder (occurring in <0.15% of the population), it affects the generation and organization of sleep and wakefulness. Narcolepsy is characterized by excessive daytime sleepiness and presence of irresistible daily attacks of refreshing sleep. Other symptoms include cataplexy (a temporary loss of muscle tone while awake), disrupted nighttime sleep, hypnopompic or hypnagogic hallucinations (visual or auditory dream-like hallucinations at the beginning or end of sleep episodes), and sleep paralysis, each

representing a component of rapid eye movement (REM) sleep that abnormally occurs during period of wakefulness.

NCS-382 – Structurally related to GHB, NCS-382 has been initially proposed as an antagonist of both high- and low-affinity GHB-specific binding sites.

Pharmacological experiments however provided contradictory results, as NCS-382 failed – in most instances – to antagonize different effects of GHB, including discriminative stimulus effects, motor-incoordination, and sedation/hypnosis. Conversely, NCS-382 has been often reported to elicit effects qualitatively similar to those of GHB or to potentiate some effects of GHB.

Succinic semialdehyde dehydrogenase deficiency – Also known as GHB aciduria, rare inborn error of GHB and GABA metabolism, resulting in GHB and GABA accumulation in brain and physiologic fluids. This disease is characterized by mental retardation, epilepsy, hypotonia, and electroencephalogram abnormalities.

γ -Hydroxybutyric acid (GHB) is a short-chain fatty acid that occurs naturally in the mammalian brain. Its primary endogenous precursor is the inhibitory neurotransmitter γ -aminobutyric acid (GABA): GABA is converted to succinic semialdehyde by mitochondrial GABA transaminase; succinic semialdehyde is then reduced to GHB by cytosolic succinic semialdehyde reductase. At the same time, GHB is also a precursor of GABA: GHB is indeed initially converted to succinic semialdehyde by GHB dehydrogenase and then succinic semialdehyde is converted to GABA by GABA transaminase. Succinic semialdehyde can also be converted to succinate by succinic semialdehyde dehydrogenase. Congenital defect in succinic semialdehyde dehydrogenase causes GHB and GABA accumulation in brain, liver, blood, and urine, and results in a rare disease – named succinic semialdehyde dehydrogenase deficiency or GHB aciduria – characterized by delayed mental, motor, and language development, epilepsy, hypotonia, and electroencephalogram abnormalities.

Before its discovery as a naturally occurring constituent of the mammalian brain, GHB was synthesized, in the early 1960s, by the French physician and scientist Henri Laborit, while attempting to produce an orally bioavailable GABA analog or precursor capable of easily crossing the blood–brain barrier.

GHB is presently recognized as (1) an endogenous constituent of the mammalian brain, where it likely acts as neurotransmitter or neuromodulator; (2) a medicine, as it exerts a large spectrum of psychopharmacological effects and is currently used for the treatment of narcolepsy and

alcoholism; and (3) a drug of abuse, illicitly used for some of its psychotropic effects. This article is intended to review the body of experimental and clinical evidence sustaining these three features of GHB.

GHB as a Neurotransmitter or a Neuromodulator

Localization of GHB in GABAergic neurons at the synaptic level, existence in brain of GHB-specific receptor sites (high- and low-affinity classes), as well as machinery for GHB synthesis, storage in synaptic terminals, depolarization-evoked and calcium-dependent release, sodium-dependent uptake, and degradation suggest a role for GHB as neurotransmitter or neuromodulator in the central nervous system.

GHB is unevenly distributed in the rat brain, with substantia nigra and hypothalamus showing the highest concentrations (*c.* 45 pmol mg⁻¹ protein), frontal cortex and cerebellum the lowest (4–8 pmol mg⁻¹ protein). High-affinity GHB-specific binding sites have been localized in specific brain structures, including hippocampus, cortex, thalamus, amygdala as well as dopaminergic nuclei (substantia nigra and ventral tegmental area) and regions innervated by dopaminergic terminals (olfactory bulbs and tubercles, nucleus accumbens, and caudate putamen), with comparable density between rat and human preparations. Although still under debate, the GHB-specific binding site has been suggested to be presynaptic and G protein coupled. As described below in greater detail, GHB may bind to the GABA_B receptor as well; however, GHB displays only a weak affinity for this receptor, and the micromolar concentrations of GHB normally present in the mammalian brain tissue are insufficient for its activation (conversely, the GABA_B receptor is more likely activated by the supraphysiological, or millimolar, concentrations of exogenously administered GHB (see below)).

The physiological function of GHB still remains to be clearly defined; data from neurochemical and electrophysiological studies suggest that GHB may (1) alter GABA and glutamate release, (2) increase serotonin and acetylcholine turnover, (3) stimulate the release of various endogenous opioids, (4) increase plasma levels of endogenous neurosteroids, and (5) participate in mechanisms controlling dopamine activity in the nigrostriatal and mesocorticolimbic pathways.

GHB as a Pharmacological Agent

In both laboratory animals and humans, GHB – when exogenously administered – is rapidly absorbed. It crosses the blood–brain barrier with relative ease, penetrates into

the brain, and exerts a number of psychopharmacological effects, including – as the dose is increased, and just to mention a few – euphoria, anxiolysis, amnesia, sedation/hypnosis, and anesthesia. Plasma levels of GHB reach their peak at approximately 40 min after oral ingestion; GHB's half-life is rather short, averaging 20–30 min.

Sites of Actions

GHB-specific binding site

Studies on rat and human brain revealed both high-affinity (K_{d1} 30–500 nM; B_{max} 0.5–1.8 pmol mg⁻¹ protein) and low-affinity (K_{d2} 1–11 μ M; B_{max} 8–46 pmol mg⁻¹ protein) GHB-specific binding-site components. The [³H]GHB-specific binding site is displaced only by GHB and GHB structural analogs, such as the putative GHB antagonist, NCS-382, and not by GABA, baclofen, muscimol, and bicuculline. Although there are contradictory data, it has been hypothesized that the high-affinity GHB-specific binding site is a G-protein-coupled presynaptic receptor whose activation induces a decrease in adenylyl cyclase. Recently, two clones encoding membrane receptors that exhibit pharmacological and functional responses in accordance with a GHB receptor have been isolated from a human frontal cortex cDNA library.

Although most of the pharmacological effects of GHB appear to be secondary to activation of the GABA_B receptor, there is also evidence suggesting that some physiological actions and effects of exogenously administered GHB may be mediated by activation of its own specific binding sites. For example, NCS-382 has been reported to antagonize GHB-induced changes in the functioning of dopaminergic, GABAergic, and glutamatergic neurons in different areas of the rat brain. Data suggesting the capacity of NCS-382 to antagonize GHB-induced sedation, catalepsy, petit mal-like absences, and reinforcement in rodents have also been reported. To date, however, the lack of potent and specific ligands to the GHB-specific binding site has hampered the understanding of the role of this binding site in GHB neurophysiological and pharmacological actions.

GABA_B receptor

Recent experimental evidence suggests that the GABA_B receptor constitutes a second – and likely the major – site of action of GHB. GHB has been found to interact, as a weak agonist, directly with the GABA_B receptor: the reported K_i values of GHB for the inhibition of different GABA_B receptor radioligands are, indeed, approximately 100 μ M. Notably, these concentrations are reached in the brain after exogenous administration of behaviorally active doses of GHB.

Besides the direct action at the GABA_B receptor, GABA_B-ergic activities of GHB may result from alternative mechanisms. First, GHB can influence GABA neurotransmission by serving as a GABA precursor: the conversion of GHB into GABA may form a pool of GABA, which, in turn, binds to GABA_B receptors. Second, moderate-to-high doses of GHB have been reported to stimulate GABA release in some brain areas: again, this GABA pool may bind to GABA_B receptors. Third, GHB – acting at the GABA_B receptor – may oppose the endocytosis of the receptor, with its retention on the cell surface and prolongation of its functionality.

Accordingly, pretreatment with GABA_B receptor antagonists – namely phaclofen, CGP 35348, and SCH 50911 – has been repeatedly reported to block several effects of GHB, including changes in neuronal activity and absence-like seizures in rats, and reinforcement, motor-incoordination, and sedation/hypnosis in mice. Treatment with GABA_B receptor antagonists resulted also in the complete antagonism of GHB-induced lethality in mice. In close agreement with these antagonism data, behavioral effects of GHB (including hypolocomotion and sedation) were virtually absent in mice with genetic deletion of the GABA_B receptor; notably affinity, density, and anatomical distribution of the GHB-specific binding site were identical in GABA_B-knockout and wild-type mice.

In addition, GHB and the prototypic GABA_B receptor agonist, baclofen, have been reported to share several pharmacological properties, including muscle relaxation, hypomotility, catalepsy, proconvulsant activity, and reduction of severity of alcohol withdrawal syndrome, and alcohol and cocaine self-administration. However, although the pharmacological profile of GHB and baclofen is similar in many respects, important differences also exist: for example, electrophysiological experiments found that GHB disinhibited, while baclofen inhibited, ventral tegmental dopamine neurons, providing a likely explanation as to why GHB is abused (see below) while baclofen is not. Possible explanations for these differences are existence of different functional GABA_B receptor subtypes, to which GHB and baclofen may bind differentially; differential activities of GHB and baclofen at GABA_B autoreceptors and heteroreceptors; and differential conformational changes induced by GHB and baclofen on the GABA_B receptor.

Further demonstrations of the contribution of the GABA_B receptor to the mediation of the pharmacological effects of GHB are provided by the results of drug discrimination studies. Indeed, it has been repeatedly observed that baclofen substituted for GHB in rats and pigeons, suggesting that GHB and baclofen have similar discriminative stimulus effects. Some of these studies also suggested that baclofen was more potent in substituting for GHB as the training dose of GHB was increased, in

agreement with the hypothesis that the psychopharmacological effects of moderate-to-high doses of GHB are primarily mediated by the stimulation of the GABA_B receptor. Additional lines of evidence demonstrated that pretreatment with GABA_B receptor antagonists blocked the discriminative stimulus effects of GHB; in some studies, these antagonists resulted in being more effective in blocking high, rather than low, training doses of GHB.

Despite the above-mentioned full substitution of baclofen for the discriminative stimulus effects of GHB, subsequent studies (1) reported solely a partial substitution and (2) demonstrated that rats could be trained to discriminate GHB from baclofen in a three-choice drug discrimination procedure, providing additional evidence that the effects of GHB and baclofen, although similar in many respects, are not identical, and multiple receptor systems may be involved in the mediation of GHB's discriminative stimulus effects.

GABA_A-benzodiazepine receptor complex

Pharmacological data implicate the GABA_A-benzodiazepine receptor complex in the mediation of some effects of GHB. For example, (1) the anxiolytic effect of GHB in rats has been reported to be prevented by the benzodiazepine receptor antagonist, flumazenil, and (2) different GABA_A receptor ligands and GABA_A receptor positive modulators (such as benzodiazepines and barbiturates) substantially substituted for the discriminative stimulus effects of GHB in rats and pigeons.

The interaction between GHB and the function of the GABA_A-benzodiazepine receptor system does not appear to be secondary to the direct activation of either GABA_A or benzodiazepine binding sites. GHB failed, indeed, to bind to both sites as well as to alter the chloride uptake through the associated channel. It is more likely that the GABAergic effects of GHB result from the conversion of GHB into GABA and/or GHB-induced stimulation of GABA release in specific brain regions; both phenomena would give rise to a GABA pool which may bind to the GABA_A-benzodiazepine receptor complex. A possible, third mechanism is the capacity of GHB to effectively increase, in different brain areas, the levels of allopregnanolone and allotetrahydrodeoxycorticosterone, two endogenous neurosteroids with potent positive modulation activity at the GABA_A-benzodiazepine receptor complex.

Interaction with the Mesocorticolimbic Dopamine System

The mesocorticolimbic dopamine system and GABA_B receptors located in the ventral tegmental area constitute the likely neural substrate of GHB's euphorogenic and addictive properties. A recent, elegant *in vitro* study by Cruz and colleagues provides evidence for a cellular

mechanism that may explain the frequently reported, opposite – and somewhat paradoxical – (1) stimulating and inhibitory effects of GHB on dopaminergic neurotransmission and (2) GHB abuse potential and anticraving properties (see below). The premise stands in the finding that the coupling efficacy of GABA_B receptor to potassium GIRK channels is lower in dopaminergic than GABAergic neurons of the ventral tegmental area. Because of its low affinity for the GABA_B receptor, GHB – at concentrations corresponding to those reached after recreational use – activated, preferentially, the GABA_B receptors in GABAergic neurons, decreasing GABA release, and, in turn, disinhibiting dopamine neurons. At higher concentrations, GHB also activated the GABA_B receptors in the dopaminergic neurons, causing their hyperpolarization. This differential action on GABA_B receptors in the ventral tegmental area apparently explains why GHB is a drug of abuse and, in some circumstances, displays anticraving effects. Conversely, baclofen (because of its high affinity for the GABA_B receptor) inhibited both GABAergic and dopaminergic neurons, thus decreasing dopamine release in the target structures; this is consistent with its well-documented anticraving properties.

Therapeutic Uses

Narcolepsy

One of the most important, current therapeutic uses of GHB is represented by the treatment of narcolepsy. The pharmacological treatment of this disorder is primarily aimed at improving the patient's quality of life (increasing alertness, improving daytime performance, and reducing the occurrence of auxiliary symptoms) with the least adverse effects. Although effective in some narcoleptic patients, central stimulants and tricyclic antidepressants often produce intolerable side effects. Conversely, a series of large double-blind studies indicated that modafinil (a stimulant agent characterized by low abuse potential and good safety profile) and sodium oxybate (the sodium salt of GHB; up to 130 mg kg⁻¹, per os, fractioned in multiple doses) are effective and generally well tolerated for the treatment of sleepiness and cataplexy attacks associated with narcolepsy. Accordingly, modafinil is used for the treatment of excessive daytime sleepiness and sodium oxybate for the treatment of cataplexy. Despite the worries for its increasingly widespread recreational use (since March 2000, GHB has been placed in Schedule I, according to the US Controlled Substances Act; see also below), sodium oxybate (known as Xyrem[®]) is currently the only medication approved by the US Food and Drug Administration (FDA) for the treatment of cataplexy associated with narcolepsy. However, it has been reported that modafinil and sodium oxybate provide, at best, only moderate improvement of sleepiness rather than full

restoration (normalization) of alertness in patients with narcolepsy. Despite the progresses in understanding the pathogenesis of narcolepsy, the ideal treatment for sleepiness due to narcolepsy is not yet available. Moreover, to date, no study has been conducted to compare the efficacy of sodium oxybate to that of tricyclic antidepressants.

Alcoholism

Alcohol dependence (a very frequent mental disorder with a lifetime risk in the general population of approximately 15%) is characterized by an obsessive and compulsive desire (craving) for alcohol and inability to control its consumption, resulting in frequent episodes of intoxication. The pharmacological treatment of alcohol dependence is aimed at helping patients to abstain from, or at least to reduce, alcohol consumption. To date, a number of studies have demonstrated some degree of efficacy of two drugs (the opioid receptor antagonist, naltrexone, and the putative GABA_A and NMDA receptor modulator, acamprosate) in reducing alcohol craving and consumption in alcoholic patients, leading to their approval for this indication in several countries.

At preclinical level, acute and/or repeated administration of GHB has been reported to suppress (1) severity of alcohol withdrawal signs in rats made physically dependent on alcohol, (2) voluntary alcohol consumption in alcohol-preferring rats exposed to the two-bottle alcohol-versus-water choice regimen (experimental model of excessive alcohol drinking), and (3) alcohol's motivational properties in alcohol-preferring rats tested in behavioral procedures modeling craving for alcohol. Although there are contradictory data, evidence from drug discrimination studies suggests that GHB and alcohol may possess similar discriminative stimulus effects.

Preliminary open and double-blind clinical surveys indicated that GHB administration reduced alcohol craving and consumption, promoted abstinence, and ameliorated alcohol withdrawal syndrome in alcoholics. Specifically, two small double-blind studies, with less than a total of 100 alcoholic patients, and seven open studies, with a total of approximately 600 alcoholic patients, demonstrated the efficacy of GHB ($50 \text{ mg kg}^{-1} \text{ day}^{-1}$, per os) in the treatment of alcohol dependence. As predictable, because of GHB's abuse potential (see below), 10–15% of the patients under GHB treatment voluntarily increased their daily GHB dosage. Finally, three double-blind studies (with a total of approximately 180 alcoholic patients) and four open studies (with a total of approximately 650 alcoholic subjects) reported that GHB ($50 \text{ mg kg}^{-1} \text{ day}^{-1}$, per os) was also effective in suppressing the symptomatology of alcohol withdrawal syndrome. On the basis of these lines of evidence, GHB is currently marketed in some European countries for the treatment of alcohol dependence and alcohol withdrawal syndrome.

In terms of mechanism of action, the similarity of the pharmacological profile of GHB and alcohol led to hypothesize that the suppressing effects exerted by GHB on alcohol withdrawal syndrome, alcohol consumption, and craving for alcohol may be due to the substitution of alcohol actions, similar to methadone in heroin addiction.

Other Indications

GHB was originally used as an adjuvant in general anesthesia because it exerts a marked hypnotic action at high doses ($75\text{--}100 \text{ mg kg}^{-1}$, per os). Namely it induces a rapid and irresistible sleep, both in healthy and narcoleptic patients, with a profile often reported to be close to that of natural sleep. Despite the positive results obtained by several studies, the use of GHB in anesthesia has never gained widespread acceptance, probably due to reports of its lacking analgesic properties and producing seizure-type EEG activity in animals that resembles petit mal epilepsy.

Over the years, GHB has also been tested for the treatment of a large number of disorders (i.e., heroin dependence, schizophrenia, depression, anxiety, and fibromyalgia), to relieve pain, anxiety, and tension during delivery and to reduce intracranial pressure. However, to date, the total number of clinical studies performed has been relatively small and most have produced contradictory results.

Recent preclinical evidence suggests that GHB may also function as tissue protectant during oxygen restriction: treatment with GHB, up to 2 h after an ischemic episode (temporary occlusion of the carotid arteries), produced indeed a highly significant protection against histological damage in the hippocampus as well as sensory-motor and cognitive impairments in rats. Preliminary studies also indicated the capacity of GHB to suppress the intravenous self-administration of cocaine, as well as acquisition and reinstatement of cocaine-induced conditioned place preference in rodents, leading to hypothesize a possible use of GHB in the treatment of cocaine addiction.

GHB as a Drug of Abuse

Animal Studies

The positive reinforcing properties and abuse potential of a drug are usually assessed in laboratory animals – with high predictive validity for abuse and dependence potential in humans – by determining its capability to be self-administered, to induce conditioned place preference, and to elicit discriminative stimulus effects similar to those produced by abused drugs. In the case of GHB, its anxiolytic, amnestic, and sedative/hypnotic effects seem also to be important, as anxiolysis, feelings of relaxation,

amnesia, sedation, and hypnosis have been reported to be frequently sought by people abusing GHB (see below).

Self-administration studies

Initial studies reported the capacity of GHB to maintain both oral and intravenous self-administration in rodents. Specifically, rats consumed pharmacologically relevant doses of an oral solution of GHB when it was continuously offered, together with water, under the homecage two-bottle free-choice regimen. All rats displayed days of preference for the GHB solution and consumed an average of approximately $700 \text{ mg kg}^{-1} \text{ day}^{-1}$ GHB, distributed in two to three discrete episodes.

In the intravenous self-administration study, mice received infusions of GHB in response to nose-pokes; the number of nose-pokes by mice actively self-administering GHB was significantly higher in comparison to yoked control mice, which passively received the drug solution. As commonly observed in self-administration studies, the amount of self-administered GHB was an inverted U-shaped function of GHB concentration. GHB self-administration was prevented by treatment with the putative antagonist of the GHB-specific binding site, NCS-382, and the GABA_B receptor agonist, baclofen.

Conversely, the few studies that tested GHB self-administration in nonhuman primates generated more equivocal results, with the number of intravenous infusions of GHB exceeding control levels only in some of the tested monkeys. A modest separation between the reinforcing and sedative/hypnotic effects of GHB might have contributed to the difficult unraveling of a more robust GHB self-administration.

Conditioned place preference studies

Two studies reported the capability of GHB to elicit conditioned place preference in laboratory rodents. In the first study, rats were exposed to the repeated pairing of a specific environment with GHB ($87.5\text{--}350 \text{ mg kg}^{-1}$, i.g.). On the test day, the time spent by GHB-treated rats in the GHB-paired compartment was significantly higher than that recorded in the pre-conditioning session. Consistently, the second study demonstrated that 250 mg kg^{-1} GHB (i.p.) induced a robust conditioned place preference in mice.

Drug discrimination studies

The drug discrimination studies conducted to date using rats and pigeons trained to discriminate GHB from vehicle suggested that alcohol and the benzodiazepines, chlordiazepoxide and diazepam, substituted – fully or only partially, depending on the different studies – for GHB. By contrast, neither *d*-amphetamine, cocaine, morphine, the uncompetitive *N*-methyl-*d*-aspartic acid (NMDA) receptor antagonists, phencyclidine, dizolcipine, and ketamine, nor the cannabinoid receptor

agonist, WIN-55,212-2, elicited clear and consistent (among the different studies) GHB-like internal cues.

When GHB was used as a testing drug (i.e., assessing its capacity to elicit discriminative stimulus effects similar to those of some abusive drugs), it failed to substitute for heroin, phencyclidine, cocaine, and Δ^9 -tetrahydrocannabinol in rats and monkeys, and substituted – although not in all studies – for alcohol in rats.

Collectively, these results suggest that GHB may elicit discriminative stimulus effects similar to those produced by alcohol and benzodiazepines, and these alcohol- and benzodiazepine-like effects of GHB may contribute to its abuse potential. This interpretation is consistent with the clinical reports on GHB producing anxiolysis, relaxation, and alcohol-like subjective effects (see below). Recent data suggest that GHB and phencyclidine potentiate reciprocally their discriminative stimulus effects in rats; translated to humans, these results would suggest that two club drugs, such as GHB and ketamine, often co-abused (see below), may potentiate each other's subjective effects.

Studies on the anxiolytic effect of GHB

The anxiolytic effect of GHB has been consistently observed in the few studies addressing this issue. Studies using mice initially kept in isolation and then exposed to contact with nonaggressive, previously group-housed mice – where the defensive responses displayed by the previously isolated mice are recorded as measures of anxiety – indicated that acutely administered GHB ($50\text{--}200 \text{ mg kg}^{-1}$, i.p.) reduced the frequency of defensive behaviors and increased the number of social interactions.

Studies using the elevated plus maze – a validated model of experimental anxiety, based on the natural avoidance and aversion of rodents for open, anxiogenic spaces and their innate preference for closed, more comforting environment; the proportion of the animal's spontaneous explorations in the open and closed spaces of the maze provides a behavioral measure of its anxiety – indicated that acutely administered GHB ($50\text{--}250 \text{ mg kg}^{-1}$, i.p.; 300 mg kg^{-1} , i.g.) exerted a robust anxiolytic effect in rats, as both time spent in and number of entries into the open arms of the maze were dose-dependently increased by GHB administration. In one of these studies, the benzodiazepine receptor antagonist, flumazenil, blocked this anxiolytic effect of GHB, suggesting the involvement of the GABA_A–benzodiazepine receptor complex in this GHB effect.

The above results, showing the capacity of GHB to produce anxiolysis in experimental models of anxiety, are in agreement with clinical observations reporting that GHB exerted anxiolytic effects in healthy subjects as well as with anecdotal reports of anxiolysis, calmness, and relaxation after its recreational use.

Studies on the amnestic effect of GHB

Different lines of experimental evidence suggest that acute or repeated administration of GHB may disrupt learning and memory processes in rodents. As an example, rats treated with 50–100 mg kg⁻¹ GHB (i.p.) and exposed to the Morris water maze took longer and swam greater distances to find the goal platform, hidden under the water surface, than saline-treated (control) rats.

Studies on the sedative/hypnotic effect of GHB

Sedation/hypnosis was historically one of the first pharmacological effects of GHB to be recognized. High doses of GHB result in the loss of the righting reflex in rats and mice, a reliable and widely used index of sedation/hypnosis. Pharmacological investigations of the receptor system(s) mediating GHB-induced sedation/hypnosis found that the putative antagonist of the GHB-specific binding site, NCS-382, increased, rather than decreased, the duration of loss of righting reflex induced by 1000 mg kg⁻¹ GHB (i.p.) in mice. Conversely, the GABA_B receptor antagonists, SCH 50911 and CGP 46381, completely prevented GHB's sedative/hypnotic effect. These results suggest a critical role of the GABA_B receptor in the mediation of GHB-induced sedation/hypnosis. In addition, SCH 50911 was capable of readily reversing the sedative/hypnotic effect of GHB in mice that had already lost the righting reflex. This finding may bear some relevance in terms of the potential therapeutic use of GABA_B receptor antagonists in reversing the episodes of coma, respiratory depression, and loss of consciousness reported to occur in an increasing number of individuals overdosing GHB.

Clinical Studies

In good agreement with the results of some animal investigations, a number of clinical observations and reports from US agencies have shown that GHB is used as a recreational drug, mostly because of its salient feelings of euphoria, disinhibition, anxiolysis, and relaxation (produced by low-to-moderate doses) and of its ability to produce – at higher doses – sedation and hypnosis, resulting in feelings of unusual refreshment on awakening. GHB effects are often described as closely resembling those of alcohol. GHB – together with its precursors, 1,4-butanediol (1,4-BD) and γ -butyrolactone (GBL) – are presently classified as club drugs, that is, recreational drugs ingested in dance clubs, circuit parties, and all-night dance parties (called raves), often in association with other drugs of abuse, most commonly alcohol, methylenedioxymethamphetamine (ecstasy), or cocaine. In the past, GHB gained some popularity among bodybuilders as a steroid alternative, due to its claimed stimulating effect on growth hormone and anabolic action.

Cases of GHB dose escalation and compulsive use, leading to signs of intoxication (i.e., nausea and vomiting,

drowsiness, dream-like state, reduced muscle tone, loss of consciousness, depressed respiration, and seizures), have been reported to occur with increasing frequency and worsened by the narrow margin of safety of the drug (doses of GHB twice those producing euphoria may indeed lead to coma). These effects appear to be exacerbated when GHB is mixed with alcohol, benzodiazepines, barbiturates, and/or other recreational drugs. Acute poisoning from GHB usually resolves within a few hours without sequelae (this rapid and uneventful recovery often creates a false sense of security in GHB users). Currently, there is no antidote to GHB intoxication; pre-clinical data suggest that potential therapies may however come from the GABA_B receptor antagonists. Instances of withdrawal syndrome (similar to that from alcohol and characterized by anxiety, insomnia, muscle cramps, tremors, hallucinations, and delirium) after discontinuation of high and chronic intake of GHB have been also reported, revealing the capacity of GHB to produce physical dependence. Benzodiazepines have been reported to effectively ameliorate GHB withdrawal syndrome.

In spite of the fact that as early as 1990 the FDA declared all GHB-containing products unsafe and banned their public sale, the use and abuse of GHB has dramatically increased. More recently, GHB has gained notoriety as ‘date rape’ drug (its combined amnestic and hypnotic effects make the victim more vulnerable to sexual assault; its solubility and lack of color and odor facilitate the surreptitious addition of GHB to the victim’s drink). The spreading use of GHB has been enhanced by several factors, including presence on the Internet and in underground literature of recipes for the household manufacturing of GHB, to the frequent mispresentation that it may act as a safe and natural sleep aid, mood- and sexually-enhancing substance with attractive names (just to cite a few: liquid ecstasy, liquid X, easy lay, grievous bodily harm, Great Hormones at Bedtime, Georgia Home Boy, liquid loving), and its low price. Further, accumulating lines of evidence suggest that GHB is frequently replaced by its precursors, 1,4-BD and GBL, which are often preferred over GHB because (1) they produce a profile of psychotropic effects similar to that of GHB (with an even shorter onset) and (2) their purchase is not prohibited as they are commonly used as industrial cleaners and solvents.

See also: Alcoholism; Animal Models of Behavior: Alcohol Addiction; Animal Tests for Anxiety; Drug Addiction; Sleep: Medical Disorders.

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Korsakoff's Syndrome

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Glossary

Anterograde amnesia – Inability to form new long-term memories. It is usually specified with reference to a certain time point (onset), such as a brain infarct. In Korsakoff's syndrome, however, the appearance is usually more gradual.

Chronosognosia – Impairment or inability to judge the time. It may manifest as a total absence of any time sense, or as a distortion in the perception of time. The distortion may be an acceleration or a prolongation (retardation) of perceived time epochs.

Confabulation – The creation of episodes or stories which have not occurred in reality in their narrated appearance, but which may be based on, or triggered by a stimulus or idea, anchored in reality.

Episodic-autobiographic memory – A memory system, introduced by Endel Tulving, that deals with personal events or episodes, can be traced back by the time (when) and the place (where) they occurred (or may occur in the future), and that is defined by the conjunction of subjective time, autonoetic consciousness, and the experiencing self. Access to this memory system is usually blocked in patients with Korsakoff's syndrome.

False memory syndrome – Self-created or implanted episodes or remembrances of events which frequently have a true core, but are otherwise the product of fantasy or free associations. Nevertheless, the creator of such memories is unable to reflect on their truth value.

Global amnesia – A term used for characterizing patients who manifest major disturbances in both the anterograde and retrograde memory domains. As memory is at present differentiated into several systems (e.g., episodic-autobiographic memory, semantic memory, and procedural memory), some of which are largely preserved in the so-called globally amnesic patients, the term is nowadays used infrequently.

Nonspecific thalamic nuclei – The thalamus is generally divided into specific and nonspecific nuclei. The specific ones have distinct connections to specific areas of the cerebral cortex, while the nonspecific ones project all over the cortex. Furthermore, the axons of specific nuclei usually reach layer IV of the cerebral cortex, while those of the nonspecific nuclei reach layer I (i.e., the top layer).

Perceptual memory – A memory system which relies on judgments of familiarity (on a presemantic level). It

allows us to distinguish between similar items or objects (e.g., apple, pear, and peach) and to identify an item or object that occurs in variations (e.g., red apple and green apple). This memory system is basically preserved in patients with Korsakoff's syndrome.

Priming – A memory system that stands for a higher likelihood to re-identify stimuli which one had perceived (nonconsciously or subconsciously) in the same or a similar way at a previous time point. This memory is preserved in patients with Korsakoff's syndrome. An old example (from 1911) for its intactness in patients with Korsakoff's syndrome is the so-called Claparede phenomenon. The French psychiatrist, Claparede, had hidden a pin in his hand when shaking hands with the patient with the consequence that she later refused to shake hands, but was apparently unaware of a reason for her behavior, or even of the fact that she had met him before.

Procedural memory – A memory system, acting principally at the subconscious or nonconscious level. It largely deals with mechanical or motor-system-related procedures such as driving a car, playing a piano, or riding a bike. This memory system is preserved in Korsakoff's syndrome.

Retrograde amnesia – Inability to retrieve (recall) memories from the past, which are considered to have been stored in the brain. Retrograde amnesia may be due to a decay of memories (usually after major cortical degeneration), to interference effects, or due to a block in recall, that is, an inability to access and activate neuronal storage places due to damage or degeneration of relevant fiber connections and/or trigger regions.

Ribot's law – The French psychiatrist Theodore Ribot formulated in 1881 a law of regression which stated that the more recent a learned information is, the more likely it is lost, while vice versa, the longer it had been stored, the more likely it is retained. In simple words, it means last in, first out – what entered the brain last is lost first and vice versa.

Semantic memory – A memory system that deals with facts (general knowledge) which are consciously available, but lacks relations with time and space. This memory may be partially preserved in patients with Korsakoff's syndrome.

Theory of mind functions – Ability of normal individuals to reflect upon other peoples' intentions or feelings and to show empathic behavior. It is assumed

that theory of mind functions develops over time and is closely related to an appropriate social interaction early in childhood. The brain region most consistently related to these forms of behavior is the medial and inferolateral (orbitofrontal) prefrontal cortex.

History and First Descriptions

The use of the term 'Korsakoff's syndrome' was suggested in 1897 by Friedrich Jolly, director of the psychiatric clinic of the Berlin Charité, at the Moscow International Medical Congress. The syndrome became popular after the appearance of several publications by Sergej S. Korsakoff (1854–1900; usually spelled Korsakow in German-language publications). Korsakoff had written four articles and one book (on alcoholic paralysis) in Russian language initially, but only due to the subsequent appearance of four further articles in German and an additional one in French language his findings and ideas were disseminated in the Western world. These last five articles appeared between 1887 and 1892. While Korsakoff himself subsumed several different etiologies under the heading of *psychosis polyneuritica seu cerebropathia psychica toxæmica*, all of which led to major cognitive and especially memory deteriorations (e.g., the existence of a decaying fetus in the uterus, puerperal septicemia, typhus, tuberculosis, diabetes mellitus, and a decaying tumor), only a few years later the term was generally (though not exclusively) reserved for an alcohol-consumption-related disease resulting – as Karl Bonhoeffer wrote in his German-language monography on the mental illnesses of chronic drinkers – in four cardinal symptoms which can be translated into anterograde amnesia, defects in remembrance, disorientation, and confabulation. Till now, these symptoms describe the core picture of the Korsakoff's symptomatology which, on the other hand (at least in principle), leaves other intellectual functions (e.g., intelligence) unchanged. The syndrome was also named 'amnesic psychosyndrome' and is of hybrid character: though caused by neurological symptoms, the patients are usually referred to psychiatric clinics. In line with the psychiatric classification of Korsakoff's patients is the use of the terms 'Korsakoff's psychosis' or 'polyneuritic psychoses' (which was even the title of a book in 1906). The older literature used the term Korsakoff's syndrome generously so that some researchers favored to speak of the Korsakoff symptom complex and included patients of various etiologies whose core symptoms were a global amnesia.

Prior to the use of the term 'Korsakoff's psychosis', Carl Wernicke in 1881 had introduced a disease

description which he termed 'acute hemorrhagic superior poliencephalitis' and soon which became known as the acute precursor of Korsakoff's disease ('Wernicke encephalopathy'). As alcohol-related diseases were quite frequent at that time, the differentiation between them was important, as some were more prone to treatment and recovery than others. (In fact, Magnus Huss emphasized already in 1852 that alcohol consumption has a major impact on psychic well-being and between his thesis and the descriptions of Wernicke and Korsakoff the use of the term 'multiple alcoholic neuritis' was common. At the beginning of the twentieth century, a 22-year-old medical student was already diagnosed as a Korsakoff's patient; he, for instance, had admitted that he once drank "prophylactically 28 glasses of schnaps against influenza.")

The alcoholic Korsakoff's syndrome is nowadays termed 'alcohol-induced persisting amnesic syndrome' (*The Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, text revision DSM-IV-TR) or 'alcoholic amnesic syndrome' (*International Classification of Diseases-10* (ICD-10)).

Differential Diagnosis and Anatomical Changes

Korsakoff himself justified his labeling of the disease as 'toxic' with his experience that in all cases investigated "the composition of the blood is altered, toxic substances have accumulated, and it is most likely that these poison the nervous system, whereby in a few cases mostly the peripheral, in others the central nervous system, but frequently both are afflicted to the same degree" (the author's translation). We know nowadays that both, the Wernicke encephalopathy and the Korsakoff's psychosis, are due to malabsorption of vitamin B₁ (thiamine) and may also consequently occur in illnesses in which vitamin B₁ deficiency is prevalent as in beriberi disease. The Wernicke encephalopathy constitutes the initial phase of a process, which may later lead to Korsakoff's psychosis or Korsakoff's syndrome. Wernicke encephalopathy is characterized by oculomotor disturbances (due to degeneration of pontine oculomotor nuclei), ataxia and further gait disturbances, dysarthria, and dysphagia; further symptoms (e.g., somnolence, insomnia, and hypothermia) may follow. Besides degeneration of pontine nuclei, the hypothalamic nuclei may also be affected.

For the Korsakoff's syndrome, a polyneuropathy of the legs (weakness due to the degeneration of peripheral axons) was already noted in the old descriptions. Other symptoms, such as fatigue and changed somatosensory perceptions (e.g., coldness), were noted early as well. However, the main symptomatology lies in the diencephalon where degenerations were noted consistently in both the dorsal and ventral diencephalon in the vicinity of

the cerebrospinal fluid (CSF). In particular, the medial portion of the mediodorsal thalamic nucleus and the medially adjacent paratenial nucleus (a nonspecific thalamic nucleus) are degenerated together with the mammillary bodies on the hypothalamic level. Other nonspecific thalamic nuclei such as the nuclei parafascicularis, submedius, and reuniens were frequently damaged as well. Less consistently, degenerations in the basal forebrain area (nucleus basalis of Meynert) and prefrontal cortical regions were found. A minority of studies also suggests some damage in medial temporal regions (e.g., hippocampal formation). Degeneration of cerebellar neurons is observed quite frequently postmortem, and that of brainstem nuclei (locus ceruleus, raphé nuclei) only occasionally.

Certainly, however, the nervous system damage in both Wernicke encephalopathy and Korsakoff's syndrome differs clearly from that of the other two main alcohol-related disease conditions, affecting the nervous system: alcoholic dementia and Marchiafava–Bignami disease. In alcoholic dementia, there is widespread damage to the neocortex, accompanied by ventricular enlargement, with the consequence of a dementic (instead of a pure amnesic) condition. In Marchiafava–Bignami disease, there is principally a degeneration of the corpus callosum fibers (with laminar cortical sclerosis); excessive drinking of specific sorts of red wine are assumed to trigger the degeneration. The condition leads to deterioration of social functioning, and dementia. In comparison to the other three disease conditions, Marchiafava–Bignami disease is very rare and most of the cases originated from Italy. While all alcohol-related disease types may be accompanied by a more general malnutrition, there are certainly several factors, including genetic variations and (related to the genetic predisposition) enzymatic variations which account for the manifestation of the diseases.

Though reduced in frequency of appearance, there is still a significant number of yearly incidences throughout the world with a preponderance of course in those countries, where alcohol (and in particular CSF) is regularly consumed. The disease can break out at any adult age, depending on the history of alcohol consumption, dietary habits of the individual, and genetic factors.

Behavioral Symptomatology

The noted degenerations accompanying Korsakoff's syndrome fit well with the behavioral symptomatology as damage to the mammillary bodies and more so to the mediodorsal thalamus is known to affect anterograde memory in particular. The mediodorsal nucleus in itself is a complex structure which has been subdivided into more than a dozen, but most commonly into three major

portions – the magnocellular, the parvocellular, and the paralamellar subnuclei, each of them projecting to different regions within the prefrontal cortex (**Figure 1**). The magnocellular subnucleus is related to the orbitofrontal cortex and is therefore related to emotional and personality dimensions. Both the mammillary and the mediodorsal nuclei are part of limbic circuits (Papez circuit and basolateral limbic circuit), which are assumed to play crucial roles in the deep encoding and consolidation of autobiographic episodes and consequently of emotional events (**Figure 2**). Already in the 1920s, the German psychiatrist Ewald Gamper emphasized the importance of the mammillary bodies for an integrated action of the brain. He noted that they constitute a center of the vegetative apparatus which controls and regulates the interaction between midbrain, thalamus, and cortex, with respect to psychic behaviors that their damage results in a disturbance or even abolition between consciousness and the experience in the present so that an increase in experience becomes impossible.

Gamper's notion was taken up in later studies and led to the repeated suggestion that the core symptomatology of Korsakoff's disease is the memory impairment, however, in relation to an underlying more basic deficit in consciousness and time perception. Patients with Korsakoff's syndrome become unable to make mental time travels between past, present, and future, and consequently are stuck in time. This basic deficit results in reduced forms of consciousness and self-awareness, together with an emotional flattening. The bases for this deficit may, in fact, not just lie in the damage of a singular structure such as the mammillary nuclei, but may be seen in an interruption of the circuits depicted in **Figure 2** and also in the relation between thalamus and (prefrontal) cortex, as shown in **Figure 1**. For instance, the mediodorsal nucleus of the thalamus certainly represents another center for both consciousness and memory processing, and other structures – such as the amygdaloid nuclei – closely connected to the mediodorsal nucleus (basolateral limbic circuit; cf. **Figure 2**) – provide (together with the septal nuclei in the basal forebrain) further prominent hubs for an integration of emotion, memory, and consciousness. Theory of mind functions, which have been associated to the orbitofrontal cortex (and also in part to the amygdala), have also been found to be impaired in Korsakoff's patients.

The memory deficit in Korsakoff's syndrome consequently is principally one in the domain of (anterograde) episodic-autobiographic memories, and much less so in the domains of the other memory systems currently defined. With respect to semantic retrograde memories, the deficit is less pronounced as can be inferred from a superior retrieval capacity under conditions of recognition compared to free recall.

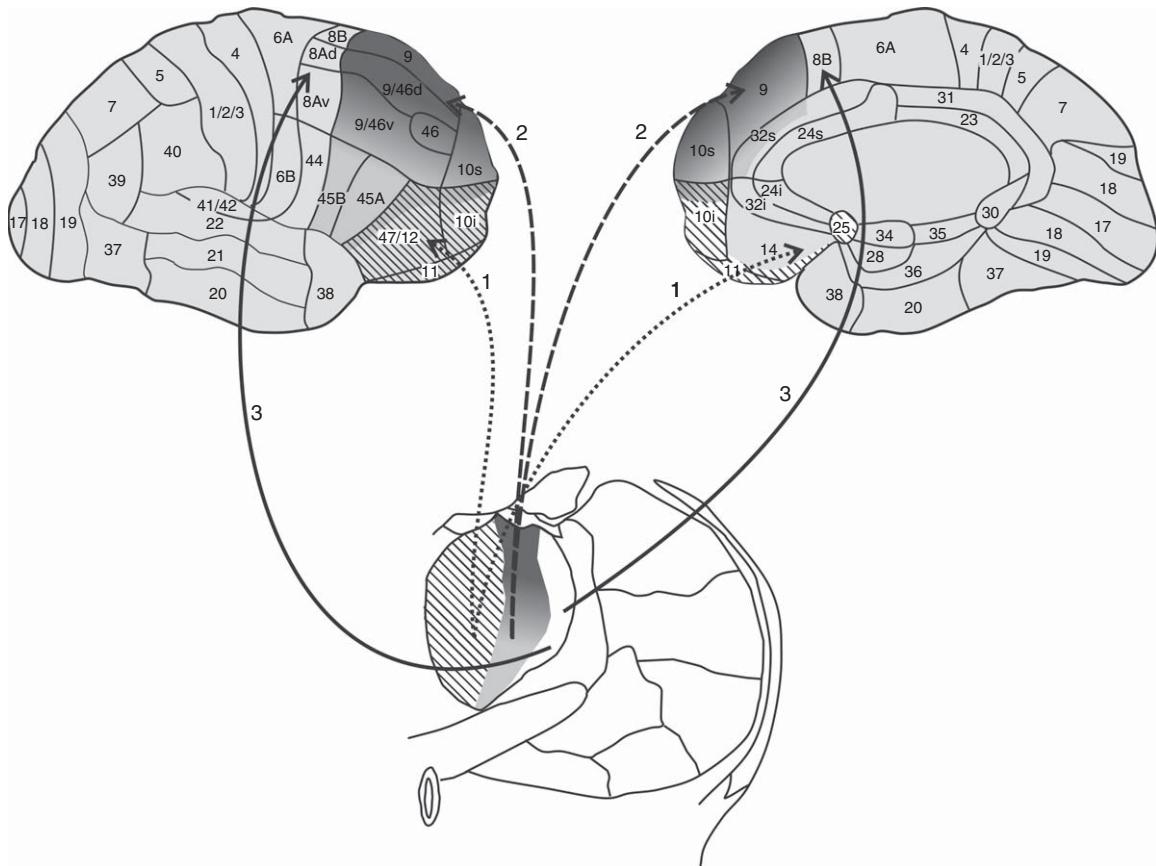


Figure 1 The relations between the mediodorsal thalamus (bottom) and the prefrontal cortex (top). The two top pictures show the lateral and medial cortex with Brodmann areas. Dorsolateral prefrontal regions are darkened, orbitofrontal/ventrofrontal ones are striped. The frontal eye field is composed of areas with the number 8. Within the mediodorsal nucleus of the thalamus the white, lateral area is named parvocellular, the central, gray one parvocellular, and the medial striped one magnocellular. The arrows 1–3 represent corresponding projections of thalamic subnuclei to regions within the prefrontal cortex.

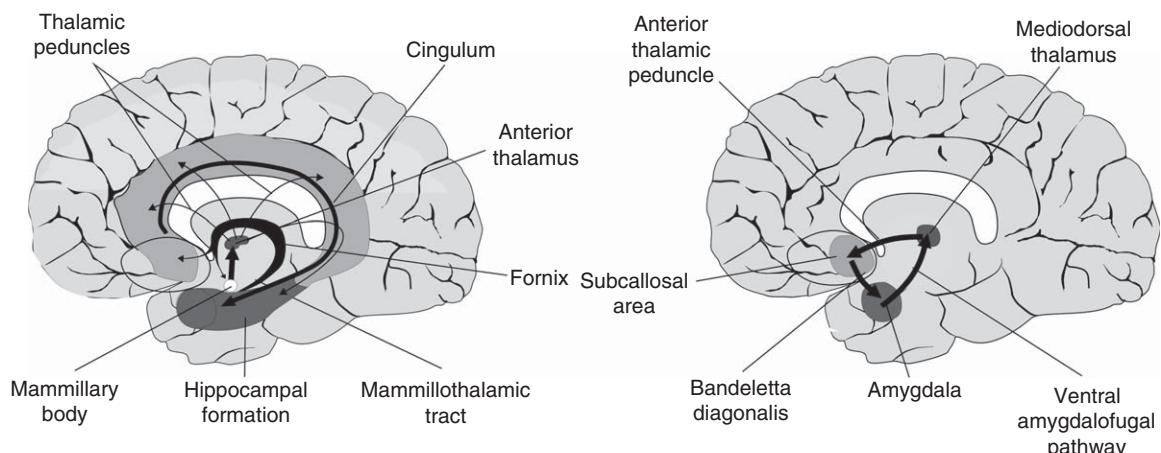


Figure 2 The Papez circuit (left) and the basolateral limbic circuit (right). Both circuits are centrally involved in the processing of conscious information, in particular episodes. They hold information online; associate and synchronize it with the already-existing comparable information and are engaged in the transformation of information for long-term storage.

A case description, given by Korsakoff himself in 1890, clearly demonstrates the differential affection of the various memory systems, showing that procedural memory (chess and card playing) is intact while episodic-autobiographic memory is severely impaired. This case also underlines Korsakoff's excellent power of observation (the author's translation):

At the beginning of a talk the mental disturbance is hardly noticeable; he gives the impression of a person with full mental powers – talking with full reflection, drawing correct deductions on the basis of given premises, playing chess, or a game of cards, in short – he behaves like a mentally healthy subject; only after longer conversation may one notice that he mixes facts quite substantially, and that he is unable to keep in mind anything of what takes place in his environment: he does not remember having eaten, or whether he got up from bed. Sometimes he immediately forgets what has happened to him. Patients of this kind may read for hours one and the same page, because they are unable to keep the contents in mind. They can repeat 20 times the same conversation without realizing the continuous repetition of their stereotyped talk. He does not know persons he met only during the time of his illness, such as the doctor or the nurse, though he sees them consistently; he is always convinced of meeting them for the first time. (Korsakoff, 1890)

Another typical case report referred to a patient with Korsakoff's syndrome who, at the age of 50 years, had retrograde amnesia dating back about three decades, but had a good remembrance of his former school friends, knew Latin syntax, and could name his University professors. He was well educated on questions of political, historical, and moral nature. This and other examples demonstrate that retrograde amnesia in Korsakoff's patients usually follows Ribot's law and that general knowledge (Latin syntax, general education, and social skills), that is, semantic memories are largely preserved. As many other patients with anterograde amnesia, short-term memory, that is, the online holding of information for a few seconds to a few minutes is preserved. An example is a German patient with Korsakoff's syndrome who was able to remember the word *omnipotnekatmehum* (which is not German) for 3 or 4 min.

Korsakoff also referred to 'pseudoreminiscences' or false memories in one of his later studies (in 1891), mentioning that these pseudoreminiscences appear in two forms – as a *déjà vu* feeling and as the appearance of ideas or conceptions which had not occurred in reality. He had noted that most of these pseudoreminiscences had some relation to a true background, and so he hypothesized that they represent a rudimentary form of memory engrams. He furthermore speculated that these rudimentary elements might then combine to constant associative

groups which are first unconscious but at a later stage may come to consciousness and thereby simulate real memories. With these statements, Korsakoff again gave quite modern descriptions of what we currently consider as confabulatory tendencies and false memory syndromes in patients with Korsakoff's syndrome.

When comparing the behavioral disturbances of patients with Korsakoff's syndrome, it becomes apparent that their deficits may vary widely, depending on the particular history of their disease and on a number of personality traits and premorbid intellectual capacities. Aside from memory defects, deficits in executive functions, attention, and emotion/mood can be noted. In most cases, however, at least older retrograde memories are preserved, in particular, when they have some emotional connotation to the patient. For instance, this was observed by Bonhoeffer already in his monography in 1901, where he wrote (the author's translation):

The inability to memorize is complete only exceptionally. Usually it is possible, even in cases with apparent total amnesia, to determine rests of a memory ability. For example, a patient was able to tell me a list of names of familiar distilleries even after a very long time, though he otherwise forgot nearly instantaneously simple, indifferent words, names, or two- or three-figure numbers. (Bonhoeffer, 1901)

With respect to a dissociation of episodic versus procedural memory, it is of interest to note that there are numerous reports of patients who could play chess quite well (e.g., winning against all other occupants of the hospital), or had superior mathematical or calculation abilities, but minutes after finishing their performance had no knowledge of having played a game or calculated. Seen in this way, there is also a disturbance of the patients' ego and of their perception of time – a deficit named 'chronosognosia' or 'amorphism' of the time sense or described as a lost sense of time. Korsakoff's patients may work with pieces of time and points of time, which implies that they may use expressions as weeks and days, but they have lost the ability to experience their duration. The actual comprehension of time and time as a principle of order are disturbed, an observation made already by Wernicke who wrote of a disturbed allopsychic orientation of these patients. In the eyes of other scientists, the principal defect of Korsakoff's patients appears to lie in the inability to relate an actual experience to the total experience of the personality. The patient is fully engaged in the momentaneously experienced situation. The ability of circumspection in the most direct sense of the word is lost. Reasons for this deficit were seen in the passive personality, lacking spontaneity and living in a suspended manner. Such descriptions fit with the neuroanatomical changes in Korsakoff's syndrome, namely the

interrupted Papez and basolateral limbic circuits (cf. [Figure 2](#)), together with an impaired or abolished thalamo-prefrontal mediation (cf. [Figure 1](#)).

Treatment

Treatment of Korsakoff's patients is, of course, based on dietary rules. Nutrition includes strict alcohol abstinence and the application of high doses of thiamine. The doses have to be high, as metabolism of vitamin B₁ is reduced in Korsakoff's patients. Problems may be that some patients may become seriously allergic toward thiamine. As in the majority of Korsakoff's patients the degree of memory loss is not complete and memory training may be helpful, though several authors favor practical work (at least at the beginning of therapeutic interventions). Pharmacological treatment (e.g., with noradrenalin agonists) may be helpful as well. Measuring the glucose metabolism in Korsakoff's patients has shown that their brain metabolism may recover after detoxification.

See also: Alcoholism; Amnesia; Animal Models of Learning and Memory; Cerebellum: Associative Learning; Conscious and the Unconscious; Declarative Memory; Episodic and Autobiographical Memory: Psychological and Neural Aspects; Implicit Learning and Memory: Psychological and Neural Aspects; Memory Consolidation; Memory Disorders in Uncomplicated Alcoholism; Social Cognition: From Behavior-Reading to Mind-Reading.

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Memory Disorders in Uncomplicated Alcoholism

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Glossary

Alcoholism – Alcoholism refers to a drinker's relationship with alcohol and encompasses alcohol abuse and alcohol dependence. Alcohol abuse is defined as repeated use despite recurrent adverse consequences. Alcohol dependence is considered alcohol abuse combined with tolerance, withdrawal, and an uncontrollable drive to drink.

Episodic memory – Episodic memory is currently described as the memory system in charge of the encoding, storage, and retrieval of personally experienced events, associated with a precise spatial and temporal context of encoding. Episodic memory allows the conscious recollection of happenings and events from one's personal past and the mental projection of anticipated events into one's subjective future. Recollection of episodic events includes autonoetic awareness, which is the impression of re-experiencing or reliving the past and mentally traveling back in subjective time.

Perceptual memory – Perceptual memory receives, stores, and makes available to other systems information about perceptual features of physical objects. It is assumed to be involved in perceptual priming in that experience-based changes in perceptual memory may implicitly manifest themselves as enhancement in the perceptual features of objects.

Procedural memory – Procedural memory is defined as the memory system in charge of the encoding, storage, and recovery of the procedures that underlie motor, visuospatial, or cognitive skills.

Semantic memory – Semantic memory refers to the memory of meaning, understanding, general knowledge about the world and other concept-based knowledge unrelated to specific experiences. The level of consciousness associated with semantic memory is noetic because it is independent of context encoding and personal relevance.

Working memory – Working memory is composed of two slave systems and a central executive, sometimes considered as similar to the executive functions. The slave systems are short-term storage systems,

comprising the phonological loop (which processes language-based information) and the visuospatial sketchpad (which processes visuospatial information). The third slave system of working memory is viewed as responsible for maintaining multimodal information and is known as the episodic buffer.

Alcohol dependence is a major public health problem with greater prevalence and severity in developing countries. Acute intoxication alters momentarily brain function while ethanol is still present in the blood but the effects of chronic alcoholism are also discernible in sober alcoholics. Among other medical consequences, chronic alcoholism can indeed lead to more persistent brain damage and attendant functional compromise involving motor, gait and balance and cognitive brain systems. These functional consequences can occur even in uncomplicated alcoholics, that is, those without clinically detectable nutritional deficiencies (e.g., Korsakoff's syndrome) or liver dysfunction often associated with chronic alcoholism. These neuropsychological deficits encompass mainly visuospatial disabilities, executive dysfunctions, and memory impairments. This section focuses on sparing and impairments of memory capacities in uncomplicated alcoholics, within the framework of a memory neostructural intersystemic model (MNESIS; **Figure 1**). Other cognitive functions will be treated only to the extent that they may influence memory functioning.

Episodic Memory

According to MNESIS, of the many components of memory, episodic memory is hierarchically the highest memory system (**Figure 1**): the most sophisticated but also the most sensitive to pathology and toxicity.

Most studies investigating episodic memory in chronic alcoholism examined learning abilities, that is, capacities to improve the level of performance with practice, by means of classical psychometric tasks. These investigations reported a nonspecific nature of learning

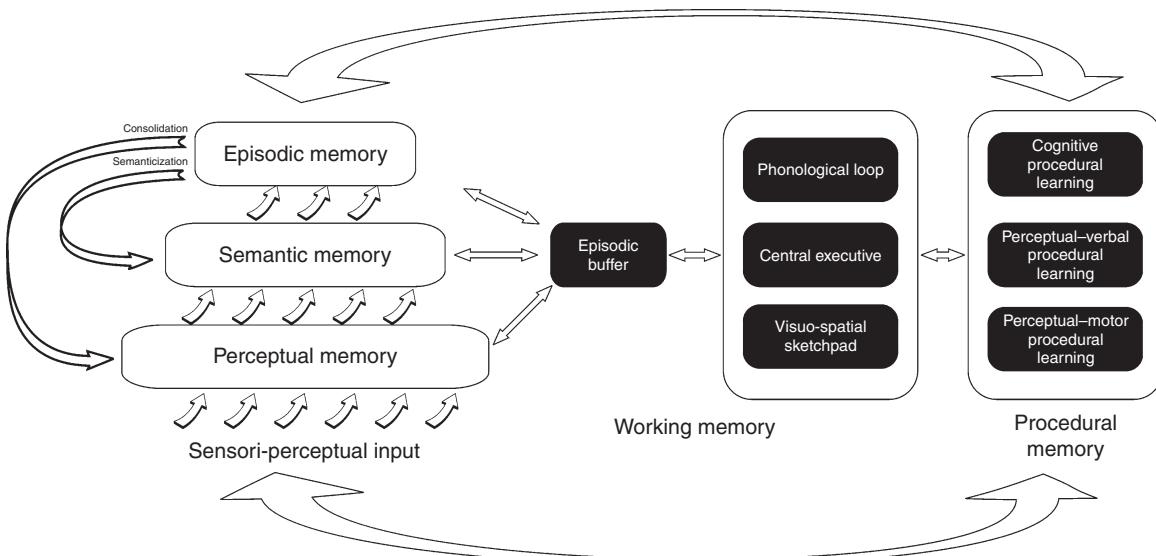


Figure 1 Memory neo-structural inter-systemic model (MNESIS). With permission from Eustache F and Desgranges B (2008) MNESIS: Towards the integration of current multisystem models of memory. *Neuropsychology Review* 18: 53–69.

impairments affecting both verbal (mainly lists of words) and nonverbal information (face-name associations, complex figure, symbols, or visual location). Although sober alcoholics commonly perform below controls, both groups can show evidence of learning over trials. Learning difficulties have sometimes been attributed to an impoverished generation of spontaneous learning strategies, which may also account for the poor performance on free recall tasks. Recently sober alcoholics, however, can show impairment in recognizing test information after spontaneous encoding as well as on a free recall after deep encoding. The timing of these deficits suggests that at alcohol treatment entry, alcoholics present an impairment of both encoding and retrieval abilities.

The spatiotemporal context of encoding is also impaired in alcoholism, affecting both temporal order and spatial context recognition. Moreover, alcoholic subjects tend not to recall complete episodes, that is, correct factual information associated with the correct spatiotemporal context of encoding, indicating that episodic memories are not complete. Source memory, which is the ability to discriminate the origin or source of information, seems also affected by chronic alcoholism. Lastly, alcoholism leads to a deficit of autoetic consciousness and a lack of confidence in the accuracy of one's decisions. Taken together, these findings suggest that most components of episodic memory are impaired in uncomplicated alcoholic subjects.

Because of the connections between episodic memory and working memory, a recent study examined whether executive dysfunctions could explain the episodic memory impairments in alcoholics, that is, whether executive dysfunction could be responsible for the deterioration of certain episodic memory processes. Findings revealed

that the episodic memory impairment observed in uncomplicated alcoholic subjects may not be linked solely to executive dysfunction. One exception was observed for learning abilities, which may draw heavily on strategies to improve performances during repeated presentations of the information. Chronic alcoholism may cause genuine episodic memory deficits and not simply an indirect consequence of executive function deficits.

There may well be a link between the episodic memory disturbance in uncomplicated alcoholic subjects and neuroanatomical abnormalities. For example, postmortem investigations have reported atrophy in the mammillary bodies and dorsomedial nucleus of the thalamus even in uncomplicated alcoholics. *In vivo* imaging studies also reported volume loss in mammillary body, hippocampus and thalamus but failed to show any correlation between gray matter shrinkage and episodic memory impairments. Rather, this memory disorder may be associated with damage of white matter fiber bundles and tracts, in particular, the cingulate bundle and the fornix, which interconnect the hippocampus, thalamus, and mammillary body in the Papez's circuit, thus leading to an interruption of this brain network. This explanation remains hypothetical and requires experimental verification.

Autobiographical memory, which is formed by different types of representation from specific personal events (episodic components) to general knowledge about one-self (semantic component), has been reported impaired in uncomplicated alcoholics. When thoroughly evaluated and compared with nonalcoholic controls, alcoholics were seen to recall specific memories less frequently and general memories more frequently, suggesting

difficulties in strategically accessing episodic autobiographical memory. Nevertheless, when a specific past event was accessed, alcoholic subjects subjectively experienced as many sensory and contextual details associated with the event as controls.

Semantic Memory

In our model of memory processes, episodic memory and semantic memory are connected to each other by two arrows (**Figure 1**). The first one reflects the fact that information is encoded in semantic memory before being encoding in episodic memory, whereas the second one reflects the process of memory semanticization of episodic memories over time.

Early in sobriety, alcoholics present with impairment in novel semantic encoding and more specifically a deficit in acquiring new labels information. Learning new categories and features can be achieved by alcoholics but at a slower rate than controls. Compared with controls, alcoholics invoke different and inefficient cognitive strategies to attempt to compensate for impaired episodic and working memory. Active and strategic learning appears therefore to be not up to par with controls, leading to the shallower (more fragile) encoding of the new information in episodic memory. Alcoholic subjects invoked specific cognitive processes, such as working memory abilities, in addition to those invoked by controls. Compensatory strategies have already been suggested in neuroimaging studies showing that alcoholics recruit higher-level cognitive systems to perform even simple tasks at control levels.

A recent investigation revealed that, in uncomplicated alcoholics, a high level of anxiety was associated with significant difficulties in inhibiting alcohol-related verbal stimuli. The authors interpreted the results as the effect of anxiety that may facilitate the activation of alcohol-related concepts in semantic memory.

Perceptual Memory

Perceptual memory includes both conscious and nonconscious processing of sensori-perceptual information. It can be considered the root of the human memory systems (**Figure 1**), because it is through perceptual memory that information is subsequently and progressively encoded in the different representation memory systems.

While deficits in basic visuospatial abilities have been consistently documented in uncomplicated alcoholic subjects, less is known about implicit perceptual learning processes. One of the implicit perceptual learning tasks is the picture fragment completion stimuli. The ability to identify the stimuli on initial testing requires intact

visuospatial abilities, whereas identification of these stimuli at later testing sessions includes an additional implicit memory component. Given that picture fragment completion tasks can be performed at normal levels by subjects with episodic memory deficits, these tests are assumed to assess implicit memory for perceptual features, that is, perceptual memory.

When using this implicit perceptual learning paradigm, alcoholic subjects were showed to present the commonly reported pattern of impairment in visuoperception but normal levels of perceptual learning, suggesting that they were able to take normal advantage of prior exposure to fragmented line drawings to enhance performance. However, although the alcoholics performed at the same level as the controls on the implicit perceptual learning task, each group used different strategies to perform the task successfully. Alcoholic subjects invoked higher-order executive processes to establish normal learning. This difference in strategy was further indication that alcoholics recruit more cognitively demanding and less efficient neural systems than controls to perform a task at normal levels, possibly because of alcoholism-related dysfunction in optimal neural systems.

Working Memory

In MNESIS (**Figure 1**), working memory is in the center, occupying therefore a strategic position between the three long-term representation systems (episodic memory, semantic memory, and perceptual memory) and procedural memory.

Regarding the nature of the information processed by this memory system, both verbal and nonverbal components can be impaired in uncomplicated alcoholics although nonverbal memory is observed as more severely altered.

The central executive of working memory is compromised in uncomplicated alcoholic subjects. This system is heavily involved in handling novel situations outside the automatic processes. It is regarded as being similar to the executive functions, which are necessary for goal-directed behavior. They include the ability to initiate and stop actions, to monitor and change behavior as needed, and to plan future behavior when faced with novel tasks and situations. Executive functions, therefore, enable us to anticipate outcomes and adapt to changing situations. Inhibition, flexibility, categorization, deduction of rules, organization, updating, and planning have mostly been found to be impaired in uncomplicated alcoholic subjects. The binding of multimodal information, reflecting the episodic buffer, is also impaired in alcoholic subjects, as are the other slave systems of working memory.

Neuroanatomical substrates of the executive dysfunctions are classically associated with compromise of

prefrontal structure or metabolism. Additional substrates of dysfunction involve damage to gray and white matter structure of frontocerebellar circuitry, which may be better predictors of executive dysfunctioning than alterations in prefrontal regions alone. More specifically, smaller white matter volume in the midbrain of alcohol-dependent subjects was related to impaired performance in executive tasks involving flexibility capacities. Uncomplicated alcoholic subjects have fewer white matter fiber tracts coursing between the midbrain and the pons and a smaller pons volume than controls. Furthermore, the number of fibers identified with neuroimaging (diffusion tensor imaging, DTI) was correlated with the alcoholics' performance in the flexibility task. Previous investigations also provided evidence of an association between certain components of executive functions (attention skills and inhibition) and white matter structure in the corpus callosum and between verbal working memory and the medial septal/diagonal band. These later findings suggest therefore a cholinergic mechanism for the working memory impairments in uncomplicated alcoholics.

Functional magnetic resonance imaging (fMRI) provides further information about the status of working memory in uncomplicated alcoholics. Such fMRI studies show that while performing a verbal working memory task, alcoholics exhibit greater activation in the left frontal and right cerebellar regions than controls, suggesting that the articulatory control system of verbal working memory requires a compensatory increase in alcoholics in order to maintain the same level of performance. Such functional reorganization was also revealed in alcoholics engaged in a visual spatial working memory task. In this case, despite equivalence in behavioural performance, uncomplicated alcoholics activated the ventral 'what' stream generally used for encoding of verbal material, whereas controls activated the dorsal 'where' stream appropriately used to encode visuospatial information.

Procedural Memory

Procedural memory is shown on the right-hand side of MNESIS (**Figure 1**). This memory system is connected to both episodic memory and working memory, which are required during encoding of a new procedure, that is, during procedural learning. Procedural learning is a dynamic process involving different phases (cognitive, associative, and autonomous) and resulting in the automation of the procedure. The cognitive stage of such acquisition involves episodic memory and working memory; impairments of these memory systems in uncomplicated alcoholics may therefore hinder cognitive procedural acquisition and hamper its automation. Early in alcohol sobriety, uncomplicated alcoholic subjects

solve cognitive procedural problems more slowly than the controls and make more moves to achieve a new cognitive procedure. Alcoholics are also slower than controls, sacrificing speed for accuracy during learning. By dint of repeating numerous trials, alcoholic subjects manage to catch up with the controls and achieve similar performance. However, alcoholics are typically not at the same stage of learning as controls would be, being still in a problem-solving mode at the end of the learning phase and implementing higher-order cognitive processes to achieve normal learning levels. Their episodic and working memory deficits may prevent them from completing the cognitive and associative stages and thus from automating the cognitive procedure. A recent sober uncomplicated alcoholic may be able to acquire a new cognitive procedure, but it would be more difficult and take longer than nonalcoholic subjects.

In addition to procedural memory, other forms of implicit memory processes have been examined. For example, alcoholism is associated with impairments in conditional responding, especially when the learned response depends on former reactions or the inhibition of the prepotent response patterns. Deficits of associative learning, using eyeblink classical conditioning, are also reported in uncomplicated alcoholics. When using reversal eyeblink classical conditioning, which involves an inversion of the two conditioned stimuli during the task, the alcoholics who learn the initial discrimination are impaired in acquiring the reversed one. These findings suggest that a factor in the maintenance of alcohol dependence is the presence of alcoholic-related associative responses that interfere with the ability to learn new adaptive associations.

Factors Contributing to the Heterogeneity of Memory Disorders in Uncomplicated Alcoholism

Human alcohol consumption is marked by considerable heterogeneity in its consequences on cognition including memory abilities. Potential sources of variability include age, gender, alcohol use pattern, smoking, nutritional status, and psychiatric comorbidity.

Normal aging disproportionately affects some brain regions, and these compromised brain areas in the healthy elderly are also vulnerable to alcohol neurotoxicity. Age and alcoholism interact thereby increasing brain vulnerability to alcohol's neurotoxicity in aging, which, in turn, increases the chances of more severely impaired cognitive capacities in the elderly who abuse alcohol.

Gender may also be a factor influencing the nature and the severity of the memory disorders in uncomplicated alcoholics. Patterns of differences occurring in the

cognitive profiles of healthy men and women may be affected by alcohol use.

The relationships between drinking history and severity of memory disorders are still a matter of debate. Drinking history can be characterized in terms of age at onset (first contact with alcohol or onset of alcoholism), quantity (number of drinks per day), and frequency (number of days drinking per month) of alcohol consumption. Studies yield heterogeneous results, and it remains unknown why some alcoholic subjects exhibit severe impairments, whereas others with measurably similar alcohol consumption levels appear to drink with impunity.

A growing body of evidence suggests chronic cigarette smoking which occurs with high prevalence in alcoholism, itself results in abnormalities in brain morphology, blood flow, neurochemistry, and neurophysiology, leading to cognitive dysfunction. Alcoholism and smoking being frequently associated, the effect of alcoholism *per se* and its interaction with smoking require further examination.

Sustained heavy drinking frequently occurs at the expense of eating, rendering alcoholics at risk for nutritional depletion. In particular, thiamine deficiency appears even in alcoholics without clinically apparent Wernicke encephalopathy and may interact with neurotoxic effects of alcohol *per se*.

Almost 60% of the alcoholics have at least one additional psychiatric (mood or anxiety) or past substance-dependence comorbidity. However, psychiatric comorbidity does not necessarily compound poor cognitive test performance associated with chronic alcohol misuse, suggesting that poorer cognitive performance is more a function of alcoholism *per se* than nonalcoholic comorbidity.

Reversibility

Some cognitive recovery may take place with sustained abstinence from alcohol consumption. The greatest recovery occurs during the first month of drinking cessation, but further improvements are detectable with prolonged abstinence. Indeed, alcoholics have been shown to present memory capacities equivalent to those of control subjects after several years of abstinence. However, some investigations report persistent cognitive impairments in alcoholics 6 months after drinking cessation and even after more than 5 years' abstinence. The lack of accuracy as to the length of abstinence required for normalization may be due to variability in the ability of different cognitive functions to recover, complicating simultaneous analysis of several domains of cognition.

Three main methodological issues inherent to the investigation of cognitive recovery with abstinence from alcohol in alcohol-dependent individuals have to be considered. First, the interaction between age and length of

abstinence may induce a slowdown in the course of recovery in the older alcoholics, notably because of a decrease in brain plasticity in aging. Second, the use of cross-sectional comparisons between groups of alcoholic subjects who are abstinent for two different lengths is not recommended. Even when patient groups are matched in demographic and alcoholism characteristics as closely as possible, cross-sectional comparisons yield less reliable results than longitudinal studies, which monitor recovery in a single sample of subjects, notably because the period of abstinence is variable and represents only a mean or a threshold, leading to an inaccurate picture of the differences between groups. The last factor, which has to be considered when evaluating normalization of abilities, is interim drinking during the follow-up. For example, interim drinking is considered differently according to the investigations, in that alcoholic subjects who have resumed drinking to a modest degree (perhaps just one drink) may be included either in the abstinent or in the relapsed group, whereas some studies consider abstinent subjects as "having not drunk to intoxication." Under conditions of strictly monitored long-term alcohol abstinence, memory capacities have been showed to return to normal in small samples of alcoholics.

Recovery of cognitive function has occasionally been associated with at least partial recovery of alcoholism-related brain shrinkage. This brain recovery may be selective according to the neuropsychological changes observed, and associations were observed between memory improvement and lateral ventricular volume change. In effect, the reduction of alcoholics' brain weight and volume, which is well documented, is largely due to an overall decrease of white matter volume, including demyelination, axonal and glial loss, and dehydration. Recent findings suggested that the reversibility of the brain damage does not only reflect a rehydration but a growth of the white matter volume associated with increased levels of *N*-acetylaspartate (a marker of neuronal integrity) and choline (a marker of cell membrane turnover) and modest increase in cortical gray matter volume. Regarding the hippocampus, a principal neural substrate of episodic memory consolidation, experiments using rodents models of binge drinking indicate that adult neurogenesis in the hippocampus is inhibited during alcohol intoxication but returns to normal with cessation of drinking. Whether substantial and functionally viable neurogenesis occurs in the human hippocampus or other brain structures remains unknown.

Relapse Prediction

Longitudinal studies of alcoholic subjects provide evidence for recovery of cognitive functions with abstinence and provide an opportunity to explore

whether cognitive abilities at initial testing are predictive of abstinence or relapse and therefore the outcome of treatment. For example, are alcoholic subjects with the lower cognitive functions at alcohol treatment entry the most likely to relapse? Conversely, are alcoholics with the highest cognitive functions the most likely to remain abstinent? Even though efficient memory functioning would appear to promote abstinence, the literature is contradictory. Some investigations reported that poor cognitive functioning, and more particularly memory disorders, are predictive of difficulty in maintaining long-term abstinence from alcohol, whereas others have failed to find any such link. Other factors, such as depressed mood, social status, and feeling of self efficacy, may be more relevant predictors than cognitive impairments. Whatever the predictive value of the neuropsychological impairments at alcohol treatment entry, they may be useful indicators when it comes to choosing the best treatment at the best time for each alcoholic subject.

Uncomplicated Alcoholism to Korsakoff's Syndrome: A Graded Effect of Memory Impairments

It was suggested many years ago that alcoholic Korsakoff patients, uncomplicated alcoholics, and heavy social drinkers represent separate points along a single scale of cognitive impairments. Continuity between alcoholic Korsakoff patients and uncomplicated alcoholics has actually been demonstrated in numerous neuropsychological investigations of cognitive functions except memory. Indeed, results of initial studies did not provide strong evidence of continuities in memory processes. Two possible explanations were therefore provided: one was that memory deficits appear when alcoholism is combined

with a thiamine deficiency, and a second was that investigators did not employ tests appropriate to gauge uncomplicated alcoholics' memory disorders and did not distinguish the different component processes of memory, each with the potential of being differentially affected. Recent works showing that the pattern of memory sparing and impairment in uncomplicated alcoholics (**Table 1**) is broadly consistent with that of alcoholic Korsakoff patients challenge the question of the specificity of the memory profile in Korsakoff's syndrome and revive the continuity hypothesis of disordered memory processes.

Most of the memory deficits in alcoholic Korsakoff subjects seem to be already present in uncomplicated alcoholics, either in an equivalent form (working memory) or in a less severe one (episodic memory). Regarding episodic memory, alcoholic Korsakoff subjects may therefore be considered as uncomplicated alcoholics, whose existing deficits due to the neurotoxic effects of ethanol on Papez circuit have been exacerbated by thiamine deficiency, leading to amnesia syndrome. Working memory impairments do not seem to be specific to Korsakoff's syndrome and may simply reflect the effects of chronic alcohol consumption, for example, on frontocerebellar circuitry.

These findings therefore lend weight to a revised continuity theory between uncomplicated alcoholics and alcoholic Korsakoff subjects. Indeed, the continuity theory postulates that alcohol has a neurotoxic effect on brain function and there is therefore a relationship between cognitive performance and drinking history. As we discussed previously, researchers have so far failed to demonstrate any robust or consistent relation between neuropsychological deficits and drinking history, notably because it is difficult to gain an accurate picture of the drinking history of alcoholic patients, let alone alcoholic Korsakoff patients. Even though their drinking history

Table 1 Synthesis of the memory disorders in uncomplicated alcoholics

<i>Memory systems</i>	<i>Memory components or nature of the information</i>	<i>Status</i>
Episodic memory	Encoding	—
	Retrieval	—
	Contextual memory	—
	Autonoetic consciousness	—
	Episodic component of autobiographical memory	—
Semantic memory	Semantic learning	— and use of different strategies ?
	Pre-existing semantic network	?
	Implicit perceptual learning	+ but use of different strategies
Perceptual memory	Central executive	—
	Phonological loop	—
	Visuospatial sketchpad	—
	Buffer episodic	—
Working memory	Cognitive procedural learning	— and use of different strategies ?
	Perceptual-verbal procedural learning	?
	Perceptual-motor procedural learning	?
Procedural memory	Cognitive procedural learning	— and use of different strategies ?
	Perceptual-verbal procedural learning	?
	Perceptual-motor procedural learning	?

— : slightly impaired; — : more severely impaired; + : preserved; ? : unexplored.

may not explain the heterogeneity of alcoholics' cognitive abilities, the theory of a continuum between non-Korsakoff alcoholics and alcoholic Korsakoff patients may at least have a heuristic value and extend all the way to alcohol dementia. These neuropsychological findings are corroborated by neuroimaging results indicating graded regional brain volume shrinkage from uncomplicated alcoholics to alcoholic Korsakoff patients.

Clinical Implications

Neuropsychological impairments, and more especially memory disorders, could impede uncomplicated alcoholic subjects from benefiting fully from cognitive and behavioral treatment approaches for alcohol dependence. Indeed, cognitive dysfunctions of alcoholic subjects have been shown to be linked with the outcome of the treatment. Successful alcohol treatment and subsequent abstinence may require high-level cognitive processes and consequently may not be feasible by alcoholic subjects with extensive impaired memory capacities. Because the classic treatment of alcoholism, notably based on cognitive behavioral treatment, may rely on the acquisition of novel semantic information or cognitive procedures, not all alcoholic subjects may be cognitively able to acquire such complex novel knowledge. Consequently, current forms of treatment may not be appropriate for alcoholic subjects with neuropsychological impairments. The assessment of neuropsychological deficits at alcohol treatment entry may prove to be useful for clinical decision making and the choice of the most appropriate treatment. Lastly, repetition of material or procedures to be learned would be a useful strategy with alcoholics who are in educational treatment programs, wherein they are expected to acquire new information that may be relevant in later settings. The neuropsychological screening of these cognitive impairments may prove to be useful at alcohol treatment entry, allowing clinicians to ascertain whether alcoholic subjects are capable of undergoing standard therapy or whether it needs to be adjusted in order to take account of memory disorders. Follow-up testing would document change in memory function that could alter therapeutic programs dynamically.

According to a recent epidemiological survey in the United States (NESARC), only about a quarter of alcoholics ever seek treatment. Some current studies indicate that those alcoholics who remain drinkers are not necessarily affected by alcoholism to a significant extent. These alcoholics may enjoy a special protection, such as relatively safe drinking pattern or safe environment for drinking (such as healthy diet or exercise).

Regarding the graded effect of memory disorders from uncomplicated alcoholics to alcoholic Korsakoff subjects, findings emphasize the need for uncomplicated alcoholics to undergo a neuropsychological assessment, focusing on memory abilities. Those with equivocal episodic memory performances, similar to those of Korsakoff subjects, may be regarded as borderline alcoholics at risk of developing Korsakoff's syndrome and should receive particular attention and preventive action before their memory disorders have harmful repercussions on their daily lives.

See also: Alcoholism; Amnesia; Animal Models of Behavior: Alcohol Addiction; Animal Models of Learning and Memory; Brain Imaging and Addiction; Episodic and Autobiographical Memory: Psychological and Neural Aspects; Implicit Learning and Memory: Psychological and Neural Aspects; Korsakoff's Syndrome.

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Molecular Neurobiology of Addiction

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Glossary

Chromatin – Material in the cell nucleus composed of DNA, histones, and non-histone proteins. The structure of chromatin (whether its open or closed) around a gene determines the rate at which that gene is transcribed.

Chromatin immunoprecipitation (ChIP) – Experimental procedure in which lightly fixed chromatin is fragmented and then immunoprecipitated with an antibody to a histone modification, transcription factor, or some other nuclear regulatory protein; the level of a given gene in the immunoprecipitate is then quantified.

Dominant-negative antagonist – A protein, which by itself is inactive biologically, inhibits the activity of another endogenous protein.

Gene expression – The process controlling the types and amounts of individual genes expressed (transcribed into mRNA and protein) in a tissue.

Histone – The major protein constituent of chromatin. Covalent modification of histones (e.g., their acetylation, methylation, and phosphorylation) controls the degree to which chromatin is active or inactive.

Histone acetyl transferase – Enzymes that add acetyl groups to histones and thereby generally promote gene expression.

Histone deacetylase – Enzymes that remove acetyl groups to histones and thereby generally inhibit gene expression.

Nucleosome – Unit of chromatin consisting of the DNA double helix wound around an octomeric complex of eight histone proteins.

Protein phosphorylation – A process by which phosphor groups are added to other proteins by enzymes called protein kinases. Phospho groups, because of their large size and charge, alter the activities of proteins, for example, they can activate TFs.

Second messenger – Signals in the cells that are induced by neurotransmitter-receptor interactions and mediate the effect of those interations on virtually all aspects of a nerve cell's function, including the regulation of gene expression.

Transcription – The process by which a gene is transcribed into its encoded mRNA (messenger RNA). mRNA is then translated into protein.

Transcription factor – A class of protein that binds to specific receptor sites present in target genes and thereby increases or decreases the extent to which that gene is expressed.

The Synapse as the Target of Drugs of Abuse

All drugs of abuse initially affect the brain by influencing the amount of a neurotransmitter present at the synapse or by interacting with specific neurotransmitter receptors. **Table 1** lists examples of such acute pharmacological actions of some commonly used drugs of abuse. The fact that drugs of abuse initially influence different neurotransmitter and receptor systems in the brain explains the very different actions produced by these drugs acutely. For example, the presence of high levels of opioid receptors in the brainstem and spinal cord explains why opiates can exert such profound effects on respiration, level of consciousness, and nociception. In contrast, the importance of noradrenergic mechanisms in the regulation of cardiac function explains why cocaine can exert potent cardiotoxic effects.

In contrast to the many disparate acute actions of drugs of abuse, the drugs exert some common behavioral effects: they are all positively reinforcing after short-term exposure and cause a similar behavioral syndrome (addiction) in vulnerable individuals following long-term exposure. This suggests that there are certain regions of the brain where the distinct, acute, pharmacological actions of these drugs converge. Indeed, we now know that activation of opioid receptors (by opiates), inhibition of monoamine reuptake (by cocaine), or facilitation of γ -aminobutyric acid (GABA)-ergic and inhibition of N-methyl-D-aspartate (NMDA) glutamatergic neurotransmission (by ethanol) elicit some common neurobiological responses – in the brain's reward circuitry – that mediate their reinforcing and addicting properties. The best-established components of the reward circuitry, as discussed elsewhere in this encyclopedia, include dopamine (DA)ergic neurons in the ventral tegmental area (VTA) of the midbrain and their projections to the nucleus accumbens (NAcc) and several other limbic forebrain regions.

Transcriptional Mechanisms of Addiction

The acute pharmacological actions of a drug of abuse *per se* do not explain the long-term effects of repeated drug exposure. To understand such long-term effects, it is

Table 1 Examples of acute pharmacologic actions of drugs of abuse

Drug	Action
Opiates	Agonist at μ , δ , and κ opioid receptors ^a
Cocaine	Inhibits monoamine reuptake transporters
Amphetamine	Stimulates monoamine release
Ethanol	Facilitates GABA _A receptor function and inhibits NMDA glutamate receptor function ^b
Nicotine	Agonist at nicotinic acetylcholine receptors
Cannabinoids	Agonist at CB ₁ cannabinoid receptors ^c
Hallucinogens	Partial agonist at 5HT _{2A} serotonin receptors
Phencyclidine (PCP)	Antagonist at NMDA glutamate receptors

^aActivity at μ and δ receptors is thought to mediate the reinforcing actions of opiates.

^bThe mechanism by which ethanol produces these effects has not been established. In addition, ethanol affects many other neurotransmitter systems in brain.

^cSeveral lipid-related molecules have been implicated as endogenous ligands for this receptor, such as anandamide.

GABA_A, γ -aminobutyric acid A; NMDA, *N*-methyl-D-aspartate; 5HT₂, 5-hydroxytryptamine (serotonin) 2.

necessary to understand adaptive mechanisms in neurons, which involve the ability of neurotransmitter–receptor interactions to regulate virtually every process in a neuron over a longer timescale. Such effects are mediated by altering the functional activity of proteins that are already present in the neuron or by regulating the amount of the proteins through the regulation of gene expression. The remainder of this article provides an overview of the mechanisms by which repeated exposure to a drug of abuse regulates gene expression in brain reward regions.

The activation or repression of specific transcriptional patterns is part of a neuron's response to virtually every cellular signal. Accordingly, transcriptional regulation can be viewed as the ultimate target of signal transduction cascades, and is a critical mediator of a wide range of neuroadaptations in neural structure, connectivity, and function, which occur in response to environmental stimuli, such as drugs of abuse.

Considering the importance placed on alterations in gene expression in addiction, it is surprising that only a small number of transcription factors have, to date, been directly implicated in addictive disorders. Moreover, even less is known about the target genes that are regulated by these transcription factors to mediate stable behavioral change. This article focuses on those transcription factors which have been shown to regulate the activity of the brain's reward pathways in animal models of drug addiction.

CREB–CREM–ATF Family

The cyclic adenosine monophosphate (cAMP) response-element-binding protein (CREB) family of transcription factors, in addition to CREB itself, include cAMP

response-element modulator (CREM) and activators of transcription (ATFs). These proteins are bZip transcription factors; bZip refers to the basic domain (b) of the proteins that binds a specific consensus sequence (cAMP response element (CRE)) in gene promoter regions, and the leucine zipper domain (Zip) which allows homo- or heterodimerization of two bZip transcription factors necessary for the transcriptional activity of these proteins. Although most proteins of this family heterodimerize selectively with other CREB–CREM–ATF transcription factors, some family members (specifically ATF2) can dimerize with bZips of other families and act on distinct response elements. To complicate matters further, there are many splice variants of these transcription factors that can act as potent repressors of transcription due to the absence of activation domains in the proteins. An example is inducible cAMP early repressor (ICER) – a product of the CREM gene, which represses CRE-mediated transcription.

Most evidence for a role of the CREB–CREM–ATF family in brain reward regions in addiction models has focused on CREB itself. CREB is activated both in the NAcc and VTA in response to acute and chronic administration of certain drugs of abuse (cocaine, amphetamine, opiates, etc.). This has been shown by direct demonstration of increases in the phosphorylation of CREB at ser133, which is required for its transcriptional activation. It has also been demonstrated by use of CRE-LacZ transgenic mice, in which CRE transcriptional activity is induced in brain reward regions under these conditions.

There is now a large body of evidence to support the view that CREB activation in the NAcc by drugs of abuse mediates a form of tolerance and dependence to drug exposure. Viral-mediated overexpression of CREB in the NAcc decreases an animal's sensitivity to the rewarding effects of several drugs of abuse, whereas blockade of CREB function in this region, via overexpression of a dominant-negative antagonist of CREB, causes the opposite effect. Studies of bitransgenic mice – in which CREB or its dominant negative is inducibly expressed in the NAcc of adult animals – as well as studies of CREB knockdown mice, generally support these conclusions. Interestingly, increased CREB activity in the NAcc also reduces an animal's sensitivity to natural rewards and induces depression-like behavior in several rodent assays. Thus, CREB activation in the NAcc by drugs of abuse could mediate some of the negative emotional symptoms seen in many drug addicts during early phases of withdrawal.

CREB activity in the VTA is more complex. Here, CREB can either decrease or increase an animal's sensitivity to a drug of abuse depending on the subregion of the VTA involved. Further work is needed to better understand these actions of CREB, as well as the influence of several other members of the CREB–CREM–ATF family in regulating responses to drugs of

abuse. There is recent evidence, for example, that drugs of abuse – in addition to activating CREB – also activate ICER as well as ATF2, ATF3, and ATF4 in the NAcc. Consequently, the net effect of drug exposure on the expression of CRE-containing target genes is likely to be very complex. For example, activation of ICER – a transcriptional repressor – may serve to dampen the functional consequences of CREB (and of some of the ATFs) induced during the course of drug exposure.

The effects of CREB and related transcription factors on NAcc function and behavior are mediated in part by the opioid peptide dynorphin, which is increased by CREB and decreased upon inactivation of CREB. Dynorphin acts on κ -opioid receptors within the NAcc and VTA to produce aversive effects by reducing DA release from presynaptic dopaminergic terminals (**Figure 1**). Thus, activation of CREB, and the resulting induction of dynorphin, in response to long-term drug exposure represents a mechanism of tolerance to drug reward as well as dysphoria during drug withdrawal (dependence).

AP1 Family

Fos and Jun proteins, like CREB proteins, are bZip transcription factors. Fos family members dimerize with Jun family members to form active AP1 complexes, which then bind to AP1 sites in gene promoters. Genes encoding Fos and Jun family proteins are immediate early genes, which means that they are induced very rapidly in

response to an acute stimulus. Accordingly, acute administration of virtually any drug of abuse induces many Fos and Jun family members in the NAcc. Not only is the induction very rapid, but it is also very transient, because the Fos and Jun mRNA's and proteins are all highly unstable.

Δ FosB is a C-terminal truncated splice variant of the FosB gene. It, too, is induced rapidly in response to acute drug exposures. However, unlike all other Fos and Jun family proteins, Δ FosB is a relatively stable protein. This stability is mediated partly by the loss of the C-terminus – which contains degron domains that are conserved in all other Fos family proteins – and by its phosphorylation. As a result, in response to repeated drug administration, levels of Δ FosB accumulate and eventually become the predominant Fos family protein in NAcc neurons. This pattern of induction is illustrated in **Figure 2**. Induction of Δ FosB occurs as a common response to many classes of addictive drugs, including cocaine, amphetamine, methamphetamine, opiates, nicotine, phencyclidine (PCP), cannabinoids, and alcohol. Δ FosB is also induced in the NAcc after repeated consumption of natural rewards, like wheel running, sucrose intake, or sexual behavior.

Studies with inducible bitransgenic mice and viral vectors show that induction of Δ FosB in the NAcc increases an animal's sensitivity to the rewarding effects of drugs of abuse, and also seems to increase incentive motivation, or drive, for the drugs. In addition, Δ FosB increases an animal's drive for natural rewards. Thus, Δ FosB may function as a sustained molecular switch,

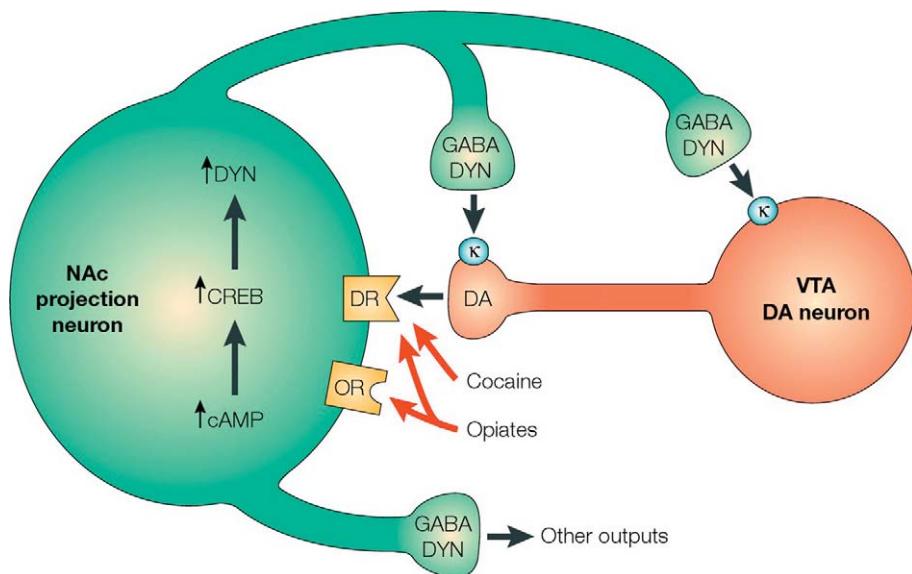


Figure 1 Feedback between the NAc and VTA via CREB activation. Cocaine and amphetamine have been shown to activate *prodynorphin* gene expression in the NAc via D₁ dopamine receptor stimulation, the cyclic adenosine monophosphate (cAMP) pathway, and the phosphorylation of CREB. The resulting dynorphin peptides are transported to presynaptic terminals including terminals that feed back on VTA dopaminergic neurons. Dynorphin peptides are agonists at inhibitory κ -opioid receptors, resulting in decreased dopamine release. From Nestler EJ (2001) Molecular basis of long-term plasticity underlying addiction. *Nature Reviews Neuroscience* 2: 119–128.

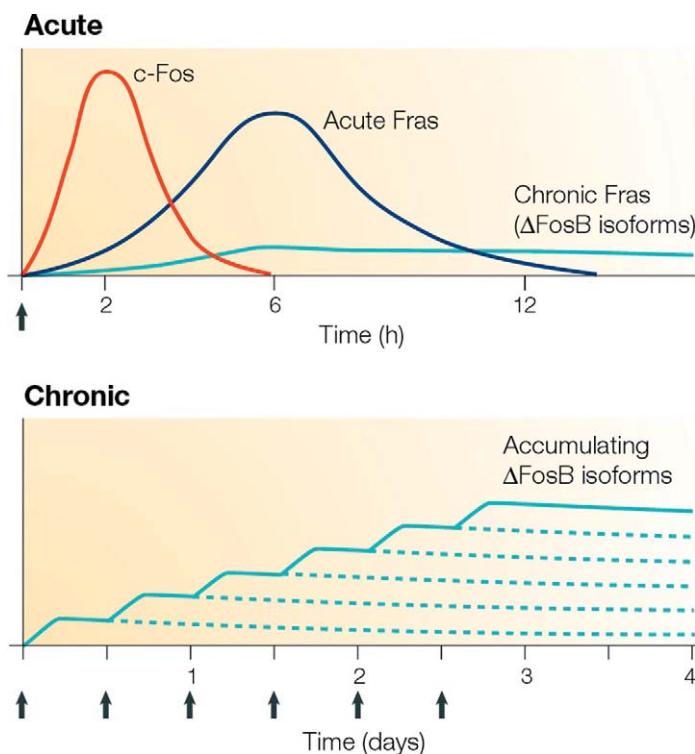


Figure 2 Regulation of Δ FosB by drugs of abuse. The figure shows the several waves of induction of Fos family proteins in the NAc after a single exposure to a drug of abuse. These proteins include c-Fos and several other Fos family proteins (FosB, Fra-1, Fra-2, etc.). Unlike all of these Fos family proteins, which are highly unstable, isoforms of Δ FosB are highly stable and, therefore, persist in the brain long after drug exposure. Because of this stability, Δ FosB accumulates uniquely with repeated drug exposures. From Nestler EJ (2001) Molecular basis of long-term plasticity underlying addiction. *Nature Reviews Neuroscience* 2: 119–128.

which first helps initiate and then maintains a state of addiction for many types of rewards for a relatively prolonged period of time.

There is early evidence that induction of Δ FosB in the NAcc may mediate the ability of a drug of abuse to increase the dendritic branching, and the number of spines on distal dendrites, in NAcc neurons. Although the precise functional role played by these morphological changes is not known, the changes have been shown to persist for at least several months after the last drug exposure, and are hypothesized to mediate the near-permanent sensitization in drug responsiveness seen in certain animal models of addiction. Δ FosB is one mediator of these drug-induced changes in dendritic structure via its regulation of several target genes. For example, chronic cocaine administration induces cyclin-dependent kinase 5 (Cdk5) and nuclear factor κ B (NF κ B) in the NAcc, effects mediated via Δ FosB, and infusion of an inhibitor of either Cdk5 or NF κ B into the NAcc prevents the ability of cocaine to increase dendritic spine density in this region. The implication of these findings is that structural changes caused by repeated cocaine administration may be mediated, in part, via induction of Δ FosB and may persist long after the Δ FosB signal itself

dissipates. While Δ FosB is the longest-lasting known molecular change in the brain seen in the context of drug exposure, and perhaps to any other perturbation of the adult brain, it nevertheless undergoes proteolysis at some finite rate: Δ FosB dissipates to normal levels within a month or two of drug withdrawal. This means that Δ FosB cannot *per se* mediate the extremely long-lived changes in brain and behavior associated with addiction and depression. One possibility is that Δ FosB causes other changes in the brain, such as the changes described in dendritic structure, which themselves are more permanent.

Other Transcription Factors that Regulate Brain Reward Pathways

Recent work has shown that several other transcription factors are also regulated in brain reward regions by drugs of abuse, although in general much less is known about the behavioral consequences of their induction compared with CREB and Δ FosB. As stated above, the transcription factor, NF κ B, has recently been shown to be induced in the NAcc by chronic cocaine. NF κ B has been the subject

of intense investigation for its widespread involvement in a multitude of disease states and for its inducible pattern of gene expression. Several types of cellular stress, including cytokines, growth factors, viruses, and environmental hazards, induce NF κ B in peripheral cells. Under normal conditions, NF κ B, which is composed of a dimer of any of several subunits, termed p50, p52, p65, c-Rel, and RelB (most abundantly p50 and p65), remains sequestered in the cytoplasm by inhibitory- κ B (I κ B). Upon phosphorylation by I- κ kinase (I κ K), I κ B releases the inactive NF κ B dimer, which can then be phosphorylated and transported to the nucleus where it undergoes further posttranslational modifications to initiate transcription of a range of genes involved in cell survival. In the brain, NF κ B has received considerably less attention, however, it has been implicated in certain forms of learning-related plasticity. Although the involvement of NF κ B in addiction and depression models is not well studied, NF κ B has been implicated in the neurotoxic effects of amphetamine and amphetamine-like drugs. Furthermore, chronic cocaine administration upregulates the NF κ B subunits p65, p105 (precursor to p50), and I κ B γ and thereby increases NF κ B-dependent transcription in the NAcc where it serves to enhance dendritic arborizations. As already mentioned, cocaine induction of the NF κ B subunits is mediated by Δ FosB. Studies are underway to better understand the target genes downstream of NF κ B upregulation in drug-addiction models.

Clock and other members of the basic helix-loop-helix-PAS (PER-ARNT-SIM) transcription factor family are best known for their control of circadian rhythms. The first evidence that this gene family may be involved in reward came from studies of *Drosophila*, where loss of Clock dramatically altered the flies' responses to cocaine. More recent work has demonstrated a role for Clock and other circadian genes in mammalian models of addiction. Thus, mice lacking Clock show dramatically increased activity of VTA DA neurons and increased behavioral responses to cocaine. Mice lacking the Clock homolog, NPAS2, or the Clock-NPAS2 target genes, Per (period), also show abnormal responses to the rewarding effects of cocaine. Current research is focused on defining the target genes through which these circadian genes act to regulate the VTA-NAcc pathway in addiction models.

Several drugs of abuse induce the release of systemic glucocorticoids, which activate glucocorticoid receptors expressed throughout the brain. These receptors, which are members of the nuclear receptor family of transcription factors, are expressed in the VTA and NAcc. As glucocorticoids are released in response to stress, they may provide one important mechanism by which stress regulates the reward pathway and addiction behavior. Estrogen and progesterone receptors, members of the nuclear receptor family as well, are also expressed in the

VTA-NAcc pathway and have been found to regulate drug-reward mechanisms. Such effects may contribute to gender differences observed in behavioral responses to drugs of abuse and stress. However, the target genes for glucocorticoid, estrogen, and progesterone receptors in the VTA and NAcc, through which steroid hormones regulate reward mechanisms, have not yet been established.

Regulation of Chromatin Remodeling in Brain Reward Pathways

Binding of transcription factors to their target genes regulates transcription via chromatin-remodeling events, which are becoming increasingly well understood. Interest in chromatin remodeling in the context of drug addiction comes from two sources. First, studies of chromatin remodeling make it possible to delineate the detailed molecular events by which drug-regulated transcription factors activate or repress target genes in the VTA and NAcc *in vivo*. Second, such changes in chromatin structure provide a novel mechanism by which exposure to a drug of abuse may cause lasting changes in gene expression that outlive changes in the transcription factors themselves.

The rate of expression of a particular gene is controlled by its location within nucleosomes and by the activity of the cell's transcriptional machinery. A nucleosome is a short span of DNA that is wound around a complex of histones and other nuclear proteins. Transcription of a gene requires the unwinding of a nucleosome, which makes the gene accessible to the basal transcription complex, comprised of RNA polymerase (which transcribes the new RNA strand) and numerous regulatory proteins (which unwind the nucleosomes). Transcription factors act by enhancing (or inhibiting) the activity of the basal transcription complex; this is achieved by altering nucleosomal structure through changes in histone acetylation, effects mediated by histone acetyltransferases (HAT) or histone deacetylases (HDAC), as well as through many other modifications of histones or the DNA directly. Transcription factors also recruit other regulatory proteins to the complex, which further modify chromatin structure. For example, chromatin remodeling involves enzymes (e.g., SWItch/Sucrose NonFermentable (SWI-SNF; mating switching and sucrose nonfermenting) complex) that reposition nucleosomes, in an adenosine triphosphate (ATP)-dependent manner, and thereby further make genes accessible for transcription.

Recent evidence has demonstrated the relevance of chromatin remodeling to drug-induced neuroadaptations within the brain's reward pathway. This work is made possible by use of chromatin immunoprecipitation (ChIP)

assays, in which brain tissue (under control or drug-treated conditions) is lightly fixed in formaldehyde to cross-link DNA to nearby histones and other proteins. The fixed chromatin is then sheared into smaller fragments, immunoprecipitated using an antibody directed against a protein of interest, the immunoprecipitate is un-cross-linked, and then individual genes of interest are analyzed by quantitative real-time polymerase chain reaction (PCR). Alternatively, the immunoprecipitated DNA is amplified and analyzed on a promoter chip, or sequenced, to obtain a genome-wide assessment of regulated genes. This approach makes it possible to identify changes that occur genome-wide within the brain reward circuit as a consequence of drug exposure.

Using ChIP assays, differential chromatin-remodeling events (such as histone acetylation and deacetylation) have been observed at various cocaine-regulated genes. For example, it was found that chronic, but not acute, cocaine administration induces histone acetylation at the Cdk5 gene promoter in the NAcc. This is consistent with earlier observations that cocaine induces Cdk5 expression in this brain region, but provides the first direct evidence that this induction is mediated via activation of the Cdk5 gene *per se*. Moreover, after chronic cocaine use, ChIP assays revealed increased binding of ΔFosB to the Cdk5

gene promoter, while inducible overexpression of ΔFosB in the NAcc of adult bitransgenic mice, which is sufficient for induction of Cdk5 expression *in vivo*, caused increased binding of ΔFosB to the Cdk5 gene. Chronic cocaine administration, or inducible overexpression of ΔFosB, also causes increased binding of Brg1 (a component of the SWI-SNF complex) to the Cdk5 gene. Together, these results support a scheme whereby the gradual accumulation of ΔFosB, in response to chronic cocaine administration, recruits chromatin-remodeling factors, such as specific HATs to induce histone acetylation as well as Brg1-containing chromatin-remodeling complexes to its target genes such as Cdk5 (**Figure 3**).

Importantly, recent work has established the behavioral relevance of histone acetylation in the NAcc. First, viral-mediated overexpression of particular HDACs in the NAcc dramatically blocked the rewarding effects of cocaine. Conversely, treatment of animals with structurally distinct HDAC inhibitors caused the opposite effect and increased an animal's sensitivity to the behavioral effects of cocaine. These HDAC inhibitors were also found to interact synergistically with cocaine to induce histone acetylation at responsive gene promoters. One of the important HDACs involved in these phenomena is HDAC5, whose function is downregulated in the NAcc

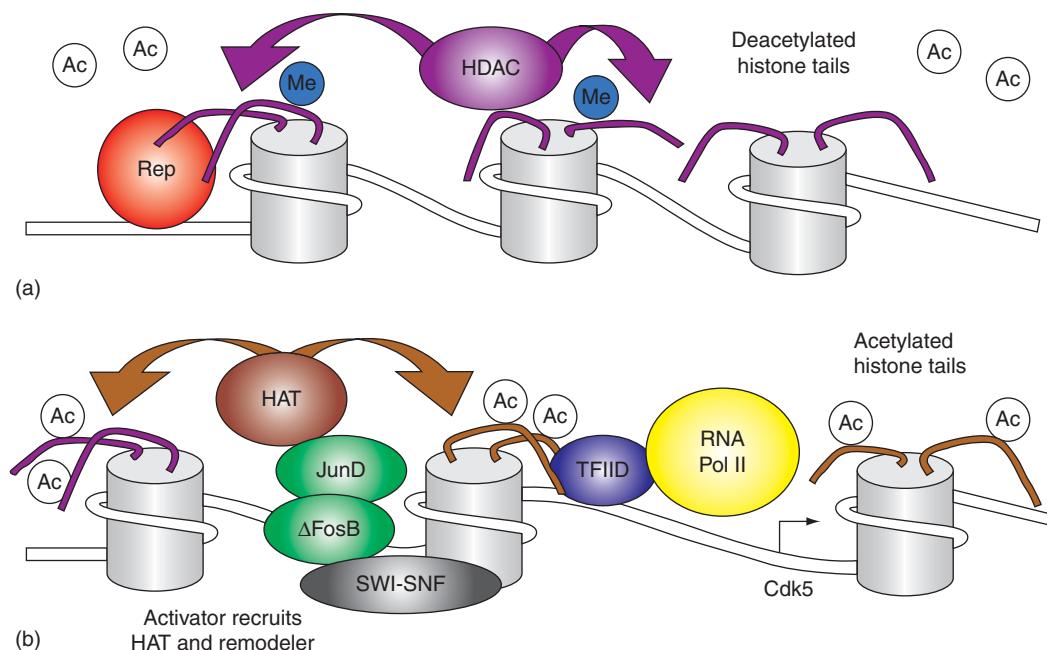


Figure 3 Scheme of proposed chromatin-remodeling events at a cocaine-activated gene. (a) shows the repressed state of chromatin, where a site-specific repressor (Rep) recruits an HDAC complex, which removes acetyl groups (Ac) from histone N-terminal tails. Gene inactivation likely involves other modifications, such as methylation (Me) of histone tails. (b) shows the active state of chromatin around a cocaine-activated gene (e.g., Cdk5), where a cocaine-induced transcriptional activator (e.g., ΔFosB-JunD) recruits a HAT and chromatin-remodeling complex (SWI-SNF), which induce acetylation (and presumably demethylation and other modifications) of histone tails and repositioning of nucleosomes. These actions facilitate the binding of general transcription factors and the basal transcriptional apparatus (e.g., TFIID and RNA polymerase II (PolII)) to the promoter. From Kumar A, Green TA, Russo SJ, Renthal W, and Nestler EJ (2009) Transcription and reward systems. In: Squire LR (ed.) *Encyclopedia of Neuroscience*, ch 195, vol. 9, pp. 1063–1070. Oxford: Academic Press.

by chronic cocaine and which normally serves to dampen cocaine's effects on gene expression and behavior. These findings demonstrate directly that histone acetylation can modify both biochemical and behavioral responses to cocaine, and implicate chromatin remodeling as a key mechanism underlying the molecular control of the brain's reward regions by drugs of abuse.

Future Research

Progress has been made in identifying transcription factors, for example, CREB, ΔFosB, NF κ B, Clock, and steroid hormone receptors, among others, that mediate the effects of drugs of abuse on reward circuits in brain. Future research is needed to better understand how drugs, presumably via regulation of DA and other receptors and of downstream intracellular signaling cascades in the reward circuitry, lead to the regulation of these transcription factors, and how these factors induce chromatin-remodeling events at particular target genes. ChIP assays now make it possible for the first time to identify the precise steps by which different transcriptional complexes are formed at each of the genes regulated in addiction models, and ChIP-chip or ChIP-seq (sequence) assays will help identify the complex gene-regulatory networks affected by these transcription factors to regulate the reward circuitry. This new dimension of molecular research will dramatically expand our understanding of the regulatory control of brain reward regions, and provide fundamentally new paths for the discovery of novel treatments for drug addiction and related conditions.

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See also: Animal Models of Bipolar Disorder; Cellular Plasticity in Cocaine and Alcohol Addiction; Drug Addiction; Neural Systems of Motivation; Neurobiology of Opioid Addiction; Neurophysiology of Drug Reward; Psychostimulants; Rewarding Brain Stimulation.

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Neurobiology of Opioid Addiction

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Glossary

Amygdala (nucleus amygdala) – Almond-shaped nuclei within the medial temporal lobes involved in emotional processing and memory.

c-Fos – A proto-oncogene member of the immediate early-gene family of transcription factors used as an index of neuronal activity.

cAMP response-element binding (CREB) – A transcription factor involved in the regulation of expression of several genes including brain-derived neurotrophic factor (BDNF), c-fos, neuropeptides, and tyrosine hydroxylase.

Conditioned place preference (CPP) – An experimental model based on the concept of classical conditioning to study the conditioned rewarding or aversive effects of drug or non-drug treatments. Animals learn to associate one part of a test chamber with a drug and another part with vehicle by repeated pairings. Later, the animal is allowed to choose between the two environments. An increase or decrease in preference toward the drug-paired context is interpreted as reinforcing versus aversive effects of the drug, respectively.

Craving (psychological dependence) – Intense desire or urge to re-use a substance. Craving is thought to cause relapse following abstinence/withdrawal.

Dependence (physical dependence) – A new equilibrium state that develops to compensate adaptations induced by persistent drug use. The dependant subject requires repeated drug administration in order to maintain normal function, and cessation of drug use causes withdrawal syndrome.

Drug self-administration – An animal model with high face and construct validity based on operant conditioning in which laboratory animals learn to lever-press or nose-poke for drug injections.

Dysphoria – A negative emotional state usually in association with depression and/or anxiety.

Extinction – A type of learning that happens when conditioned stimuli that were previously paired with reinforcing stimulus (food, drug, etc.) are presented in the absence of the reinforcing stimuli. In the drug self-administration procedure, extinction refers to lever pressing in the absence of the drug and/or cues that had previously been paired with drug injections. This leads to a decline in a previously established conditioned response.

Hippocampus – A part of the three-layer archicortex inside the medial temporal lobe that is related to the limbic system and plays important roles in long-term memory formation and spatial navigation.

Locus ceruleus – A brainstem nucleus which is the main source of brain noradrenaline.

Long-term potentiation (LTP) – A form of synaptic plasticity that is thought to be involved in learning and memory processes. In this process, high-frequency, patterned stimulation of cells results in prolonged increase in synaptic transmission efficacy.

Mesocorticolimbic pathway – A major dopamine pathway of the brain connecting the ventral tegmental area to limbic areas (such as the nucleus accumbens, amygdala, and hippocampus) and the cerebral cortex (especially the frontal cortex). It is known to be involved in motivational processing and cognitive performance and the pathogenesis of mental disorders such as addiction, depression, and schizophrenia.

Neuroplasticity – Change in morphology and connections of neurons by external stimuli that cause long-lasting alterations in neural function.

Nucleus accumbens (NAcc) – A pair of forebrain nuclei composed of core and shell parts with distinct morphology and function. Nucleus accumbens is important in reward processing and addictive processes.

Orexin (hypocretin) – A neurotransmitter (made of two neuropeptides – Orexin-A and B) produced exclusively by hypothalamic neurons that send projections throughout the brain. The orexin peptides bind to two G-protein-coupled receptors, OX₁R and OX₂R, and play roles in awareness and reward.

Prefrontal cortex – The anterior part of the frontal lobes involved in higher cognitive and executive functions, such as decision making, goal-directed behavior planning, personality, and social behavior.

Priming – In the drug self-administration paradigm, it refers to re-exposure to a previously experienced drug which usually results in resumption of drug seeking/taking.

Reinstatement – A process that refers to the retrieval of an extinguished memory and, thereby, recovery of a previously learnt response. In the drug-seeking context, mostly refers to the resumption of drug seeking/taking following extinction induced by drugs, drug-associated cues, or stress.

Relapse – A clinical term that refers to the resumption of drug administration following abstinence.

Reward – A stimulus that is interpreted as intrinsically positive by the brain and, therefore, is worked for.

Tolerance – Reduction in subject's responsiveness to a drug with repeated exposure in a way that higher doses are required to achieve the same effect.

Ventral tegmental area (VTA; ventral tegmentum) – A midbrain nucleus that plays an important role in reward processing and the development of many mental disorders.

Withdrawal syndrome – A collection of signs and symptoms associated with the discontinuation of drug intake (or blockage of drug actions by antagonist). Symptoms are usually the opposite of acute drug actions. Withdrawal is thought to play an important role in relapse to addiction.

Among the remedies which it has pleased Almighty God to give to man to relieve his sufferings, none is so universal and so efficacious as opium.

Thomas Sydenham, 17th Century

Definition

Opioids (substance with a morphine-like effect; synonymous here with opiates) include the natural drug opium and its derivatives (such as morphine and codeine), semi-synthetic compounds made of morphine (such as heroin, oxycodone, and oxymorphone), and synthetic drugs (such as fentanyl, levorphanol, meperidine, and methadone), in addition to endogenous opioids that are the naturally occurring ligands for opioid receptors (such as endorphins and enkephalins). Opium is traditionally made by drying the juice produced by lacerating the immature seed capsule of the plant *Papaver somniferum* (opium poppies). Opioids have been the mainstay of pain treatment for thousands of years, and they remain so today. The term narcotic refers to the sleep-producing effects of opioids, although it is not their major therapeutic use today.

Opioids: Past to Present

History

Opium has been used since prehistoric times. Cultivation of opium dates back to the Neolithic Age (4th millennium BC). The first undisputed reference to opium is found in the writings of Theophrastus (third century BC). Physicians of ancient civilizations used opium for the treatment of pain, sedation of crying children, anesthesia, euthanasia,

diarrhea, and melancholy. Opium has been mentioned in important medical texts of the ancient world (such as the writings of Galen and Canon of Avicenna). Sometime between AD 400 and 1200, opium was introduced to the Far East and Europe from the Middle East. Opium lost popularity in inquisitional Europe (AD 1300–1500), when it was believed to be a tool of the devil. Paracelsus reintroduced opium to Western medicine in 1527. In 1680, laudanum (a compound containing opium, sherry wine, and herbs) was introduced by Thomas Sydenham to treat pain, insomnia, and diarrhea. The smoking of pure opium became popular in the eighteenth century and it was brought to North American cities by Chinese workers. Morphine (named after Morpheus, the god of dreams) was isolated from opium in 1806, and was followed by the isolation of other alkaloids. By the middle of the nineteenth century, the use of pure alkaloids in place of crude opium preparations began to spread throughout the medical world. Heroin, the first semi-synthetic opioid, was synthesized in 1874. From 1898 to 1910, heroin was marketed as a nonaddictive morphine substitute and cough medicine for children. Soon, however, it became apparent that it caused profound dependence.

Abuse Potential

To avoid confusion with physical dependence, the term addiction used in this article refers specifically to drug craving and compulsive use. In contrast to physical dependence (a natural consequence of chronic drug exposure), addiction is a chronic mental illness characterized by drug craving and impaired control over compulsive drug taking despite obvious harm to self and society. Hence, addiction and physical dependence can exist independent of each other and, although opioid addicts often show evidence of physical dependence, this is neither sufficient nor necessary to make a diagnosis for addiction. Importantly, the brain areas responsible for physical dependence and withdrawal are, to a considerable extent, anatomically distinct from the neural circuitry that underlies the habit-forming effects of drugs.

Theories of addiction

Different theories based on neurobiological data have been proposed to explain addiction. In the opponent-process theory, chronic drug use produces disequilibrium in brain reward systems causing a vicious cycle of altered hedonic set-point changes. This transition results in compulsive drug use and vulnerability to relapse. Alternatively, in the incentive sensitization theory of addiction, it is postulated that continuous drug exposure alters brain areas involved in incentive motivation for reward, resulting in hypersensitivity to the drug and drug-associated cues and, thereby, a shift from drug liking to drug wanting. Learning-based theories of addiction

propose that repeated drug exposure is associated with particularly strong memories, mediated by drug-induced changes in brain reward regions. Accordingly, drug taking is a learned response to conditioned stimuli, such as drug-associated cues. It has been proposed that impaired decision making due to prefrontal cortex dysfunction contributes to compulsive drug use. Different theories of addiction overlap in some aspects and are not mutually exclusive. None of them alone fully explains all aspects of addiction. It is likely that a combination of factors proposed in each of these theories contributes to the neural and behavioral pathology that underlies addiction.

Abuse vulnerability

Opioids are highly addictive and addiction to opioid drugs is a serious clinical and social problem. Opioids are one of the most commonly prescribed analgesics. It is noteworthy that only a small proportion of individuals who use opioids progress to addiction. The rate of addiction is higher among illicit users than patients using opioids for clinical purposes. Interactions among several internal (i.e., genes) and external (i.e., drug and environment) factors contribute to the transition from abuse to addiction and relapse following abstinence.

Genetic variations have been shown to affect an individual's response to drugs of abuse, vulnerability to addiction, relapse, and treatment outcome for opioid addiction. For instance, specific polymorphisms in genes encoding the cytochrome P450 system, catechol-*O*-methyl-transferase (COMT; an enzyme involved in dopamine (DA) catabolism), DA and serotonin transporters, DA D₂-like receptor, and especially opioid receptors have been related to amounts of heroin use, development of heroin addiction, and craving and relapse to heroin seeking. In addition, the genetic background of an individual is linked to impulsivity, risk-taking and novelty-seeking behaviors, response to stress, as well as other comorbid disorders (such as schizophrenia, mood disorders, and attention-deficit hyperactivity disorder (ADHD)). These factors may increase the risk of abuse and addiction and are associated with an unfavorable prognosis. Abuse of other illicit drugs is common among opioid addicts. Pharmacological properties of drugs are also important. For instance, heroin is more addictive than morphine because it is more potent, has a faster onset of action (due to its higher lipid-solubility), and produces more abrupt and intense syndromes of withdrawal (due to its short half-life).

Epidemiology

Addiction to opioids is a major health problem in the world. Heroin is the most commonly abused opioid. The prevalence of opioid addiction in many developed

countries is less than that of addiction to stimulants or other illicit drugs (the lifetime rate of heroin use in the US is less than 2%). However, the percentage of people addicted to heroin out of those who ever used is much higher (23.1%) than for other drugs of abuse (except tobacco). Heroin addiction occurs most commonly in low socioeconomic populations. Heroin is widely available on the illicit market with a continual decline in price and increase in purity over the last decade. This may be relevant to the doubling of heroin use observed in high school students. Opioid-associated mortality (especially due to heroin overdose, drug-related violence, or human immunodeficiency virus (HIV) infection) is also higher compared to other drugs of abuse such as cocaine. Infection with HIV or viral hepatitis is a very common comorbidity among heroin addicts. Although there are no statistics that provide an accurate number of heroin addicts, it is estimated that over 3.5 million Americans have used heroin and from 800 000 to 1 million heroin addicts reside in the United States of America. The prevalence of heroin use has increased among youths in the last decade. Heroin addiction is seen more commonly in males (male/female ratio: 3:1) in the age range of 30–50 years of age. The advent of newer prescription opioid analgesics (such as oxycontin and other oxycodone derivatives, methadone, fentanyl, and buprenorphine) has led to the abuse of these agents consequent to medical treatment with opioids.

Physiological Effects and Medical Uses of Opioids

Opioid Receptors

Opioids act by binding to and activating specific membrane receptor proteins. Three main subtypes of opioid receptors have been recognized: μ , δ , and κ . These receptors couple to G proteins and thereby modulate the activity of adenylyl cyclase (AC), voltage-gated calcium ion (Ca^{2+}) channels and G-protein-gated inwardly rectifying potassium ion (K^+) (GIRK) currents. Opioid receptors can also couple to other second-messenger systems such as mitogen-activated protein (MAP) kinases and the phospholipase C (PLC). More recently, a fourth subtype, the nociceptin/orphanin FQ (N/OFQ) receptor, has been cloned – based on extensive sequence homology, but not function. Opioid receptors are widely distributed throughout the body and brain including reward-related areas, and each subtype has a unique anatomical distribution in the nervous system. Actions of different subtypes of opioid receptors are summarized in **Table 1**. Most of the clinically used opioids work mainly through μ -opioid receptors (MORs).

Table 1 Physiological actions of opioid receptors

Receptor subtype	Location in CNS	Major Actions	Agonists	Antagonists
μ	Amygdala, caudate, cortex, nucleus accumbens (NAcc), periaqueductal gray (PAG), spinal cord, thalamus	analgesia, ↓ cough, euphoria, ↑ feeding, ↓ gastrointestinal motility, ↑ prolactin and GH release, ↓ immune function, miosis, physical dependence, respiratory depression, sedation	DAMGO Morphine Methadone Endomorphin Met-enkephalin β -endorphin Levorphanol	CTOP CTAP Naloxonazine Naloxone naltrexone
κ	Claustrum, cortex, hypothalamus, NAcc, PAG, spinal cord	analgesia, ↓ antidiuretic hormone release, ↑ feeding, ↓ gastrointestinal motility, miosis, muscle rigidity, sedation	Dynorphin U50,488 Fentanyl Butorphanol	Nor-BNI Naloxone Naltrexone
δ	amygdala, caudate, cortex, NAcc, olfactory bulb, Pontine nucleus	Analgesia, ↑ feeding	DPDPE Deltorphin Leu-enkephalin Etorphine	Naltrindole Naloxone naltrexone

Clinical Effects and Uses

In clinics, opioids are widely used as relatively selective painkillers without affecting other sensory modalities. They are more effective in the relief of nociceptive than neuropathic pain. Agonists acting at μ - and κ -receptors cause miosis (constriction of the pupils) – an effect that is more resistant to development of tolerance than other physical responses. Opioids depress respiration and the cough reflex by directly affecting respiratory and cough centers of the brainstem, and cause nausea and vomiting by stimulation of chemoreceptors in the medulla. Opioids can act in the hypothalamus to change the set point for body temperature, inhibit the release of pituitary trophic hormones, increase prolactin release, and decrease plasma levels of testosterone and cortisol. However, with chronic use, tolerance develops to these hormonal effects of opioids. In the gastrointestinal tract, morphine and other μ -agonists decrease acid secretion, gastric motility, biliary and pancreatic secretions, peristalsis of the large bowel, and increase the tone of the anal sphincter and sphincter of Oddi. These effects may lead to gastroesophageal reflux, epigastric pain, delay in absorption of food and drugs taken orally, biliary colic, increase in plasma levels of amylase and lipase, and, more commonly, constipation. Morphine can normalize the oxytocin-induced contractions of the uterus. In the skin, morphine causes flushing and pruritus. Nearly all of these opioid effects can be reversed by the MOR antagonist, naloxone. In general, opioids suppress the immune system; therefore, the drugs increase the risk of infections and spread of malignancies. Morphine-like drugs may also cause catalepsy, seizures, muscle rigidity, and stereotypical behavior especially at higher doses. Death from opioid overdose is almost always due to respiratory arrest and may occur when purity or potency of the supply is unexpectedly high or after recent abstinence (due to reduced tolerance).

Transition from Abuse to Addiction

Acute Rewarding Properties of Opioids

Subjective effects depend on the drug, dose, route, and rate of administration. In the human, acute intravenous heroin administration induces an intense pleasurable effect or euphoria (rush) in a few seconds that has been described as a total-body orgasm. The heroin rush lasts for a few minutes. The opioid-induced sense of well-being (high) lasts for a few hours after the rush and is, sometimes, followed by a state of de-realization and inattention to the external world. The acute rewarding effects of heroin disappear after a few hours, leading addicts to inject several times a day. Therefore, addicts typically oscillate between a euphoric high (during which they are relatively calm) and early withdrawal symptoms (which make them irritable and sometimes aggressive). In laboratory animals, opioids induce conditioned place preference (CPP) and are readily self-administered.

Accumulating evidence indicates that the acute rewarding properties of opioids are, at least in part, mediated by the mesocorticolimbic DA system. This system has two parts that work in parallel. The mesolimbic pathway consists of DA projections from neurons of the ventral tegmental area (VTA) to limbic structures such as the nucleus accumbens (NAcc), ventral pallidum (VP), amygdala, and hippocampus. These target areas of the mesolimbic pathway are involved in the primary reinforcing properties of drugs, memory formation, and conditioned and contextual learning. The mesocortical pathway is made of DA projections from VTA to cortical regions such as the prefrontal cortex (PFC), orbitofrontal cortex, and anterior cingulate cortex, and is thought to mediate the cognitive aspects of the drug experience, craving, altered executive function, and loss of control

over compulsive drug use. Additionally, DA has been proposed to be involved in reward anticipation and associative learning. Opioid agonists acting at MORs inhibit γ -aminobutyric acid (GABA)-ergic inhibitory interneurons in VTA, resulting in disinhibition of DA neurons and increased DA release in terminal regions. Accordingly, self-administration of MOR agonists into the VTA and NAcc increases DA in the shell subregion of the NAcc, and lesions of the NAcc, VTA, and VP impair heroin self-administration. However, animals will continue intra-NAcc self-administration of opioids despite DA blockade in the NAcc, indicating the presence of DA-independent mechanisms in the acute rewarding effects of opioids.

The endogenous opioid system (especially the MOR) is also implicated in the mediation of opioid reward. In fact, although DA neurotransmission plays a role in the rewarding properties of many drugs of abuse, MORs are central to the rewarding effects of opioids and DA has only a modest role. The opioid system is also important in the reinforcing effects of other drugs of abuse such as cannabinoids, alcohol, and nicotine. Involvement of other pathways in opioid reward has also been suggested. For instance, opioid self-administration and CPP is abolished in cannabinoid CB₁-receptor knock-out mice or by cannabinoid antagonist administration.

Tolerance and Withdrawal

With repeated exposure to opioids, adaptations occur that lead to chronic and endurable changes at the level of cells and circuits.

Tolerance

Tolerance is defined as a reduced response to a drug for a desired effect after repeated administration. Therefore, increasing doses and/or frequency of drug use are required to achieve the same effect. Tolerance develops rapidly to some effects of opioids such as analgesia and reward and the dose can even increase hundreds of folds. However, tolerance to opioid-induced miosis and constipation develops slowly. Development of tolerance depends on the dose and pattern of drug administration. In addition, tolerance is affected by conditioned cues (such as context) and the administration of the usual dose in a different context may lead to overdose (conditioned tolerance). Different types of tolerance by various mechanisms exist. Tolerance may be innate (genetic sensitivity to a drug) or acquired (pharmacokinetic, pharmacodynamic, or learned tolerance). Pharmacokinetic tolerance is mostly due to increased metabolism rate of a drug. Pharmacodynamic tolerance is related to adaptive changes that occur within target systems of a drug that decrease the response to it. Learned tolerance is a decline in the effects of a drug made by previous learned mechanisms. Situation-specific (conditioned) tolerance may occur

if drug taking is always paired with distinct environmental cues. Hence, cues predict the effects of the drug and less effect of drug is observed when environmental cues are available.

Adaptive mechanisms underlying tolerance may occur at any level, from MORs (receptor tolerance) to neurons (cellular tolerance) to networks (system tolerance). Tolerance may develop acutely upon a single exposure to opioids (acute tolerance); however, this is different from the classical tolerance seen after chronic administration of opioids. Desensitization and internalization of MORs are considered to be involved in the development of receptor tolerance. It has been proposed that activation by agonist induces phosphorylation of MORs and increases their affinity for binding to arrestin proteins (β -arrestin2), which eventually uncouples MORs from G-protein signaling and starts the process of internalization. Accordingly, β -arrestin2 knock-out blocks the development of tolerance to antinociceptive effects of acute and chronic morphine. However, the importance of these mechanisms for the development of tolerance is still uncertain. Desensitization is a distinct process that precedes internalization. Morphine, unlike more potent agonists, has low efficacy for desensitization and a very low efficacy for internalization of MOR in most neurons. Therefore, it seems that the reduced coupling of MOR to its intracellular effectors over time (desensitization) is a more potent mechanism for tolerance than decreased cell-surface expression of MOR, although it is unlikely that these mechanisms can fully account for the greater magnitude of tolerance observed at behavioral levels. Possibly, tolerance to the effects of morphine results, in part, from ineffective desensitization, which causes prolonged receptor activation leading to further downstream cellular adaptations that increase development of tolerance. The importance of interactions of different neurotransmitters (such as the glutamatergic system) with opioid pathways in the development of tolerance to morphine has been demonstrated.

Withdrawal

A prototypical withdrawal syndrome occurs when drug administration is withheld after dependence has developed with chronic exposure. Signs and symptoms of opioid withdrawal are very unpleasant but are not life threatening, and are often opposite to the original effects produced by the drug before the development of tolerance. They include: yawning, lacrimation, mydriasis (dilated pupils), rhinorrhea, perspiration, gooseflesh, muscle spasm, hot and cold flashes, tremor, anorexia, nausea, emesis, diarrhea, restlessness, insomnia, weight loss, dehydration, hyperglycemia, hyperpyrexia, and hypertension, as well as dysphoria (a negative emotional state containing depression and anxiety-like symptoms). The symptoms of withdrawal depend on the last dose and

type of drug. For long-acting agents (such as methadone) withdrawal occurs more slowly, is less intense, and is more prolonged than for short-acting drugs (heroin). Although avoidance of withdrawal seems to be an important motivation to take drug, considerable evidence indicates that the rewarding properties of drugs play the major role in the continuation of drug seeking and administration.

The locus ceruleus (LC) – the major noradrenergic (NA) nucleus of the brain – has been studied extensively in opioid withdrawal. Acute administration of opioids inhibits AC (subtypes I and VII) and the production of cyclic adenosine monophosphate (cAMP) levels in LC neurons and also decreases the firing rate of LC neurons. However, with continued administration of opioids the inhibition of AC and firing rate recovers to normal as a homeostatic compensatory response, and they increase several times above normal levels during withdrawal. Evidence indicates that enhanced excitatory inputs and intrinsic unregulated excitability lead to the observed hyperactivity of LC neurons during opioid withdrawal. More recently, it has been shown that withdrawal symptoms as well as the spontaneous firing activity of LC neurons are highly attenuated in GIRK knock-out mice. However, the causal relationship between this LC hyperactivity and behavioral symptoms of withdrawal is still unclear. Although surgical lesion of the LC area or decreased expression of transcription factor cAMP response-element-binding protein (CREB) in the LC attenuates the withdrawal syndrome, pharmacological ablation of noradrenergic LC function does not prevent the expression of withdrawal behavior.

Evidence indicates that the dysphoria associated with opioid withdrawal can be dissociated from the physical symptoms listed above. For example, studies in our laboratory found that blockade of β -NA receptors in the ventrolateral bed nucleus of stria terminalis (vBNST), or lesion of the ventral NA bundle which originates in the caudal medullary NA cell groups, strongly attenuates the aversion associated with morphine withdrawal but has very little effect on the physical withdrawal syndrome. Thus, different circuits may be involved in the affective and somatic responses to withdrawal. These results also point to the caudal NA cell groups, rather than the LC, as an important site mediating the affective response to withdrawal (**Figure 1**).

Chronic opioid exposure also upregulates the cAMP pathway in regions outside the LC, including the NAcc. One target for CREB in the NAcc is the opioid peptide dynorphin. During withdrawal, dynorphin released from neurons of the NAcc binds to κ -opioid receptors on VTA dopaminergic neurons, inhibits their activity and decreases DA release in the NAcc. This phenomenon seems to contribute to the dysphoria seen during acute withdrawal. Consistently, mutations of the CREB gene

decrease morphine withdrawal symptoms, and increased CREB expression decreases the rewarding effects of opioids. Drug withdrawal activates stress systems other than Norepinephrine (NE) neurons, and increased levels of corticotropin-releasing factor (CRF) in the amygdala have been observed. In addition, blockade of the CRF₁ receptor blocks the development of place aversion for precipitated opiate withdrawal.

Protracted withdrawal/abstinence

Prolonged exposure to drugs causes neuronal adaptations that continue long after symptoms of acute withdrawal have dissipated. These adaptations cause anhedonia, depression, dysphoria, anxiety, and decreased interest in natural rewards during protracted withdrawal (weeks or longer). Our knowledge with regard to changes in the brain during protracted withdrawal is limited. However, it seems that plasticity in the extended amygdala results in prolonged elevated anxiety as well as altered hedonic processing during this period that may contribute to increased vulnerability to drug seeking and relapse. In a series of studies in our laboratory, morphine-dependent or naive rats were subjected to abstinence withdrawal for 2–5 weeks, followed by training for morphine or food CPP. Postdependent animals showed enhanced morphine preference and attenuated food preference, compared to nondependent animals. This altered hedonic processing (decreased valence for natural rewards in addition to enhanced incentive value of drugs) was correlated with Fos activation in some stress-related areas such as ventral BNST and central amygdala (CeA), and their major NA afferent in the nucleus tractus solitarius (NTS). These alterations and the resulting changes in hedonic analysis could be attributed to changes in afferents from these areas to the VTA, or to plasticity within the VTA. Postdependent rats also exhibited more anxiety and increased sensitivity to stress during the protracted withdrawal (**Figure 2**).

Other brain changes were observed that may contribute to altered hedonic processing during protracted withdrawal. For example, Fos induction in the BLA, NAcc, and lateral hypothalamus (LH) mirrored the changes in preference in postdependent animals – being increased for increased morphine preference but decreased with decreased food preference. These changes in the LH were determined to occur in orexin (hypocretin) neurons; the role of the orexin system in opioid abuse will be discussed further in this article. Changes in the NAcc shell may be particularly involved, as FosB expression in this region (but not in other areas examined) 5 weeks after withdrawal correlated with increased drug preference.

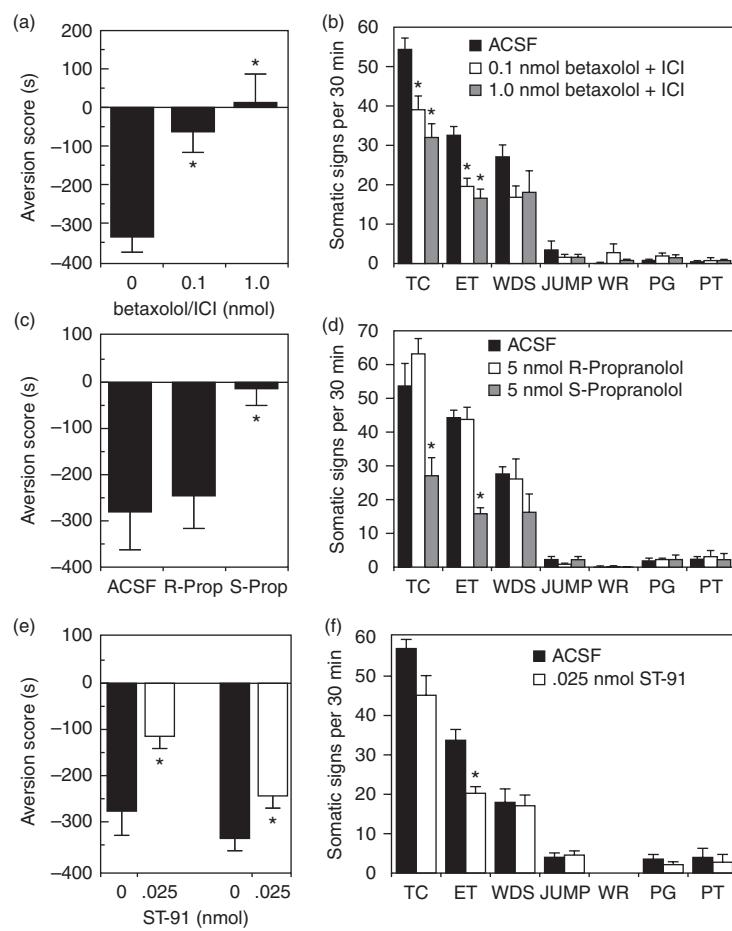


Figure 1 Effects of the β -antagonists betaxolol + ICI 118,551 ((a) and (b)), propranolol ((c) and (d)) or the α_2 agonist ST-91 ((e) and (f)) on aversion scores and somatic withdrawal. Infusion of noradrenergic drugs into the BNST blocked conditioned place aversion but had minimal effects on somatic signs of acute opioid withdrawal. TC = teeth chattering; ET = eye twitch; WDS = wet dog shakes; JUMP = jumping; WR = writhing; PG = penile grooming; PT = paw tremor. Aversion score for the withdrawal-paired environment is the mean time in seconds spent in the withdrawal side minus the nonwithdrawal side on test day. * $P < 0.05$. With permission from Delfs et al. (2000) Noradrenaline in the ventral forebrain is critical for opiate withdrawal induced aversion. *Nature* 403: 430–434.

Neural Plasticity

Neural plasticity (the brain's ability to change over time) is thought to contribute to normal learning and memory, as well as to the development of psychiatric diseases. Evidence suggests that long-term adaptations that are induced by chronic exposure to drugs in reward-related areas of the brain are the neural basis of the transition from social drug use to compulsive intake and relapse. In particular, adaptations in the VTA and the NAcc have been studied extensively and are thought to be relevant to long-lasting drug-induced plasticity. The behavioral relevance for many of these changes is yet to be determined.

Acutely, opioids increase the firing rate of DA neurons in the VTA by hyperpolarizing inhibitory GABAergic synapses onto these cells. This phosphorylates the transcription factor CREB which, in turn, increases the expression of the GluR1 subunit of the α -amino-3-hydroxy-5-methyl-4-isoxazole-propionate

(AMPA) glutamatergic receptor. It has been shown that even a single injection of morphine is capable of inducing long-term potentiation (LTP)-like enhancement of glutamatergic responses in the VTA DA neurons. For cocaine, such VTA plasticity has been shown to be dependent on orexin neurons originating from the hypothalamus. The orexin system has been shown to affect the addictive properties of morphine in several studies. For example, Fos induction in LH orexin cells correlates strongly with morphine preference. In addition, application of the orexin A peptide in the VTA, or stimulation of LH orexin neurons, induces reinstatement of extinguished morphine CPP; 'this reinstatement' is blocked by an orexin 1 receptor antagonist.

Neurotrophic factors also seem to play a role in opioid addiction. The VTA DA neurons express neurotrophic factors such as the brain-derived neurotrophic factor (BDNF). Serum BDNF is altered in human opioid addicts; and in the rat, morphine acutely increases expression of

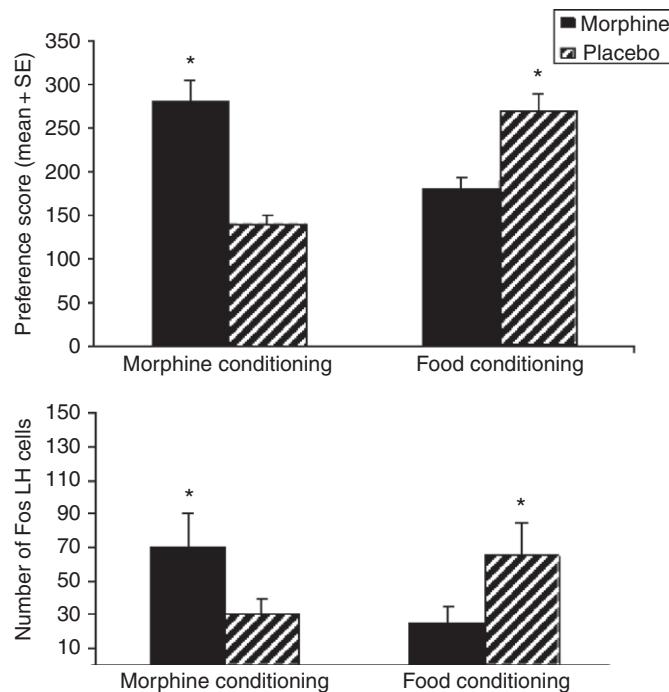


Figure 2 Upper panel: Previously morphine-dependent animals showed decreased preference for the environment associated with food but increased morphine CPP after 5 weeks of abstinence, as compared to nondependent animals (placebo). Preference for food- or morphine-paired environments was measured as the difference in times spent in the rewarded side minus the nonrewarded side on test day. Lower panel: More Fos+ neurons were found in the LH of morphine-abstinent, than placebo-pretreated, rats that underwent morphine CPP, and conversely significant – more Fos+ neurons were found in the LH for placebo than abstinent animals that underwent food conditioning. Other studies from our laboratory show that many of these Fos+ LH neurons contain the neuropeptide orexin. Modified with permission from Aston-Jones and Harris (2004) Brain substrates for increased drug seeking during protracted withdrawal. *Neuropharmacology* 47: 167–179.

BDNF in the VTA, NAcc, and frontal cortex. Chronic exposure to escalating doses of morphine (but not subcutaneous implants) downregulates BDNF function in the VTA. In addition, chronic morphine differentially modulates the downstream signaling pathways from BDNF in the VTA. The effect of chronic morphine exposure on the extracellular signal-regulated kinase (ERK) pathway is not clear, and both decreased and increased activity of this pathway have been reported. However, IRS-PI3K-Akt signaling is decreased by chronic opioid exposure in both VTA and NAcc, which could be related to the decreased size of DA cells (in the VTA) or decreased arborization and dendritic spine density (in the NAcc). The PLC γ pathway is activated by chronic morphine. In addition to BDNF, another neurotrophic factor called glial cell line-derived neurotrophic factor (GDNF) is increased in the VTA following chronic opioid use.

In the NAcc, acute administration of opioids rapidly increases the expression of the Fos family of transcription factors which lasts for a few hours. Δ -FosB is a member of Fos family that is extremely stable; and, therefore, chronic drug use leads to gradual accumulation of Δ -FosB in NAcc over time. Overexpression of Δ -FosB in the NAcc has been associated with increased rewarding

properties of morphine. However, the levels of Δ -FosB return to normal values after 8 weeks of abstinence, and so these alone cannot explain relapse to addiction after longer periods of abstinence (months or years). Nonetheless, Δ -FosB or similar proteins can possibly act as a molecular switch between transient acute effects of drugs and persistent long-lasting features of addiction (such as prolonged changes in gene expression and synaptic remodeling).

Morphologically, chronic morphine decreases the size of the VTA DA neurons – an effect that can be prevented by intra-VTA infusion of BDNF. In addition, both contingent and noncontingent chronic administration of opioids decrease the arborization of dendrites and the spine density of medium spiny neurons of the NAcc in rats. Fewer studies have shown the effect of chronic opioid use in other areas of the brain. For instance, it is known that chronic exposure to opioids diminishes the number and complexity of dendritic spines (mPFC and the hippocampus) and neurogenesis (hippocampus). In human opioid addicts, metabolism and blood flow is decreased in the PFC and this hypofrontality is thought to be involved in relapse to addiction.

Craving and Relapse

Long-term exposure to exogenous opioids induces adaptive changes in the brain that disrupts natural reward seeking and increases drug craving and vulnerability to relapse. The major problem in opioid addiction is return to drug seeking and taking, even years after detoxification. The relapse rate for heroin addicts is about 75–85% in the first year of abstinence and is still 25% after 15 years of abstinence.

During abstinence, craving and relapse to drug seeking can be induced by re-exposure to the drug or cues previously associated with drug use, or by stressors. In the last few decades, several laboratories have been using animal reinstatement models to study the underlying neural mechanisms of relapse. The most commonly used methods are reinstatement of self-administration and CPP. Animals are trained to press a lever for drug infusion (self-administration) or to associate a distinct environment with the drug experience (CPP), and are then subjected to abstinence or extinction training during which the drug is not available. Reinstatement of lever pressing or preference is then tested upon exposure to a noncontingent priming injection of the drug, cues associated with drug intake, or stress. The neural basis of relapse to opioid seeking has not been studied extensively, compared to studies on stimulant seeking. Recent findings indicate that, although there are many commonalities in the brain areas mediating reinstatement of cocaine and heroin seeking, a more diffuse neurocircuitry seems to underlie heroin seeking.

Drug Priming

A wide range of areas is engaged in drug-induced reinstatement. Inactivation of the VTA, NAcc (both shell and core), BLA and CeA, PFC (both dorsomedial (dmPFC) and ventromedial (vmPFC)), BNST, and even the dorsolateral caudate-putamen (dlCP), substantia nigra (SN), and ventral pallidum (VP) attenuate heroin-primed reinstatement of drug seeking in rats. Several neurotransmitter pathways are involved. For instance, systemic or intra-VTA injection of MOR agonists, systemic or intra-NAcc application of indirect DA agonists, and systemic D₂ – but not D₁ – agonists reinstate heroin seeking. On the other hand, neurochemically specific lesions of DA neurons and terminals in the VTA or NAcc block morphine-primed reinstatement of CPP, and D₂ antagonists block heroin-induced reinstatement. Both heroin- and intra-VTA morphine-induced reinstatement of heroin seeking are blocked by naltrexone. In addition, it has been shown that NA depletion in the PFC blocks morphine-primed CPP reinstatement.

More recently, it has been suggested that glutamatergic input to the NAcc core from the PFC or amygdala contributes to the reinstatement of heroin seeking. Heroin- (and cue-)induced reinstatement increases glutamate in the NAcc core, and inhibition of PFC or amygdala afferents attenuate heroin-induced reinstatement. The stimulation of presynaptic group II metabotropic glutamate (mGluR2/3) receptors, or cystine–glutamate exchange, can inhibit synaptic release of glutamate and also attenuate reinstatement of heroin seeking. A role for the endocannabinoid system in relapse to heroin seeking has also been shown, for example, CB₁ receptor agonists can trigger reinstatement of heroin self-administration. In addition, CRF₁ – but not CRF₂ – receptor antagonists attenuate reactivation of morphine CPP.

Cues

Afferents from the medial PFC and amygdala to the NAcc core play a major role in the reinstatement of heroin seeking upon exposure to cues previously associated with heroin. In addition, inactivation of several other areas have been shown to impair cue-induced reinstatement of heroin seeking in the rat, including the medio-posterior parts of the BNST, dlCP, SN, and VP. Dopaminergic and glutamatergic neurotransmission in the NAcc are known to contribute to cue-induced reinstatement of heroin seeking and inhibiting either glutamate or DA receptors blocks both cue- and drug-induced reinstatement of heroin seeking. Importantly, the NAcc core mediates discrete cue-induced reinstatement while the shell subregion is involved in context-induced reinstatement. Akin to heroin-primed reinstatement, stimulation of the mGluR2/3 receptors (systemic or intra-VTA and NAcc shell), which decreases synaptic glutamate release, attenuates context-induced reinstatement of heroin seeking. In addition, chronic administration of *N*-acetyl-cysteine – which increases cystine–glutamate exchanger activity – has been shown to block cue (and heroin-)induced reinstatement of heroin seeking. Cholinergic transmission in the VTA versus the NAcc has been shown to provoke and block reinstatement of heroin seeking upon exposure to heroin-associated cues, respectively. A role for cannabinoid receptors (in the PFC and NAcc) in cue-induced reinstatement has also been proposed. In addition, as mentioned above, activation of orexin neurons in the LH, or intra-VTA administration of the orexin A peptide, can reinstate morphine CPP (Figure 3), an effect that may mimic stimulus-induced reinstatement as drug-associated stimuli stimulate orexin neurons. This orexin-driven reinstatement is completely blocked by pretreatment with an orexin 1 receptor antagonist, as is cue-induced reinstatement of cocaine-seeking behavior.

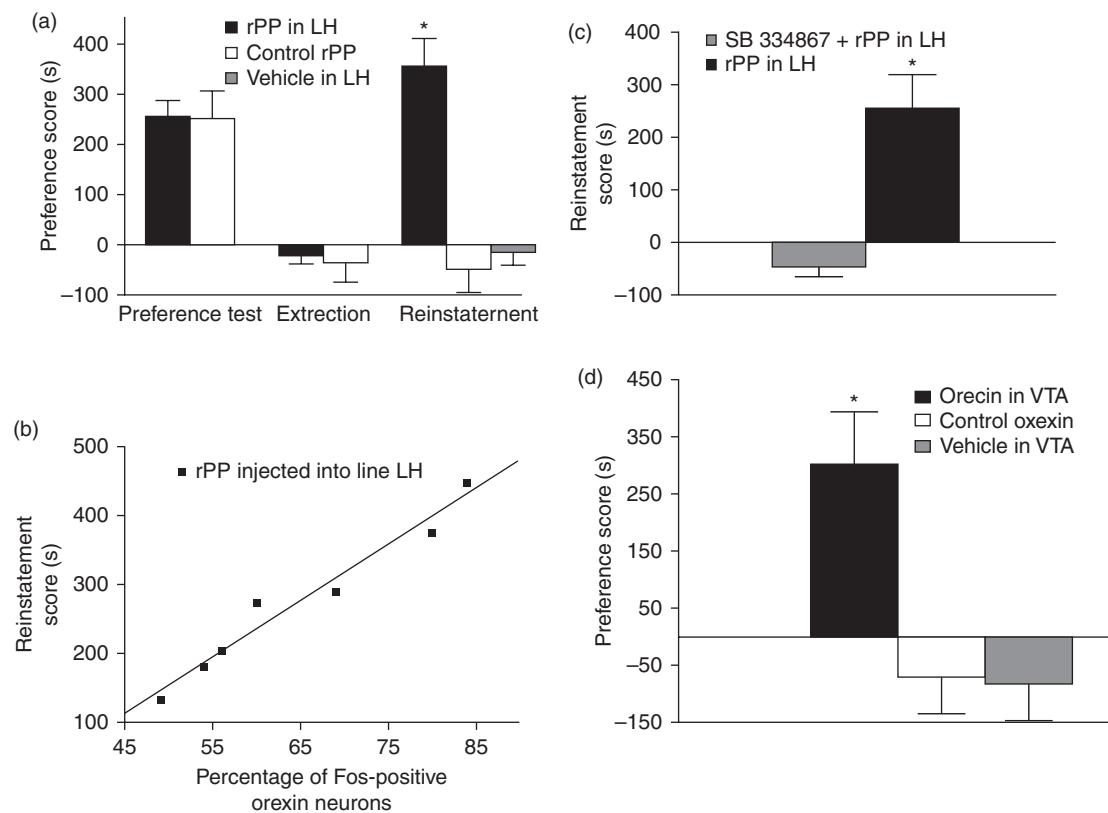


Figure 3 Activation of lateral hypothalamus orexin neurons by rPP reinstated an extinguished preference for morphine.

(a) Preference scores were measured as the difference in time spent in the morphine-paired side minus that spent in the saline-paired side. (b) The selective orexin A antagonist, SB 334867 ($20\text{--}30\text{ mg kg}^{-1}$), blocked reinstatement induced by rPP injected into the lateral hypothalamus. (c) Correlation between reinstatement scores and percentages of lateral hypothalamus orexin neurons that were Fos activated in rPP-reinstated animals. (d) Administration of orexin (140 nM) into the VTA reinstated an extinguished morphine place preference. Modified with permission from Harris *et al.* (2005) A role for lateral hypothalamic orexin neurons in reward seeking. *Nature* 437: 556–559.

Stress

Stressors are important factors in relapse to drug seeking and taking both in the human and animal models. The most commonly used stressors in laboratory animals are footshock, food-deprivation, and pharmacological blockade of α_2 -adrenoceptors. The CeA and BNST are major areas involved in this regard. It has been suggested that unlike drug- and cue-induced reinstatement, the dopaminergic system plays an indirect role in footshock-induced reinstatement of heroin seeking; this reinstatement is attenuated by the chronic administration of nonselective DA receptor antagonists (but not selective D₁ or D₂ antagonists). However, CRF and central noradrenergic pathways are critically involved in stress-induced reinstatement of heroin seeking. For instance, antagonists of CRF receptors (especially the CRF₁ receptor) and α_2 -adrenergic agonists have been shown to attenuate footshock-induced reinstatement of heroin seeking. It appears that NA neurons in the lateral tegmental area and their projections through the ventral noradrenergic bundle, but not LC neurons, are involved in the effects of

the NA system on stress-induced reinstatement of heroin seeking, similar to the findings for stress-induced reinstatement of cocaine seeking. Leptin is involved in heroin seeking reinstated by food deprivation.

Acknowledgments

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See also: Acute Dependence; Cellular Plasticity in Cocaine and Alcohol Addiction; Drug Addiction; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Drug Priming; Drug Sensitization and Drug Abuse; Drug Withdrawal – Motivational View; Molecular Neurobiology of Addiction; Motivation; Neurophysiology of Drug Reward; Pain and Addiction; Psychostimulants; Stress and Drug Craving; Transition to Addiction; Vulnerability Factors in Addiction Disorders.

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Neurophysiology of Drug Reward

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Glossary

Action potential – Also known as a nerve impulse, spike, or firing, it is a change in neuronal membrane potential that is propagated down the length of the axon. Membrane potential refers to the difference in electrical charge across the neuronal membrane, between the inside of the cell and the extracellular fluid surrounding the neuron. In order for a neuron to generate an action potential, the membrane potential must change from resting (-70 mV) to the threshold for firing (-50 mV). The change in membrane potential from resting threshold depends on neurochemical actions at membrane receptor proteins located on the neuron soma and dendrites, which lead to the opening of ion channels and the influx of positive ions into the neuron.

Electrophysiology – It is the study of the electrical properties of biological cells and tissues. It involves measurements of voltage change or electrical current on a wide variety of scales from single ion channel proteins to whole organs such as the heart. In neuroscience, it includes measurements of the electrical activity of neurons, and particularly action potential activity.

Extracellular single-neuron recording – It is an electrophysiological method. The technique is used to measure changes in current that occur in the extracellular fluid caused by movement of ions across the membrane of a single neuron in association with the occurrence of action potentials.

Neurophysiology – It refers to neuronal electrical properties and events that underlie biological functions regulated by the brain. These functions are diverse in nature (e.g., breathing, heart rate, movement, sensory perception, goal-oriented behavior, and emotion).

Neuroplasticity – Also referred to as neuroadaptations, it is the changing of neurons and the organization of neuronal networks and hence the function of the neurons and networks. Neuroplasticity can be induced by various experiential events, such as those associated with learning (e.g., repeated stimulation of particular synapses during repeated exposure to particular environmental stimuli and behavioral events). It can also be caused by exposing the neuron to pharmacological agents that affect neural function via actions at either membrane or intracellular receptors. Examples of drug-

induced neuroplasticity include neuronal changes that underlie drug tolerance and sensitization.

Operant behavior – Also referred to as goal-directed or reward-directed behavior, it is a term used in reference to operant (instrumental) conditioning (i.e., learning). The operant is a behavior upon which a biologically significant event (i.e., reward) is contingent. Rewards that are contingent upon the occurrence of a particular behavior are referred to as reinforcers if the behavior-reward contingency is associated with an increase in the probability of the operant behavior.

Overview

A reward is a biologically significant event, such as food or a receptive mate. Through Pavlovian and instrumental conditioning, an individual can learn predictive relationships between rewards, stimuli, and instrumental behavior. These associations evoke, maintain, and direct behavior, which lead organisms to contact rewards. Certain drugs, particularly those having abuse potential, share properties of natural rewards. However, given adequate drug exposure, drug-directed behaviors can become compulsive and uncontrollable. Understanding the mechanisms that mediate instrumental behavior directed toward drug reward is not only relevant to understanding the mechanisms that contribute to reward-directed behavior generally but is also relevant to understanding mechanisms that contribute to drug addiction.

Neuropsychopharmacological studies show that the acquisition and expression of instrumental behavior directed toward natural rewards and drug rewards are mediated by overlapping neural circuitry. Electrophysiological recordings of single-neuron activity in behaving animals have refined our understanding of the functional contribution of particular brain regions to natural reward-directed behavior. The recordings have also identified underlying neurophysiological mechanisms. During the late 1980s, researchers began to conduct similar studies of drug-directed behavior. The present article reviews some of this latter work. The article also compares studies of drug-directed behavior to similar investigations of natural reward-directed behavior.

The reviewed research shows that some of the neurophysiological correlates of drug-directed behavior are comparable to those of natural reward-directed behavior. The research has also led to the discovery of novel patterns of neural activity, some of which are unique to drug-directed behavior and which may be relevant to drug addiction.

Investigations of Drug-Directed Behavior: Research Method

Drug Self-Administration Paradigm

To conduct electrophysiological studies of drug-directed behavior, researchers have integrated chronic extracellular recording methods with the intravenous drug self-administration model. The intravenous drug self-administration model is a well-validated model of human drug self-administration. In the simplest case, animals (e.g., rats or nonhuman primates) are implanted with an intravenous catheter and then trained to engage in operant behavior, typically a lever press response, according to a fixed-ratio 1 (FR1) schedule of reinforcement. Each press of the lever is followed by a single drug infusion, which is typically paired with a conditioned reinforcer such as a light or a tone. Although the experimenter controls the drug concentration and volume of each infusion, animals control the timing of infusions. The patterns of drug taking exhibited by animals in the self-administration paradigm are quite similar to those observed in humans.

If exposed to short daily sessions (i.e., 1–2 h per day) and moderate drug doses, animals exhibit stable rates of intake across many days. In this circumstance, the drug-directed behavior is sensitive to the same associative variables as is natural reward-directed behavior. However, if animals are exposed to high doses of drug and/or long daily sessions, animals exhibit escalation of drug intake across repeated self-administration sessions and exhibit other behavioral changes reminiscent of drug addiction symptoms, such as a decreased ability to withhold drug-directed behavior despite adverse consequences. The paradigm is thus used to study both drug reward and drug addiction.

Chronic Extracellular Recording

To conduct electrophysiological recordings of single neurons during drug self-administration, animals are chronically implanted with either a bundle or an array of insulated microwires (e.g., 50 µm diameter stainless steel wires with Teflon coating). After surgery, extracellular recording procedures are used to record the activity of individual neurons. To conduct these recordings, a connector cemented to the skull, which contains electrical contacts to the microwires is connected through a flexible cable to a computer-controlled amplification and filtering

system. Animals can move freely and exhibit typical operant behavior. During recording sessions, the occurrence of action potentials can be tracked with a 1-ms resolution. The recordings are highly stable such that the activity of a single neuron can be followed for many hours and sometimes for multiple days. Given the temporal resolution and stability of the recording procedure, it is possible to characterize changes in neuronal firing that occur time locked to rapid discrete events, such as the cocaine-reinforced operant response. One can also concurrently evaluate slow and long-lasting changes in firing that might be associated with changes in either drug exposure or the motivational state of the animal (i.e., time frame of minutes to hours). With histological procedures, the location of recording wires can be identified with a resolution of $\approx 100\text{ }\mu\text{m}$.

Focus of Most Research Studies of Drug-Directed Behavior

Brain areas implicated in mediating self-administration of addictive drugs in animals are consistent with regions implicated in drug self-administration and addiction in humans. These brain areas include the ventral tegmentum, and regions in the prefrontal cortex, striatum, particularly the nucleus accumbens, and limbic regions such as the amygdala. As noted already, these regions also overlap with those implicated in natural reward-directed behavior. Most of the electrophysiological studies of drug self-administration have thus far focused on characterizing the activity of individual neurons in the nucleus accumbens during periods in which rats self-administer cocaine. However, there have also been characterizations of accumbal activity during ethanol and heroin self-administration, and studies of neural activity in the prefrontal cortex and amygdala. Many of these investigations involve a characterization of the topography and behavioral correlates of single-neuron firing patterns during the drug self-administration session. A smaller but growing number of studies have investigated the changes in neural firing patterns that occur across repeated days of drug self-administration and subsequent periods of abstinence. Both types of studies are relevant to understanding drug reward. The latter type of study is also relevant in identifying lasting neuroadaptations that contribute to drug addiction.

Firing Patterns of Individual Neurons during Drug Self-Administration Sessions

Overview

One focus of recording studies of cocaine-directed behavior has involved the cataloguing of firing patterns that occur during FR1 cocaine self-administration sessions.

Another has been investigating the nonpharmacological factors that contribute to those firing patterns. This section reviews some of this research.

Short-Duration Changes in NAcc Firing Time Locked to Cocaine-Reinforced Behavior

The firing patterns

Some of the first extracellular recordings conducted during FR1 cocaine self-administration sessions characterized phasic firing patterns time locked to the operant behavior. These studies showed that at a cocaine dose of 0.75 mg kg^{-1} per infusion (inf), approximately 25–40% of NAcc neurons exhibit rapid phasic changes in firing during the seconds which bracket the cocaine-reinforced

lever presses (referred to herein as lever-press firing patterns) (Figure 1(a)). The firing patterns have been subtyped into three main groups: (1) prepress, (2) post-press, and (3) prepost press. A small subset of additional neurons exhibit similar changes in firing with a slightly longer duration (e.g., 30–60 s). The broad range of firing-pattern time courses suggests that, during cocaine self-administration, NAcc neurons track multiple variables or combinations thereof during the seconds that precede and follow the reinforced operant response.

Lever-press firing patterns have been observed under a range of experimental conditions but have been consistently reported as predominantly excitatory. There is evidence that the firing patterns are more prevalent in the core subterritory of the NAcc than in the shell

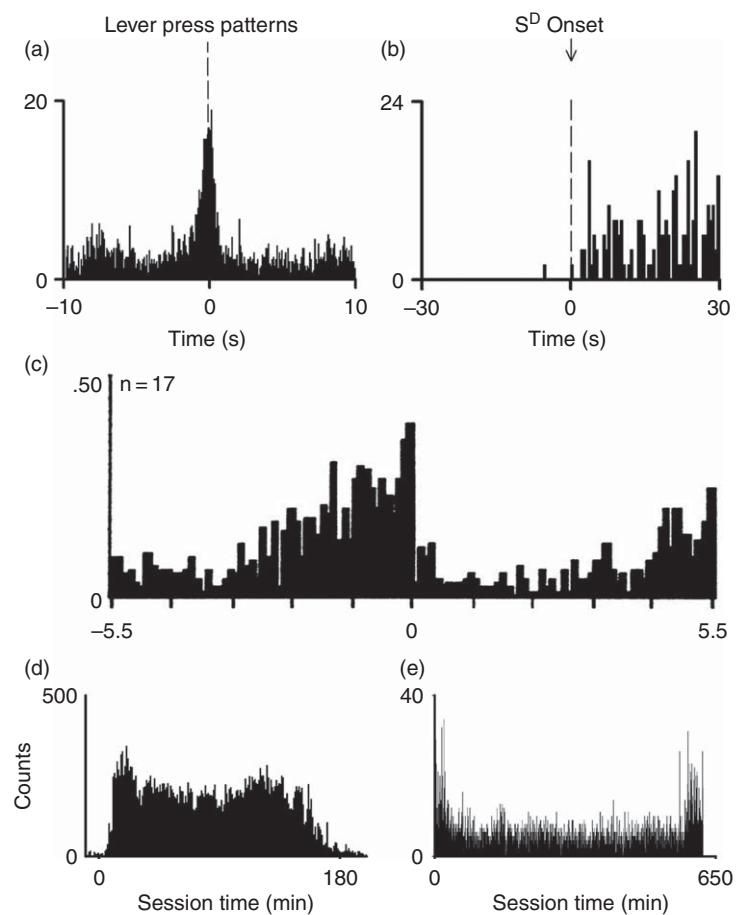


Figure 1 Single-neuron firing patterns during FR1 cocaine self-administration sessions. (a) Average phasic change in firing time locked to the cocaine-reinforced lever press. Average firing rate (Hz per 0.1-s bin) is plotted during the 12 s before and after presses made during the maintenance phase of a self-administration session (i.e., 10th to 27th reinforced lever presses). (b) Change in firing time locked to presentation of cues that signaled the onset of an FR1 cocaine self-administration session (firing pattern referred to as an S^D response). Firing rate (counts per 0.5-s bin) is plotted for the 30 s before and after onset of the cue. Cue onset is shown at Time 0 on the abscissa. (c) Average change in firing time locked to the cocaine-reinforced press (firing pattern referred to as progressive-reversal firing patterns). Average firing rate (Hz per 0.6-s bin) is plotted during minutes before and after press (i.e., all presses excluding the first 10 of the session). The time base of the histogram corresponds to the average inter-infusion interval exhibited by the individual animal from which the neural activity was recorded. (d, e) Session-long changes in firing during a FR1 cocaine self-administration session. In each histogram, firing (Hz per 30-s bin) is plotted during an entire FR1 cocaine self-administration session, as well as during a pre- and postsession baseline period. Beginning and end of self-administration is indicated by arrows at top of histograms.

subterritory of the NAcc (i.e., particularly posterior shell), though this anatomical difference has not always been observed.

Correlates of the firing patterns

The offset of the short-duration lever-press firing patterns precedes the expected latency for pharmacological actions post-infusion. There are numerous nonpharmacological events that occur within the seconds, which lead up to and follow the reinforced lever press during typical FR1 cocaine self-administration sessions. These include, but are not limited to, the following: approach to the lever, the lever-press operant, the conditioned reinforcer cues, and delivery of the primary reinforcer. To isolate the event(s) to which the firing is correlated, researchers recorded responses of lever-press responsive neurons to individual events in isolation of other events. These studies showed that, for all neurons, the prepress firing is associated with the occurrence of the operant behavior, though not the movements which make up the lever-press response *per se*. For about half the neurons showing postpress activity (i.e., for prepost neurons and post neurons), the neurons consistently showed a response to the occurrence of a lever press, even when it occurred in the absence of either a cocaine infusion or the cues normally paired with delivery of the infusion. However, the same neurons did not respond to the infusion or cues when they occurred in the absence of the operant behavior. These data indicate that for about half of the neurons showing postpress activity, the neural response is specifically associated with the operant behavior, though again, not with the execution of the movements required to make the lever press. For most of the remaining neurons that showed postpress activity, the firing is associated with both the operant and the conditioned cues typically paired with delivery of the cocaine infusion.

Several studies showed that the lever-press firing patterns are specific for the expected reward. In these studies, NAcc activity was recorded during multiphase sessions in which the reinforcer varied between phases and was either a natural reward (water or food) or cocaine. During these sessions, most neurons that showed a phasic response time locked to cocaine-reinforced lever presses did not show a phasic response time locked to lever presses reinforced by the natural reward. Similarly, other neurons that exhibited responses time locked to lever presses reinforced by the natural reward rarely did the same when the operant was cocaine reinforced. In some of these experiments, the animals pressed the same lever during both phases so the between-phase difference in firing could not have reflected the movements associated with either the operant or the spatial location of the lever. These findings are consistent with the interpretation that the lever-press firing patterns are related in some

way to expectation of a particular reward. Additional studies are required to further specify the information encoded by the phasic firing patterns.

Electrophysiological studies of natural reward-directed behavior have progressed farther than studies of drug-directed behavior. The natural reward studies have shown that NAcc neurons exhibit phasic firing patterns in association with reward-predictive stimuli and behavior. The majority of the firing patterns involve a short-duration increase in firing. The firing patterns are dissociable from both specific movements and the physical properties of the stimuli, and can reflect various characteristics of the expected reward, including the following: (1) the reward predicted by the cue; (2) the magnitude of the predicted reward; and (3) the temporal proximity of the reward. Based on available data, NAcc firing patterns during drug self-administration will be shown to share similar characteristics.

Studies of natural rewards have also shown that NAcc neurons exhibit changes in firing during reward consumption. In contrast to the phasic activity time locked to reward-predictive cues and operant behavior, the majority of the changes in firing during reward consumption are inhibitory. Though cocaine self-administration is not associated with specific consummatory behaviors, a high percentage (50%) of neurons show an inhibition in firing during the minute(s) that follow each cocaine infusion. The nature of these decreases is not yet understood; however, it is possible that the change in firing is analogous to the inhibitory response exhibited by NAcc neurons during consumption of natural rewards. These findings suggest that increases in NAcc neural activity are associated with instrumental events, which lead animals to contact reward (referred to as preparatory behavior), whereas decreases are predominantly associated with reward consumption.

NAcc Firing Patterns during Cocaine Seeking

In addition to characterizing firing patterns during FR1 drug self-administration sessions, researchers have begun to document and investigate firing patterns during periods in which animals engage in drug-directed behavior under drug-free conditions. There are a number of reasons for conducting these types of studies. First, neuropharmacological studies show that the mechanisms that mediate drug-directed behavior under drug-free and drug-exposed conditions are not identical. Second, drug-directed behavior under drug-free conditions (referred to as drug seeking) is thought to better model conditions associated with relapse in humans, which is a primary therapeutic target. Third, the research also represents an opportunity to compare patterns of firing during drug-directed and natural reward-directed behavior in the absence of pharmacological effects, which can sometimes complicate comparisons of neural firing patterns associated with natural reward-directed versus drug-directed behavior.

These studies have been conducted in a number of ways, though all the studies thus far have focused on NAcc neural activity in animals with a history of cocaine self-administration. Several studies have characterized the response of neurons to cocaine-predictive cues (e.g., cues typically paired with cocaine infusions) in the absence of operant behavior in drug-free animals. Studies have additionally or alternatively characterized NAcc activity during periods in which operant behavior is sustained for a period by response-contingent presentation of only conditioned cues, which have previously been paired with cocaine delivery. The findings of the various experiments are quite consistent in showing that a portion of NAcc neurons (e.g., 25%) exhibit changes in firing in response to the presentation of cocaine-predictive cues (**Figure 1(b)**). The NAcc responses are predominantly excitatory. If operant responding occurs, the cue-evoked neural responses are typically maintained during the period of drug-directed behavior. In cases in which drug is ultimately delivered, some neurons show a significant decrease in average firing relative to firing during a presession baseline period that preceded the operant session (see session-long changes described below). Similar findings have been obtained in other experiments in which animals were exposed to a priming infusion of cocaine concurrently with the conditioned cue. The functional correlates of NAcc firing patterns during drug seeking have not yet been characterized. However, given the partial overlap between neurons which are responsive during drug seeking and drug self-administration, it is likely that there are at least some shared functional characteristics of NAcc firing patterns during drug-directed behavior in drug-free and drug-exposed situations.

Long-Duration Changes in Firing Time Locked to Operant Behavior during Cocaine Self-Administration Sessions

The firing patterns

NAcc neurons exhibit another category of firing pattern time locked to the cocaine-reinforced lever-press. At a cocaine dose of $0.75 \text{ mg kg}^{-1} \text{ inf}^{-1}$, approximately 65% of all neurons show a change in firing that occurs slowly across the interval between successive self-infusions. For most neurons (i.e., 50% of all recorded neurons), firing rate decreases during the first-minute postpress and then progressively increases until the time of the next lever press (i.e., decrease + progressive reversal) (**Figure 1(c)**). For the remaining neurons, firing rate increases postpress and progressively decreases until the time of the next press. Interestingly, the majority of the neurons that show a lever-press firing pattern also show a decrease + progressive reversal firing pattern. Consistent with this

observation, the progressive reversal firing patterns are significantly more prevalent in the rostral and lateral regions of the core than in the posterior parts of the shell.

Determinants of the firing patterns

In considering possible reward-related determinants of the decrease + progressive reversal firing pattern, it is relevant to note that the firing pattern mirrors changes in drug and DA levels in the NAcc during the cocaine self-administration session. The changes in drug level that occur between successive self-infusions are expected to engender interoceptive cues, including those that develop progressively over the course of the interval, in conjunction with drug metabolism. These interoceptive cues could include or become associated with changes in the motivational state of the animal that occur with changes in drug level. It is possible that the progressive increase in firing reflects excitatory afferent input related to the cues and motivational state of the animal and that the rapid decrease in firing that occurs following the press reflects the cessation of that afferent input. An alternative, but not mutually exclusive, interpretation of the firing pattern is that it reflects more direct and acute actions of cocaine in the NAcc. The relative contribution of pharmacological and nonpharmacological variables to the decrease + progressive reversal firing patterns remains to be further delineated.

Similar firing patterns do not appear to occur in either the amygdala or prefrontal cortical regions. The firing patterns were also not observed in a study of accumbal neural activity during nicotine self-administration. The patterns may thus be relatively specific to accumbens and psychomotor stimulants, though further research is required to evaluate this possibility.

Session-Long Changes in Average NAcc Firing Rate during Cocaine Self-Administration Sessions

The firing patterns

Extracellular recordings during intravenous FR1 cocaine self-administration ($0.75 \text{ mg kg}^{-1} \text{ inf}^{-1}$) sessions have shown that most ($\approx 90\%$) NAcc neurons exhibit a change in average firing rate during the self-administration session relative to pre- and postsession baseline periods. These firing patterns have been referred to as session-long changes or session changes (**Figures 1(d)** and **1(e)**). The session changes in firing are exhibited by neurons that exhibit a phasic lever-press firing pattern, a progressive reversal firing pattern, or both. However, the patterns are also exhibited by neurons that show no other change in firing. At the dose of $0.75 \text{ mg kg}^{-1} \text{ inf}^{-1}$, the majority of the session-long changes in firing (i.e., 60% of all recorded neurons) are decreases.

Correlates of the firing pattern

The session-change firing pattern is defined by a sustained change in average firing during the self-administration session relative to the drug-free baseline period. It thus involves a difference in firing between a drug-free period and the entire period of drug exposure and potentially reflects an acute drug effect. Though the firing pattern has a time course consistent with a potential pharmacological origin, it is also possible that the firing patterns reflect NAcc encoding of nonpharmacological events. Available data are indicative of a combined role of pharmacological and nonpharmacological events in mediating the firing patterns.

Two lines of evidence are consistent with the hypothesis that at least some portion of the session-long changes in firing are nonpharmacological. First, the onset of some of the changes in firing precedes the delivery of the first drug infusion and occurs in association with the cues that signal the onset of drug availability and the onset of the first operant response. Second, NAcc neurons exhibit session-long changes in firing during FR1 sucrose-reinforced operant behavior. In considering possible nonpharmacological determinants of the firing patterns, researchers tested the hypothesis that the patterns reflect primarily an effect of particular behaviors (e.g., locomotion and operant response), which are present during the self-administration session, but not during baseline periods, when animals are typically at rest. Several lines of evidence led to the rejection of this hypothesis.

An alternative nonpharmacological determinant of the firing patterns could include reward-related events or states that are maintained throughout the operant session. Investigations of sucrose-directed behavior are consistent with this idea. In these studies, average firing of NAcc neurons was characterized across alternating phases of operant behavior reinforced on an FR1 schedule of conditioned reinforcer presentation and an FR1 schedule of sucrose plus conditioned reinforcement. Of the neurons that showed a session-long change in firing during one type of phase, approximately two-thirds showed session-long changes that were unique to the particular phases. Behavior during the two phases was comparable, except for the differential presence of consummatory behavior. Control studies showed that this behavioral difference could not explain the change in firing between the conditioned and primary reinforcement phases. These data suggest that the phase-specific session-long changes in firing reflect the response-reinforcer contingency (i.e., different expectations related to reward), which was stable throughout the particular phases but differed between the phases. These NAcc data are comparable with findings of a similar experiment, which recorded the activity of orbitofrontal cortex neurons during sucrose-directed behavior and with preliminary findings of an ongoing study of NAcc neurons during cocaine self-administration (Figure 2).

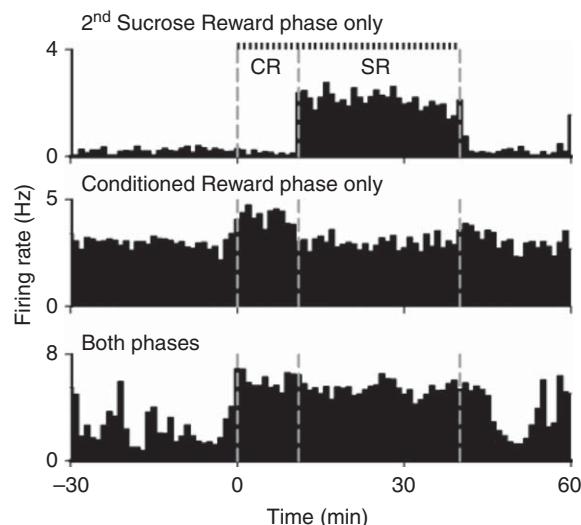


Figure 2 Individual-neuron examples of firing patterns during conditioned reinforcement (CR) and sucrose reinforcement (SR). Vertical dashed lines represent (from left to right) the start of conditioned reward phase, the start of the sucrose reward phase, and the end of the sucrose reward phase. Tick marks above the top histogram represent lever presses. Each row corresponds to the firing of a single neuron. Reproduced with permission from Kravitz AV and Peoples LL (2008) Background firing rates of orbitofrontal neurons reflect specific characteristics of operant sessions and modulate phasic responses to reward-associated cues and behavior. *Journal of Neuroscience* 28: 1009–1018.

Several lines of evidence suggest that during cocaine self-administration, some of the session-long changes in firing additionally reflect an influence of acute inhibitory pharmacological actions of cocaine. First, session-long decreases are the predominant change in average firing during cocaine self-administration sessions and are significantly more prevalent during cocaine self-administration sessions than during sucrose self-administration sessions. By contrast, the prevalence of session-long increases in firing is comparable between the two rewards. Second, decreases in average firing are dose dependent, increasing in prevalence, and in some cases magnitude, as drug level increases (Figure 3). Average firing of NAcc neurons does not show a similar concentration dependence when animals are trained to self-administer different concentrations of sucrose (Figure 3). Third, electrophysiological recordings in slice and anesthetized animal preparations show that experimenter-delivered cocaine inhibits spontaneous and glutamate-evoked firing of NAcc neurons.

Further investigations of the cocaine-induced inhibition in behaving animals showed that the acute inhibition induced by self-administered cocaine is activity dependent. Specifically, as a group, neurons that show a phasic increase in firing time-locked to the cocaine-reinforced lever-press show significantly less inhibition in firing

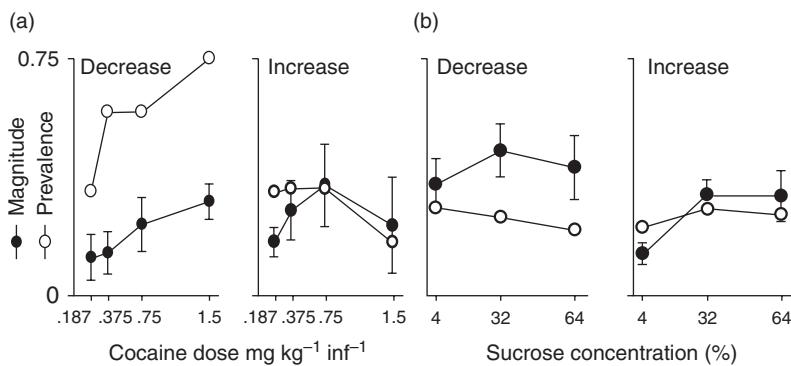


Figure 3 Changes in average firing of NAcc neurons during exposure to either different doses of cocaine or different concentrations of sucrose. (a) Animals trained to self-administered cocaine (FR1 to 60 s; 0.75 mg kg⁻¹) were exposed to one of three cocaine doses (0.75, 1.5, or 3.0 mg kg⁻¹ inf⁻¹). Prevalence and average magnitude of session-decrease firing patterns and session-increase firing patterns are plotted as a function of infusion type. Average magnitude of change in firing rate during the 2–6 min post-infusion relative to baseline. Index is defined as $|A - B|/(A + B)$, where A and B = average firing rate (Hz) during the self-administration session and the presession baseline, respectively. (b) Separate groups of rats were trained to self-administer sucrose on an FR1 schedule of reinforcement. The sucrose concentration varied across the groups. Prevalence and average magnitude of session-decrease firing patterns and session-increase firing patterns are plotted as a function of infusion type.

during the self-administration session than do neurons which show no change in firing time-locked to the behavior. The differential inhibition can be observed not only in the overall average firing rate during self-administration but also across multiple specific behaviors (e.g., stereotypy or locomotion), which occur during the self-administration session. This latter finding indicates that the differential change in firing rate reflects a general differential susceptibility of the neurons to cocaine-induced inhibition. The differential inhibition causes a net strengthening of firing during drug-directed behavior exhibited by the phasically active neurons (i.e., signal) relative to average background of all neurons (i.e., firing during periods in which animals are not engaged in drug-directed behavior). The differential inhibition and increases in signal:background ratio are not observed in animals trained to self-administer sucrose. It is hypothesized that the drug-induced increase in signal:background ratio may strengthen drug-directed behavior during periods of drug exposure.

Changes in NAcc Firing Patterns, Which Occur Across Repeated Cocaine Self-Administration Sessions, Drug Abstinence, and Reexposure to Cocaine Self-Administration

Multiple electrophysiological studies conducted in slice and anesthetized animal preparations have shown that a history of repeated experimenter-delivered cocaine exposure induces NAcc neuronal hypoactivity. Consistent with this observation, researchers have shown that a history of repeated cocaine self-administration is associated with decreases in average firing of NAcc neurons. These

decreases in firing are observed across multiple behavioral and pharmacological conditions and thus appear to reflect a general hypoactivity (e.g., reduced neuronal excitability). A similar decrease in average firing does not occur in animals exposed to a history of sucrose self-administration, which has been matched to the cocaine self-administration history (i.e., same number of reinforcers per session and same number of training sessions). The decrease in average firing is correlated with increases in the propensity of animals to seek and take cocaine, and thus potentially contribute to the change in behavior.

Interestingly, the decreases in average firing that occur in cocaine-exposed animals are only observed in a subset of accumbal neurons. Specifically, lever-press nonresponsive neurons (i.e., neurons that do not show a phasic change in firing time-locked to the operant) show an emergent hypoactivity. Lever-press responsive neurons do not exhibit this change in average firing. The differential change in average firing between the two groups of neurons may reflect a net increase in NAcc signaling related to drug-directed behavior and a corresponding NAcc-mediated facilitation of drug-directed behavior. The differential neuron susceptibility to the hypoactivity which develops across repeated drug self-administration sessions parallels the differential acute inhibition of NAcc neurons during daily self-administration sessions. It is hypothesized that the acute cocaine-induced activity-dependent inhibition of NAcc neurons during daily self-administration sessions alters the susceptibility of neurons to certain types of drug-induced plasticity, which in turn contributes to the activity-dependent changes in firing that occur after repeated exposure to cocaine self-administration.

A number of other studies have tested for changes in NAcc firing patterns that occur in animals that have a history of cocaine self-administration and an extended period of drug abstinence. Comparisons of firing before versus after abstinence show that phasic firing patterns time locked to cocaine-predictive cues, and cocaine-directed operant behavior are significantly increased after a 30-day period of abstinence relative to before the abstinence period. This increase in phasic responses to events which are predictive of cocaine persists when animals are reexposed to repeated daily cocaine self-administration sessions. In addition, the hypoactivity of the task-non-activated neurons which develops across initial pre-abstinence self-administration sessions is absent after 30 days of abstinence but quickly reemerges after only 3 days of reexposure to daily periods of self-administration training. Importantly, none of the changes in average or phasic firing that have been observed in cocaine-trained animals has been observed in similarly treated control animals trained to self-administer sucrose. This observation suggests that the changes in firing observed in cocaine-trained animals reflect cocaine-induced neuroadaptations.

The plasticity studies show that NAcc neurons undergo drug-induced adaptations which are stage dependent (i.e., ongoing periods of drug taking versus abstinence) and activity dependent (e.g., occur depending on whether neurons are lever-press responsive vs. lever-press nonresponsive). The adaptations in firing are associated with a progressive increase in the difference and ratio between average firing at the time of the drug-directed operant and average background firing (i.e., firing during periods in which animals are not engaged in drug-directed behavior). This type of change could potentially contribute to the progressive and chronic increases in drug-directed behavior and the decreased interest in nondrug rewards, which are associated with the development of drug addiction. Finally, the pattern of plasticity observed in behaving animals, which have a history of self-administered cocaine, is more complicated in several respects relative to that detected in electrophysiological slice and anesthetized animals studies, which have been primarily conducted in animals with a history of experimenter-delivered cocaine. This observation suggests that certain cocaine-induced adaptations depend on behavioral conditions present during periods of drug exposure and/or during electrophysiological studies.

Other Brain Regions and Other Addictive Drugs

Operant-locked phasic firing patterns during FR1 drug self-administration sessions have been observed during nicotine, ethanol, and heroin self-administration. The

firing patterns also occur in multiple regions connected with the NAcc, including the orbitofrontal cortex, medial prefrontal cortex, and the amygdala. The subtypes of firing patterns are similar across drugs and brain regions, though some differences in prevalence and functional characteristics of the firing patterns have been observed.

Session-long changes are exhibited by NAcc neurons during nicotine and heroin self-administration sessions. The firing patterns are also observed in the medial prefrontal cortex during cocaine and heroin self-administration, and in the orbitofrontal cortex and insula cortex during both cocaine and sucrose self-administration. The prevalence of the firing patterns differs across brain regions and drugs but available evidence indicates that in all cases session-long changes in firing during drug self-administration reflect a combined effect of pharmacological actions and normal afferent input associated with simple FR1 operant sessions. The inverse relationship between the direction and magnitude of session-long changes and phasic responses time locked to the operant behavior is observable in orbitofrontal cortex as well as the NAcc, suggesting that the pattern of activity may reflect a general mechanism associated with reward-directed operant behavior.

The studies of changes in neural activity across repeated self-administration sessions and abstinence are particularly challenging experiments, and as of yet few such studies have been completed. Only one study has characterized a drug other than cocaine. Interestingly, the adaptations in firing were identical to those observed in cocaine-trained animals.

Summary

Available evidence shows that there are similarities between NAcc firing patterns exhibited during individual sessions of cocaine- and natural reward-directed instrumental behavior. Some of the similarities include the following: (1) the predominance of excitatory changes in firing time locked to predictive cues and instrumental behavior, (2) evidence that the firing patterns reflect reward-related expectations, and (3) the occurrence of session-long changes in firing.

At least one similarity between drug and natural rewards is unexpected, which is the occurrence of session-long changes in average, background firing, and the relationship between these changes in background and phasic responses of neurons to discrete reward-predictive events such as the reinforced operant. Historically, electrophysiological studies of natural reward and reward-directed behavior have focused on short duration changes in firing time-locked to discrete events such as conditioned cues, operant behavior, and reward delivery. Many variables (e.g., context or motivation), which

influence reward-directed behavior are sustained over extended periods of time and can be critical to directing and maintaining behavior toward rewards, which are often distal in space and time and can require a long sequence of instrumental behavior. It is difficult to see how the short-duration phasic responses to discrete events could track or mediate the effects of the longer-duration determinants of reward-directed behavior. Instead, the session-long changes in firing could quite plausibly contribute to coding and mediating sustained influences on reward-directed behavior, such as the influence of context and motivation on behavioral responses to particular reward-predictive stimuli. The firing patterns could also potentially contribute to the persistence of behavior directed toward distal rewards.

The accumbal recording studies of cocaine-directed behavior have also demonstrated differences between the neurophysiological events associated with drug-directed and natural reward-directed behavior. Thus far, the most prominent differences in firing of NAcc neurons during cocaine versus food sessions include a greater prevalence of session-long decreases in firing during cocaine sessions than during sucrose sessions and a dissociation between neurons that are lever-press responsive during cocaine-directed versus natural reward-directed behavior. Comparisons of firing patterns across repeated self-administration sessions and periods of extended abstinence and reexposure also show that NAcc firing patterns in cocaine-trained animals exhibit a number of adaptations that are not observed in sucrose-trained animals. The majority of the differences in firing patterns between natural rewards and cocaine appear to reflect the differential presence of acute and chronic pharmacological actions of cocaine and are relevant to the search for mechanisms that contribute to drug addiction.

Studies of drugs other than cocaine and regions other than the NAcc have shown that firing patterns time locked to conditioned cues and operant behavior, as well as session-long changes in firing are general characteristics of neural activity during periods of drug-directed behavior. The presence of similar types of firing patterns between drugs and natural rewards is consistent with the evidence that drug-directed behavior of animals exposed to a limited history of drug self-administration is sensitive to many of the same variables that have an impact on natural reward-directed behavior. Moreover, the neural circuitry that contributes to both drug- and natural reward-directed behavior are similar. Continued studies

of drug reward and comparisons to natural reward are likely to further enhance our understanding of natural reward as well as drug reward and drug addiction.

See also: Animal Models of Behavior: Alcohol Addiction; Basal Ganglia; Cellular Plasticity in Cocaine and Alcohol Addiction; Drug Addiction; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Incentive Motivation and Incentive Salience; Motivation; Neural Systems of Motivation; Nicotine; Psychostimulants.

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Nicotine

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Glossary

Abstinence/withdrawal – A syndrome of uncomfortable psychological and biological effects that result when the use of an addictive drug is discontinued.

Cigarette – French for small cigar (cigar + -ette). A manufactured product consisting of cured tobacco leaf and other additives that are rolled into a paper cylinder that may have a filter at one end. The product is ignited at the nonfiltered end and smoke is inhaled through the filtered end.

Cotinine – The primary chemical byproduct ($C_{10}H_{12}N_2O$) of nicotine metabolism that is often used as a marker of tobacco use because of its long half-life.

Drug addiction – A behavior pattern of psychoactive drug use that involves overwhelming involvement with use of the drug, securing its supply, and a high likelihood to relapse to drug use after abstaining from use. Addiction often is accompanied by tolerance and withdrawal effects.

Drug dependence – The highly controlled or compulsive use of a psychoactive drug that is reinforced by the effects of that drug. Drug dependence often is accompanied by drug tolerance and withdrawal.

Negative reinforcement – The increased likelihood that a behavior will be repeated when that behavior is followed by the removal of an unpleasant or aversive state.

Nicotiana tabacum – Genus and species name for the cultivated tobacco plant most commonly used to make commercial tobacco products.

Nicotine – A chemical alkaloid ($C_{10}H_{14}N_2$) that is found naturally in tobacco.

Nicotine replacement therapy (NRT) – Any of several medicinal nicotine-containing products intended to help a person abstain from tobacco use by offsetting withdrawal symptoms.

Nicotinic acetylcholine receptors (nAChRs) – Ligand-gated ion channels found in the cell membranes of various cell types, including neurons and muscles that open in response to the endogenous neurotransmitter acetylcholine and to nicotine.

Passive administration/involuntary smoking/second-hand smoking – A mixture of the side-stream smoke from a burning cigarette, cigar, or pipe, and the smoke exhaled by a smoker, that is inhaled by a nonsmoker.

Positive reinforcement – The increased likelihood that a behavior will be repeated when that behavior is followed by a pleasant or rewarding consequence.

Sympathomimetic – The activation of the sympathetic nervous system branch of the autonomic nervous system.

Tobacco – An herbaceous plant native to North and South America, Australia, southwest Africa, and the South Pacific that contains nicotine, and is used to make consumer products such as cigarettes, cigars, pipe tobacco, chewing tobacco, and snuff.

Nicotine is a fascinating drug with a long history. It is highly addictive, can be arousing, as well as calming, but is usually self-administered through deadly tobacco products. The study of nicotine has revealed important information about receptor biology underlying drug actions in the body, neurochemical pathways in the brain, and mechanisms of positive and negative reinforcement. This primary drug of addiction in tobacco products also may be used or may inspire the development of new medications to treat anxiety, mood, thought, and skeletal muscle disorders.

History of Tobacco and Nicotine

Historical Use

Tobacco use and the self-administration of nicotine through tobacco products date back centuries. Mayan stone carvings (c. 600–900 AD) indicate tobacco use in the New World and other archeological evidence indicates tobacco use dating back several millennia earlier. European explorers to the New World in the late fifteenth and sixteenth centuries discovered native people of the American and Caribbean islands smoking or chewing dried tobacco leaves in spiritual practices, for medicinal reasons, and to achieve desired effects, including appetite regulation, energy enhancement, and relaxation. The word tobacco is derived from the Y-shaped tube (called a taboca or tobago) that was used to smoke tobacco leaves in the Caribbean islands. Several species of wild tobacco plant grew indigenously in North and South America, including *Nicotiana petunoides*, *Nicotiana rustica*, and *Nicotiana tabacum*. The tall,

broad-leaved *N. tabacum* became the tobacco species that is primarily cultivated and sold for commercial use throughout the world. Today, tobacco is grown in over 100 countries worldwide, including the United States. In every country and culture where tobacco has been introduced, self-administration of tobacco products has become common and persistent.

Isolation and Synthesis

As tobacco use spread to Europe, interest in the plant and its actions grew. In the early 1800s, Cerioli and Vauquelin discovered that the major active ingredient in tobacco was an oily material which was named ‘nicotianine’ after Jean Nicot de Villemain, the French ambassador to Portugal who introduced tobacco to the French court as a universal cure-all herb. Several decades later, Posselt and Reimann at the University of Heidelberg isolated nicotianine from *N. tabacum* and changed the name to ‘nikotin.’ In the mid-1800s, Melsens determined the chemical formula for nikotin ($C_{10}H_{14}N_2$), and Schloesing determined its molecular weight (162.23 g mol⁻¹). In the late 1800s, Pinner discovered the structure of what became known as ‘nicotine,’ an Anglicized spelling of the German word. Also in the late 1800s, Langley and Dickinson discovered that nicotine acted to stimulate autonomic ganglia and investigations of these actions led to the idea that chemicals (including nicotine) act at specific sites or receptors on cells which, in turn, release chemicals that transmit information between neurons. Pharmacological study of nicotine became active in the mid-twentieth century and continues to the present day.

Physical Chemistry

Nicotine (3-(1-methyl-2-pyrrolidinyl)-pyridine) is a highly toxic liquid alkaloid found naturally in several plant species, including *N. tabacum*, and is the key addictive component of tobacco products. Nicotine is a bicyclic compound with pyridine and pyrrolidine rings. The compound possesses one asymmetric carbon and can exist in two enantiomeric forms. In nature, nicotine exists in the S-shape, or levorotary form. Nicotine is a colorless and odorless base, with a dissociation constant (pK_a) of approximately 8.0. It forms water-soluble solid salts when mixed with acids. Nicotine is hydrophilic and lipophilic and readily distributes throughout the body because it is absorbed through the skin, mucous membranes, lungs, and gastrointestinal tract. Free-base nicotine combusts at approximately 35 °C (95 °F), so much of the nicotine in a cigarette burns off when the cigarette is lit but the amount that is inhaled exerts powerful effects in the brain, nervous system, and at other sites in the body.

Forms of Nicotine and Their Use

Not only is nicotine most commonly self-administered through tobacco products, but it also is self-administered in an ever-increasing variety of nicotine replacement products.

Tobacco Products

Tobacco cigarettes are the most common nicotine-containing products. Most cigarettes are commercially produced, but some people continue to roll their own cigarettes. Smoking cigarettes is the most common way to self-administer nicotine and it is the most effective way to get nicotine to the brain. Tobacco also is smoked in pipes, cigars, flavored cigarettes, bidis, kreteks, and hookahs. Pipe and cigar smoking is more common among men than women, but cigar smoking is increasing in popularity among women. Flavored cigarettes are most common among teenage and young adult smokers. Flavored cigarettes usually have lower nicotine contents than conventional cigarettes, and have distinctive flavors such as cherry, mocha, or vanilla. Bidis are small, hand-rolled cigarettes primarily smoked in or imported from India and Southeastern Asian countries. Bidis may or may not be flavored. Kreteks are made from a mixture of tobacco, cloves, and other herbs. Bidis and kreteks deliver more nicotine than the standard tobacco cigarette. Hookah (also known as water pipe, nargeela, arghileh, and nargile) is a traditional Middle Eastern or Asian device that operates by water filtration and indirect heat to cool the tobacco smoke as it is inhaled by the smoker. Nicotine-containing tobacco products also include smokeless products, including chewing tobacco and snuff. Chewing tobacco is usually kept under the lip or in the cheek. Snuff can be inhaled through the nasal cavity.

Nicotine Replacement Products

In addition to tobacco products, nicotine is now available in various nicotine replacement products or nicotine replacement therapy (NRT). Nicotine polacrilex gum was developed by Ove Ferno and colleagues in Sweden in the 1970s to help people quit smoking tobacco cigarettes. Nicotine gum became available in the United States as a prescription medication in the 1980s to help alleviate withdrawal symptoms in abstinent smokers. By the twenty-first century several dosages and flavors of nicotine gum became available over the counter (OTC) and no longer required a prescription. Currently, nicotine is available in several other forms, including nicotine transdermal patches, nicotine vapor inhaler, nicotine lozenges, nicotine microtabs (small sublingual tablets that slowly dissolve under the tongue), nicotine nasal spray, and nicotine in water.

Epidemiology

Approximately 20% of adults (1.3 billion people) smoke cigarettes worldwide and more men than women smoke. Similarly, in the United States, 20.8% of adults (45.3 million people) currently smoke tobacco cigarettes, and prevalence is higher among men (23.9%) than among women (18.0%). In the United States, tobacco use by numbers and by percentage of the population has decreased over the past 20 years, but global numbers of smokers have increased. In the United States, it is estimated that 4000 children under the age of 18 try smoking each day, that about 2000 of them continue to smoke, and that almost 20% of high school students smoke cigarettes everyday.

Prevalence of smokeless tobacco use among adults in the US is about 3% (6% of men vs. 0.4% of women). About 8% of high school students in the US use smokeless tobacco (13.6% of males vs. 2.2% of females). Worldwide 11% of children use tobacco products other than cigarettes.

Administration, Absorption, and Distribution

The chemical and physical properties of nicotine allow it to be readily absorbed through the skin, mucous membranes, and the respiratory tract. These multiple sites of absorption and ready passage of nicotine allow it to be self-administered or administered medically through several different methods.

Self-Administration of Nicotine-Containing Tobacco Products

The most common way to self-administer nicotine is by smoking tobacco products, primarily commercially made cigarettes. When a person smokes a tobacco product, the inhaled smoke travels through the respiratory tract and is absorbed by the alveoli in the lungs. The absorbed nicotine passes from the alveoli through the capillary walls and into the bloodstream. The pulmonary circuit of the cardiovascular system carries nicotine directly to the brain within seconds of inhalation and the nicotine rapidly crosses the blood-brain barrier through passive diffusion because of its small size and lipophilicity and through active transport by the choroid plexus. As a result, the smoker receives a bolus of nicotine into the brain soon after taking a puff on the cigarette. That bolus infusion of nicotine sets off a cascade of neurochemical and neurophysiological events (discussed below under the section titled 'Actions') which result in reward, dependence, and other reinforcing effects.

Nicotine also is self-administered through smokeless tobacco products, but the time course for nicotine to reach the brain is much slower than with smoked tobacco products. Nicotine from smokeless tobacco either is absorbed in blood vessels in the mucous membranes of the mouth or travels to the stomach with saliva and enters the bloodstream through the digestive system. The amount of nicotine that reaches the brain from smokeless tobacco products is relatively small and some of it is metabolized in the liver before reaching the nervous system. The addiction liability to smokeless tobacco products is markedly less than the addiction liability to smoked tobacco products because of the slower time course and reduced quantities of nicotine that reach the brain.

Self-administration of Nicotine-Containing Medications

Nicotine is available in several products that are used as medications (NRT) to help smokers abstain from tobacco use and to attenuate withdrawal symptoms that accompany abstinence from regular tobacco self-administration. NRTs use several different routes of administration.

Nicotine polacrilex gum, or simply nicotine gum, was the first nicotine replacement product. Nicotine gum became available in Europe in the 1970s and seemed to be a valuable adjunctive pharmacological therapy to behavioral smoking cessation approaches. It was hailed as a new effective treatment to aid in smoking cessation but subsequent studies in the United States in the 1980s showed modest efficacy. Later in the 1980s and 1990s as nicotine addiction became better understood, comparison of nicotine gum clinical trials revealed that higher dosages were being used in the European versus American clinical trials. Now several dosages and flavors of nicotine gum are available. These gums are, indeed, useful ways to administer small amounts of nicotine and are valuable adjunctive therapies to behavioral and cognitive smoking cessation techniques.

Similar to nicotine gum, nicotine lozenges and nicotine microtabs administer nicotine orally. Some nicotine crosses the mucous membranes in the mouth and some nicotine travels through the gastrointestinal tract and is absorbed into the circulation. Much of this nicotine is metabolized in the liver, so reduced amounts of the drug reach the brain.

Nicotine can also be inhaled using an NRT vapor inhaler. Although the administration device is called a nicotine inhaler, most of the nicotine actually stays within the oral cavity and is absorbed through the oral mucosa rather than through the lungs. Similar to the gum, lozenge, and microtab, much lower plasma concentrations of nicotine are achieved from the NRT vapor inhaler than by smoking cigarettes, so many doses are necessary

throughout the day to satisfy the smoker and to attenuate withdrawal.

Nicotine patches and nicotine nasal spray deliver nicotine through different routes than the other products. Nicotine skin patches are now available in several dosages and from several manufacturers. Nicotine is slowly absorbed across the skin over many hours (up to 24 h per patch). The nicotine nasal spray administers the drug intranasally and it is absorbed through the blood vessels in the mucous membrane of the nose. As with the other NRTs, dosages of nicotine are much lower and follow a slower time course than smoking tobacco products, which is why these products have a low addiction liability compared with smoking nicotine-containing tobacco.

Passive Administration

It is important to realize that nicotine can be administered unknowingly. Environmental tobacco smoke (also known as ETS, second-hand smoke, involuntary smoking, or passive smoking) from side-stream smoke of burning tobacco products and from exhaled smoke from tobacco smokers is absorbed by inhalation and transdermally by nonsmokers who are in the proximity of smokers. In fact, a nonsmoker who lives with a two-pack-a-day smoker can have urinary levels of cotinine (the primary metabolite of nicotine) equivalent to smoking several cigarettes per day. The amount of ETS exposure increases in spaces that are enclosed, poorly ventilated, or where the air is recirculated. That is why airplanes and many restaurants and enclosed public places have banned tobacco smoking. Children are at particular risk to develop smoking-related pulmonary health problems as a result of ETS.

Distribution

Following absorption, nicotine is rapidly distributed in many tissues and organs throughout the body because it is lipophilic and readily passes through cell membranes. In

cigarette smokers and other tobacco users, nicotine reaches its peak plasma concentration within 30 min of administration, and then slowly declines during the next several hours. Smoking cigarettes delivers nicotine to the brain within seconds of inhalation. NRTs have different time courses for distribution of nicotine based on the specific route of administration. Nicotine nasal spray, gum, and lozenges deliver and distribute nicotine quickly to the peripheral bloodstream, but nicotine levels also decline quickly using these methods. In contrast, nicotine is delivered slowly by the nicotine transdermal patch. For the 24-h patch, plasma concentrations of nicotine rise slowly during the first 6–10 h, plateau after 8–12 h, and then decline slowly during the subsequent 6 h. The slow distribution and clearance of the patch allow for its once daily use, as opposed to the high dosing frequency of other NRTs.

Actions

Table 1 presents a summary of neural substrates underlying key actions of nicotine.

Nicotine Receptor Biology

Nicotine acts at chemical receptors located throughout the body which partially explains its wide-ranging biological and psychological effects. Nicotine is an agonist at nicotinic acetylcholine receptors (nAChRs) in the central and peripheral nervous systems (nAChR_N), but has little or no effect at similar nicotinic acetylcholine receptors in the muscle (nAChR_M). The receptors are presynaptic ligand-gated ion channels which are composed of five subunits. There are several classes of subunits found in the nervous system (including α and β), each of which has several different subtypes (e.g., $\alpha 2-10$ and $\beta 2-4$). α -Subunits are differentiated from the β -subunits because they have two adjacent cysteine residues that are necessary for the binding of acetylcholine. There is

Table 1 Summary of neural substrates underlying key actions of nicotine

<i>Nicotine action</i>	<i>Neural substrate</i>
Reinforcing and rewarding effects	Mesocorticolimbic dopamine pathway (including PFC, NAcc and VTA) via $\alpha 4\beta 2$
Increase attention, alertness, vigilance, and arousal	Decreased slow-wave EEG, increased fast-wave EEG
Enhance memory	Hippocampus via $\alpha 7$
Enhance movement	SNC
Sympathomimetic effects (including effects on respiration, heart rate, and blood pressure)	Sympathetic and parasympathetic branches of the autonomic nervous system
Mood modulator	Via increased serotonin release
Maintenance of self-administration and conscious urges	Insula and mesocorticolimbic dopamine pathway
Induce nausea and vomiting	Emetic trigger zone in area postrema
Induce vasopressin secretion	Supraoptic nucleus of hypothalamus

growing evidence that the various effects of nicotine (e.g., addiction, appetite regulation, mood modulation, and attention effects) in humans and animals may be explained by activation of different nicotine receptor subunits and various combinations of these subunits.

Nicotine receptors are found on dopaminergic ($\alpha 4\beta 2$, $\alpha 6\beta 2$, and $\alpha 6\beta 3$), glutamatergic ($\alpha 4\beta 2$ and $\alpha 7$), γ -aminobutyric acid (GABA)ergic ($\alpha 4\beta 2$), adrenergic ($\alpha 3\beta 2$ and $\alpha 7$), and cholinergic ($\alpha 3\beta 4$) nerve terminals. The receptors are located in several areas of the brain relevant to reward, addiction, and psychoactive effects, including the substantia nigra pars compacta (SNc), ventral tegmental area (VTA), nucleus accumbens (NAcc), midbrain tegmentum, striatum, and various regions of the cerebral cortex. The receptors are located presynaptically in the brain. Nicotine binding increases depolarization of the nerve terminal, leads to an influx of Ca^{2+} , and enhances neurotransmission at that terminal. Stimulation of nAChRs in the VTA, for example, releases dopamine in the NAcc, an area responsible for the reinforcing effects of many drugs. Mice genetically bred with the $\alpha 4$ - or the $\beta 2$ -subunit knocked out do not exhibit increased dopamine release in response to nicotine, and do not maintain nicotine self-administration. Further, $\alpha 4$ knock-in mice (which express a defective $\alpha 4$ -subunit) show reduced sensitization, reinforcement, and tolerance to nicotine. These data suggest that the $\alpha 4\beta 2$ receptor is necessary for dopamine release in the NAcc and is a key receptor underlying nicotine self-administration. The $\alpha 7$ -subunit seems to be involved in effects of nicotine on attention and memory and also may be involved in axogenesis and neuroprotection. Ongoing studies of nicotine subunits are focusing on the development of new medications to treat nicotine dependence and to treat other conditions that may be helped by nicotine agonists, including cognitive dementias; anxiety, mood, and thought disorders; and skeletomuscular problems.

Nervous System and Neurotransmitters

Nicotine acts at many sites in the brain, including the medial habenula, interpeduncular nucleus, SNc, and VTA. These sites are relevant to nicotine's physical (movement), cognitive (attention), motivational (reinforcement and reward), and therapeutic effects (e.g., to treat schizophrenia, dementia, or Parkinson's disease). Nicotine acts in the central nervous system (CNS) and peripheral nervous system (PNS). It is classified as a sympathomimetic pharmacological agent but it actually stimulates both the sympathetic and parasympathetic branches of the autonomic nervous system (ANS). Nicotine induces the release of norepinephrine in the sympathetic branch and acetylcholine in the parasympathetic branch of the ANS. Because the two branches have

opposing actions, nicotine can exert differential effects depending on which branch has greater activation. These opposing effects may help explain why nicotine seems to be unique in that it can stimulate or relax, arouse, or calm. Colloquially stated, nicotine can bring people up when they are down and down when they are up. Although these opposite physiological effects have been well known to scientists and smokers for a century, psychologist Stanley Schachter (in the late 1970s) referred to this phenomenon as Nesbitt's paradox, based on research in his laboratory being conducted by Paul Nesbitt, and the nickname stuck.

Nicotine acts on mesolimbic and mesocortical dopaminergic pathways that are involved in reward and addiction. The major dopaminergic neurons of these pathways originate in the VTA and project to the NAcc, pallidum, hippocampus, amygdala, and medial prefrontal cortex (PFC) in the mesolimbic circuit, or to other areas of the frontal cortex in the mesocortical pathway. The mesocorticolimbic dopamine pathway is involved in reinforcing or rewarding effects of pleasurable experiences or activities including drug use. After smoking a cigarette, dopamine levels in the NAcc increase, which increases the likelihood that the person will smoke (self-administer nicotine) again in the future. Nicotine also increases release of serotonin, which may underlie nicotine's effects to help modulate mood. The insula, sometimes called the fifth lobe of the brain, is involved in conscious urges, and also may be involved in maintenance of nicotine self-administration and smoking. Nicotine increases the respiration rate through receptors in the carotid body and aortic arch, induces nausea and vomiting through receptors in the emetic trigger zone of the midbrain's area postrema (which is why first-time smokers often feel sick), and induces vasopressin secretion by acting in the supraoptic nucleus of the hypothalamus.

Physiology

Nicotine is considered a CNS stimulant or sympathomimetic because it increases heart rate, respiratory rate, blood pressure, and causes peripheral vasoconstriction. Increased arousal is evident in the electroencephalogram (EEG). Nicotine decreases slow-wave electrical activity in the brain; increases faster, high-voltage waves that are involved in conscious, active thought; and enhances responses to auditory and evoked potential responses reflecting increased attention and information processing. Nicotine decreases skeletal muscle tone as reflected by electromyogram (EMG) recordings, which may contribute to feelings of relaxation when smoking. Nicotine also decreases body weight by decreasing appetite and by increasing metabolism.

Behavior

Nicotine's ready passage into the CNS, especially when self-administered in tobacco smoke, produces marked central effects that increase subsequent self-administration of nicotine-containing products. The close temporal association between nicotine self-administration and central actions and the rewarding effects of nicotine (including increased arousal, attention and mood, and reduced anxiety, appetite, and body weight) increase the likelihood of nicotine self-administration.

Cognition

Nicotine increases selective attention, sustained attention, alertness, and vigilance. It also decreases distraction. Clinical studies suggest that nicotine can improve cognitive function in cases of senile dementia and Alzheimer's disease.

Motivation

Nicotine acts in several ways to alter motivation for nicotine self-administration. Nicotine decreases general and specific hunger (to sweet tastes) and increases desire for nicotine itself. It is likely that these motivational effects are related and that both involve mechanisms of the mesocorticolimbic dopamine pathway. These effects include alterations in hedonic tone, so it is likely that the limbic system also is involved in the pleasant experience associated with nicotine administration with repeated exposure. Further, it is likely that endogenous opioid systems and serotonergic mechanisms act in concert with the dopaminergic pathways to underlie reinforcement of nicotine self-administration. Both positive and negative reinforcement also contribute to motivation to self-administer nicotine. Nicotine's neurochemical actions (especially on dopamine, serotonin, and endogenous opioid peptides), appetite-controlling effects, and attention-enhancing effects, all act as positive reinforcers to increase the likelihood of nicotine self-administration. The unpleasant withdrawal effects following nicotine abstinence act through negative reinforcement to motivate nicotine self-administration to offset the abstinence effects (see the section titled 'Abstinence effects'). The fact that nicotine self-administration accompanies stress also may reflect a motivational effect and seems to involve the corticotropin-releasing factor (CRF) system.

Toxicity

Nicotine is a poisonous chemical and is used as a powerful pesticide. It is toxic at 60 mg for the average human adult and roughly 10 mg for a child. Exposures to toxic levels of nicotine are most common in tobacco farmers and in

people exposed to high amounts of nicotine-containing pesticides. Symptoms of acute nicotine poisoning include nausea, vomiting, salivation, cold sweat, pallor, abdominal pain, diarrhea, and respiratory distress as a result of over-stimulation of nAChRs in the ANS. Prolonged exposure to high dosages of nicotine results in severe CNS depression, muscle paralysis, and respiratory failure. The leading cause of death from nicotine poisoning is respiratory failure.

Metabolism

The liver is the major site of nicotine metabolism in the body, accounting for 80–90% of its metabolism. Approximately 70–80% of nicotine metabolized by the liver is converted to the nicotine iminium ion and 5'-hydroxynicotine by the cytochrome P450 (CYP) enzymes 2A6 and 2B6, and then to cotinine by aldehyde oxidase. Sixty percent of cotinine is further metabolized. Whereas it is clear that nicotine is an active drug, there is some evidence that cotinine also may have physiological and psychopharmacological actions. A small percentage of nicotine is converted to nicotine-1'-N-oxide (4%) by flavin-containing monooxygenase, nicotine glucuronide (4%) by *N*-glucuronidation, and nornicotine (<1%) by *N*-demethylation. Some nicotine is excreted unchanged. Minor sites of nicotine metabolism include the brain, lungs, and kidneys.

Elimination

Nicotine has an elimination half-life ($t_{1/2}$) of approximately 2 h in humans, but this value varies from 1 to 4 h among people. This relatively short $t_{1/2}$, and the relative pharmacological inactivity of nicotine's metabolites contribute to frequent smoking by people who are addicted to nicotine. The $t_{1/2}$ for cotinine is 17 h, and cotinine clearance is highly correlated with nicotine clearance. Concentrations of cotinine in the saliva, plasma, and urine are correlated, so salivary cotinine serves as a good biomarker for tobacco or nicotine exposure.

The major site of elimination for nicotine and its metabolites is in the urine, through the kidneys. Approximately 10% of nicotine and 10% of cotinine are excreted unmetabolized in the urine, although the process is pH dependent. When the pH of urine is acidic, then nicotine reabsorption from the kidney decreases which results in increased renal clearance of nicotine. Certain foodstuffs as well as physical and psychological stress can acidify the urine and may thereby contribute pharmacokinetically to increased nicotine intake through cigarette smoking.

Trace amounts of nicotine and its metabolites remain in other body fluids, such as the saliva. Nursing mothers also secrete nicotine in breast milk which can expose nursing children to nicotine.

See also: Animal Tests for Anxiety; Cellular Plasticity in Cocaine and Alcohol Addiction; Depression; Drug Addiction; Drug Withdrawal – Motivational View; Ethanol and Nicotine Interactions; Feeding; Hallucinogens; Motivation; Neurobiology of Opioid Addiction; Parkinson's Disease; Psychostimulants; Schizophrenia.

Abstinence Effects

Abstinence from nicotine self-administration in a drug-dependent or addicted individual results in withdrawal effects. Symptoms of nicotine abstinence include craving for nicotine, irritability, difficulty concentrating and paying attention, sleep difficulties, dysphoria, impatience, increased appetite, and weight gain. Most of these symptoms begin within 24 h after nicotine abstinence, peak between 36 and 72 h, and gradually subside after several days. However, urges or craving for nicotine and increased appetite and weight gain can persist for 6 months to a year. The withdrawal symptoms, severity, and length of time symptoms last vary widely among people, and may be influenced by gender, age, ethnicity, duration of nicotine self-administration, and amount of nicotine self-administration.

Administration of nicotine will attenuate or offset the unpleasant withdrawal symptoms and will enhance dependence on nicotine via negative reinforcement. NRTs are used to offset withdrawal symptoms and to complement behavioral, cognitive, and motivational strategies and techniques to help people successfully give up tobacco use.

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Pain and Addiction

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Glossary

Aberrant drug-related behaviors – Behaviors such as unauthorized dose escalations or obtaining controlled prescriptions from multiple physicians may signal the presence of abuse or addiction. However, such behaviors sometimes reflect inadequate analgesia (pseudoaddiction) or the misuse of opioid analgesics to treat mood or other psychiatric symptoms.

Buprenorphine – Partial μ -opioid agonist and kappa antagonist, listed on Schedule II and approved by the Food and Drug Administration (FDA) for the treatment of opioid dependence since 2002. The combination tablet Suboxone (buprenorphine/naloxone) is the abuse-deterrent formulation recommended for the treatment of opioid abuse. Suboxone can be used for the concurrent treatment of opioid dependence and chronic pain.

Medication agreement – Written agreements delineating rules for patients receiving opioid analgesic therapy. Intended to be a communication tool, such agreements specify parameters for adherence, methods for monitoring compliance (e.g., random urine testing, pill counts), and general goals of treatment. Medication agreements typically call for periodic reassessment and a clear exit strategy for opioid tapering and termination, in the event of either lack of therapeutic response or nonadherence.

Methadone – Full μ -opioid agonist with a long half-life (17–128 h). When used to prevent opioid withdrawal, once-daily dosing is sufficient. Analgesic effects only last for 6–10 h, however. Methadone must be prescribed at a low dose initially and then titrated slowly to avoid toxicity.

Prescription-opioid addiction – Distinguished from simple physiological dependence, opioid addiction consists of one or more of the following behaviors: impaired control over drug use, continued use despite harm, and craving. Rates of the nonmedical use and abuse of prescription opioids have risen dramatically in the United States of America (USA) in the past two decades.

Pseudoaddiction – Reversible opioid-seeking behavior occurring in a patient with under-treated pain whose behavior normalizes once the pain is under control. This behavior is not maladaptive and, thus, does not represent drug addiction but may be misinterpreted as opioid abuse.

Epidemiology of Pain in the United States of America

Pain that persists or recurs for more than 6 months is defined as chronic and is one of the largest medical problems in the developed world. Chronic pain is a major health issue in the United States of America (USA), affecting approximately 76 million Americans. There is, thus, an enormous population at risk for becoming involved in prescription-opioid misuse. Its economic toll, including healthcare costs and lost work productivity, is about \$100 billion per year. These individuals experience decreased quality of life and frequently suffer from sleep disturbance, depression, and anxiety. A recent cross-sectional survey found that 13% of the total US workforce experienced a loss in productive time in a 2-week period due to a common pain condition. Headache is the most common (5.4%) pain condition resulting in lost productive time, followed by back pain (3.2%), arthritis pain (2.0%), and other musculoskeletal pain (2.0%). A large study in the Southwest found that 30% of hospitalized patients reported being under-treated for pain. In addition, in the ambulatory population, it is estimated that 50–70 million people in the USA suffer from pain that is under-treated or not treated at all.

Despite the prevalence and importance of chronic pain, many healthcare providers wish to avoid becoming involved in its treatment for several reasons. Persons with chronic pain who have seen many physicians without relief

may be perceived as less than honest concerning their symptoms. The treatment of pain is complicated and requires a multidisciplinary approach, which often receives poor reimbursement. In addition, chronic pain does not produce sympathetic arousal, and the absence of objective signs of physiological stress often leads to potential skepticism about the reported symptoms. Finally, in the face of increased regulatory scrutiny and an epidemic of prescription drug abuse, many clinicians have shied away from using opioids. This position of real or perceived vulnerability in which healthcare professionals find themselves places patients at risk for suboptimal management of an often treatable medical condition.

Epidemiology of Prescription-Opioid Abuse in the USA

In the past two decades, the nonmedical use and abuse of prescription opioids has risen dramatically in the USA. Between 1990 and 2003, the rate at which young adults (age range: 18–25) initiated prescription-opioid abuse tripled from 10.2 per thousand-person-years in 1990 to 31.6 per thousand person-years in 2003. From 1992 to 2005, the US population increased by 15%, whereas during this period the number of adults abusing controlled substances increased by 98%. A 2006 survey by the Partnership for a Drug-Free America showed that 19% of US teens (4.5 million) reported using prescription opioids that were not prescribed to them. In 2005, 2.4% of the US population in the age range 18–25 initiated the use of a pain reliever for a nonmedical purpose. The abuse of prescription opiates starts later (21.9 years) on average than that of marijuana (17.4 years) and alcohol (16.6 years). ‘Generation Rx’ is the popular name given by the Partnership for a Drug-Free America for these young, adult, prescription-opioid abusers.

Young adults are much more likely to start abusing prescription opioids than illegal opioids such as heroin. Dependence on or abuse of prescription opioids is now as common as dependence/abuse of cocaine, and more common than dependence/abuse of any other drug except marijuana. Compared to heroin abusers, prescription-opioid abusers are more likely to be white, be younger, have higher incomes, and use less opioid per day. Prescription-opioid users also seek treatment earlier, are more likely to complete treatment, and have better outcomes than patients using heroin. In addition, prescription-opioid users are less likely to have hepatitis C infection.

The Drug Abuse Warning Network – another indicator of problem drug use – revealed that overall narcotic analgesic emergency department (ED) visits rose from 42 857 in 1995 to 160 363 in 2005. Methadone, oxycodone, and hydrocodone were the most frequent opioids. During the period 1992–2002, ED visits involving narcotic analgesics increased for all age groups, except those in

the age range 12–17, and the 45–54 age group experienced the largest increase (298%). From 1995 to 2002, increases in drug abuse-related ED visits were observed for several individual opioid analgesics: oxycodone (512%), methadone (176%), hydrocodone (159%), and morphine (116%). The most frequently mentioned motive underlying drug abuse-related ED visits involving narcotic analgesics was dependence (47%; 50 623 visits), followed by suicide (22%; 24 308 visits), and psychic effects (15%; 16 153 visits). Further, among reported drug-induced and drug-related deaths, a narcotic analgesic ranked in the top three most frequently reported drugs in New York and in seven other US cities. Taken together, these data reveal that the continuing trend toward increasing nonmedical use and abuse of prescription opioids in the USA has resulted in sharp recent rises in morbidity and mortality at the local and national levels.

While prescription opioid abuse is increasingly common among young adults, its initiation is also closely tied to the relief of pain. A 2004 survey of patients presenting for methadone therapy found that 83% had been using prescription opioids (with or without heroin), and 24% had been using prescription opioids exclusively. The majority of patients using opioids only (or initially) reported that their primary reason for starting to use opioids was to treat pain: 86% (and 62%, respectively). Most (61%) of those using prescription opioids obtained at least some of their supply from physicians. However, most prescription-opioid abusers obtain their drugs from family members or friends, and not directly from physicians. The most common source for nonmedical users to obtain pain-relievers is from a friend or relative for free; 53% of past-year prescription-drug users said they received the prescription drug in this way, while 13% obtained prescriptions from one physician, and 11% bought the pills from a friend or relative. In a sample population of nearly 28 000 patients presenting for addiction-treatment programs in 2001–04, 78% of those who reported OxyContin use had not received a prescription for any medical reason.

Neurobiology of Opioid Addiction

Like other types of substance dependence, opioid addiction is a chronic neurobiological disease arising from repeated exposure to an addictive drug and characterized by loss of control over drug use. Neuroadaptive dysregulations within the brain reward systems and emotional/stress systems occur with chronic abuse to produce the profound dependence that characterizes opioid addiction. The ‘reward circuits’ that play a pivotal role in maintaining a pattern of compulsive drug taking are comprised of neuronal pathways that originate within the ventral tegmental area (VTA) and project to the nucleus accumbens

(NAcc), amygdala, and prefrontal cortex. Opioids act to release dopamine (DA) directly via the opioid receptors in the NAcc, as well as indirectly by decreasing γ -aminobutyric acid (GABA) inhibition in the VTA.

Certain neuropathological changes have been found in individuals with a history of opioid abuse. These include reductions in gray matter in the prefrontal, insular, and temporal cortices in patients on methadone maintenance compared with age- and sex-matched controls. Lower-than-normal levels of *N*-acetylaspartate, a marker of neuronal viability, have also been detected by magnetic resonance spectroscopy in frontal gray matter of patients maintained on methadone, buprenorphine, or heroin. To date, these studies do not allow the differentiation of pre-existing conditions from possible effects of opioid abuse.

Several recent studies have provided some insight into the poor behavioral regulation that is a core feature of substance abuse. Chronic pain patients demonstrate cognitive deficits including decision-making abnormalities and impaired prospective short-term memory. Functional magnetic resonance imaging (fMRI) studies have shown that chronic pain patients display reduced deactivation in several key regions of the 'default mode network' (DMN), representing areas of the cortex known to be active at rest. Absent or diminished error-related activation has been found in the anterior cingulated cortex in opiate users, with compensatory recruitment in the parietal and temporal areas in order to perform the task. Heroin addicts also display disadvantageous decision making and prolonged deliberation times while making risky decisions. Evidence from neuropsychological and neuroimaging studies suggests that impairments in prefrontally mediated cognitive functions, namely decision making and inhibitory control, are associated with addiction. These deficits increase the risk for impulsive decisions and may underlie the 'loss of control' pattern characteristic of opioid addicts, who display poor inhibitory control of drug-related behavior despite adverse consequences.

Recent US Trends in Opioid Prescribing

Opioid prescribing practices play an important role in the supply of abusable opioids and hence the rise in prescription-opioid dependence. Opioid prescriptions have increased substantially from 1997 to 2005, with increases of 933% in methadone prescriptions, of 588% in oxycodone prescriptions, and of 198% in hydrocodone prescriptions. Much of the increase in methadone prescriptions occurred because of escalating abuse and diversion of OxyContin. Physicians looked for a suitable long-acting analgesic and erroneously chose methadone, which has a duration of analgesic action only slightly longer than morphine. In EDs, opioid prescribing for pain-related visits increased from 23% in 1993 to 37% (95% CI: 34–39%)

in 2003 ($p < 0.001$ for trend). This trend was more pronounced in 2001–05 ($p = 0.02$), following national quality-improvement initiatives in the late 1990s which emphasized the need for adequate treatment of pain. Similarly, a nationally representative stratified cluster sample of 30 000 physician office visits in the decade 1993–2003 revealed a significant increase in the visit rate over the decade from 0.126 opioid visits per person in 1993 to 0.1666 – a 32% increase ($p < 0.001$ for trend). During this same period, there was a large shift in the types of opioids prescribed; while codeine and propoxyphene visit rates declined by 40% and 28% respectively, substantial increases occurred in visit rates for higher-potency opioids such as hydrocodone and oxycodone (115% and 156%). The co-occurring increases in opioid abuse and prescribing in the last decade suggest that both EDs and office visits are channels for the supply of abused opioids. Regulatory actions by the Drug Enforcement Administration (DEA) in 2006–07 have substantially curtailed the availability of prescription-opioid analgesics via Internet pharmacies.

In pain-management settings, at present up to 90% of patients receive opioids for chronic pain management. While chronic opioid analgesic therapy will lead to opioid abuse or addiction in a small percentage of chronic pain patients (3.3%), a larger percentage will demonstrate aberrant drug-related behaviors or illicit drug use (11.5%). While these percentages are considerably lower (0.2% and 0.6%, respectively) if chronic pain patients are preselected for the absence of a current or past history of alcohol or illicit substance use or abuse/addiction, the presence of an addictive disorder does not adequately predict prescription-opioid abuse.

Education with regard to the risks attendant in prescribing controlled substances is currently lacking in schools of medicine and pharmacy. Surveys have shown that less than 40% of physicians have received any training in medical school in identifying prescription-drug abuse or diversion. In addition, only 50% of pharmacists receive any training in recognizing prescription drug abuse, addiction, or diversion. Moreover, at present it is not known whether the risk of iatrogenic addiction in patients treated with opioid analgesics is relatively high (>10%) or low (<0.1%). Randomized trials and comparative longitudinal studies have been lacking, and methodologically weak studies have yielded conflicting results.

Psychiatric Comorbidities in Chronic Pain Patients

Chronic pain patients frequently present with psychiatric comorbidities including depression, anxiety, substance-use disorders, somatization, and personality disorders. Among chronic pain patients, the prevalence of drug or alcohol

abuse has been noted to be 3–19%. Some recent studies have suggested even higher rates. A prospective cohort study among opioid-treated chronic pain patients reported a 1-year incidence of 32%. Although the direction of causality remains controversial, there is some recent evidence to suggest that psychiatric disorders lead to substance abuse among prescription-opioid users, rather than prescription opioids posing an iatrogenic risk for substance abuse.

By contrast, the rates of new-onset opioid abuse in patients introduced to opiates medically appear to be quite low. Of the total of 15 000 veterans who were initiated and maintained on opiates for at least 3 months, only 2% developed an opioid abuse diagnosis. Another recent prospective study found a new opioid abuse incidence of about 6% in individuals previously treated for pain. Of note, this study found prior amphetamine use to be the single best predictor of future nonmedical opioid use. This association is striking in light of the recent finding that in patients treated for the first time with opioids for chronic pain, a subjective feeling of stimulation was predictive of eventual addiction.

Assessment of Substance Abuse in Long-term Opioid Therapy

Given that many patients taking opioids may not exhibit psychological dependence such as craving or other overt addictive behaviors, special attention is needed to assess potential opioid abuse in pain patients. In a study of opioid-abuse criteria among 404 chronic pain patients enrolled in a pain clinic, neither past opioid or alcohol abuse, nor psychosocial testing on admission reliably predicted who would become an opioid abuser. Aberrant drug-related behaviors that suggest an addiction disorder in pain patients include: (1) selling prescription drugs; (2) forging prescriptions; (3) stealing drugs; (4) injecting oral formulations; (5) obtaining prescription drugs from non-medical sources; (6) concurrent abuse of alcohol or illicit drugs; (7) repeated dose escalations or otherwise failing to comply with the prescribed regimen despite warnings; (8) losing prescribed medication on multiple occasions; (9) repeatedly seeking prescriptions from other clinicians or from emergency rooms without informing the prescriber or after warnings to desist; (10) showing evidence of deterioration in the ability to function (at work, in the family, or socially) that appears related to drug use; and (11) repeatedly resisting changes in opioid therapy despite evidence of adverse effects from the drug.

Another screening tool for the assessment of addiction in patients with chronic pain – the Prescription Drug Use Questionnaire (PDUQ) – evaluates patterns of opioid use, social and family factors, and substance-abuse and psychiatric history. Screening indicators identified as strong predictors of the presence of addictive disease in patients

with chronic pain were: a tendency to hoard unused medications, using analgesics to relieve symptoms other than pain, supplementing analgesics with alcohol or other psychoactive drugs, and a history of being terminated from care by a healthcare professional because of concerns about analgesic use. Further, responses to three questions were identified as key predictors of addictive disease: (1) patient believes he/she is addicted; (2) increases analgesic dose/frequency; and (3) route of administration preference. These preliminary findings, while promising, have not been validated in larger samples of chronic pain patients. At present, there are no clear clinical guidelines for identifying chronic opioid users in pain treatment who are abusing their prescription opioids. The most objective methodology developed to date for identifying such patients has been the Pain Assessment and Documentation Tool (PADT) which assesses four domains of patient response during long-term opioid therapy: pain relief, patient functioning, adverse events, and drug-related behaviors.

The term ‘pseudoaddiction’ refers to reversible opioid-seeking behavior occurring in a patient with under-treated pain whose behavior normalizes once the pain is under control. Because the behavior is not maladaptive, it does not meet the criteria for drug addiction. Drug-seeking behavior may be seen with either active addiction or pseudoaddiction, or as part of deviant behavior such as drug diversion. A way to distinguish between these conditions is by giving the patient appropriate pain medication and subsequently observing the pattern of behavior to determine the cause of the drug-seeking behaviors.

There are difficulties associated with applying the definitions and criteria developed for addiction in illicit drug users to pain patients. Depending on the measure of abuse cited in various studies (i.e., opioid abuse vs. any substance use), reported rates of drug abuse among chronic pain patients maintained on opioid therapy may be relatively low or quite high. Further, opioid-maintained pain patients often develop physical dependence and analgesic tolerance without apparent behavioral change. In pain patients, the clinical picture of progression from simple dependence through problematic use to addiction is more subtle and insidious than that observed in illicit users. Addiction is thus far more difficult to identify in this population.

Treatment Strategies for Prescribing Narcotics to Pain Patients

The use of prescription opioids for the treatment of pain is associated with the need to monitor patients carefully for signs of the misuse or abuse of medications. A common strategy employed in the clinical setting is the use of a narcotic protocol, consisting of a narcotic contract,

consent, psychological evaluation, and random urine-toxicology testing to ensure that controlled substances are being used safely and appropriately. Opioid abuse or other substance abuse appears to be relatively common in patients maintained on chronic opioid therapy. A retrospective 3-year study of such patients found that 43% had either positive urine toxicology or one or more aberrant drug-taking behaviors. Of patients with no behavioral issues, 21% had a positive urine screen for either an illicit drug or a nonprescribed controlled medication. In addition, in patients with no evidence of drug abuse on urine toxicology, 14% displayed one or more problematic behaviors. Thus, monitoring both urine toxicology and aberrant behaviors in chronic pain patients treated with opioids will detect more patients with inappropriate drug taking than either strategy alone.

Since there is no definitive test to predict which patients will do well with a therapeutic trial of opioids for chronic pain, it is advisable to take a universal precautions approach to all patients. This uniform approach reduces stigma, improves patient care, and reduces risks. Universal precautions include asking patients about personal and family history of substance abuse (often using screening tools), obtaining informed consent for treatment (usually with a formal, written treatment agreement), ongoing reassessment of benefits from a trial of opioid therapy, and complete documentation of the evaluation and reassessments.

By using universal precautions, clinicians can triage individuals to different categories (low-, medium-, and high-risk) in terms of addiction liability. Low-risk patients with chronic noncancer pain have no history of substance abuse and lack any major psychiatric comorbidity. There are no indications of aberrant behaviors in such individuals, and they can be managed in a primary care setting. Medium-risk patients may have a prior history of substance abuse or dependence, family history of substance abuse, or may have psychiatric comorbidity. These individuals can be managed in a primary care setting, particularly with consultation from a specialist (e.g., an addiction specialist or psychiatrist). High-risk patients are those with active addictive disorders. These individuals are at an increased risk for aberrant behaviors and should be referred to a tertiary clinic that specializes in pain management. In this setting, it is recommended that clinicians, together with the patient, prepare treatment agreements that delineate rules such as having no early refills and requirements for urine toxicology.

Sometimes referred to as a contract, a medication agreement is intended to be a communication tool rather than a legally binding contract. Medication agreements usually specify parameters for adherence, means by which compliance will be monitored, and general goals of treatment. In a recent study, monitoring adherence was associated with a 50% reduction in opioid abuse among patients in

chronic pain management. Adherence monitoring is carried out by obtaining an appropriate history and using an initial medication agreement to communicate the parameters for periodic evaluation of appropriate medication use, random drug testing, and pill counts.

The appropriateness of ongoing opioid use should be periodically evaluated, especially if there is little or no objective evidence of improvement in terms of pain relief or function. In any trial of opioid pain-management therapy, there must be a clear exit strategy which may include opioid tapering and termination, in the event of either a lack of therapeutic response or nonadherence to the treatment contract.

Treatment of Chronic Pain in Substance Abusers

Both methadone and buprenorphine are medications available for the treatment of opioid dependence, and can also be used for the treatment of chronic pain. They are recommended for patients with a history of substance abuse, especially opioid abuse or dependence. Methadone is a full opioid-agonist with a long half-life (17–128 h). While once-daily dosing is sufficient to prevent opioid withdrawal, analgesic effects last only for 6–10 h. Caution should be used with titrating the dose of methadone. It should be prescribed at a low dose initially, and then increased slowly to avoid toxicity.

Buprenorphine is a semisynthetic thebaine derivative. It is classified as a partial μ -opioid agonist and κ -opioid antagonist. Its analgesic potency is 25–40 times that of morphine and it slowly dissociates from opioid receptors, which is in part responsible for its long duration of action. Buprenorphine is well absorbed sublingually, with 60–70% of the bioavailability of intravenous doses. At higher doses, its agonist effect plateaus and it begins to behave more like an antagonist, thus limiting the maximal analgesic effect and respiratory depression. Analgesic doses of buprenorphine are usually given in divided daily doses because the analgesic duration is 6–9 h.

Buprenorphine, listed on Schedule III, was approved by the FDA for the treatment of opioid dependence in 2002. It is the first agent available in the USA for office-based treatment of opioid dependence under the Drug Abuse Treatment Act of 2000. The main objective of this law was to expand access to treatment for opioid dependence by incorporating its management into primary care medicine. The ‘ceiling effect’ of buprenorphine as a partial agonist confers a high safety profile clinically and results in a low level of physical dependence and only mild-to-moderate withdrawal symptoms upon cessation after prolonged administration. The lower total daily doses of sublingual buprenorphine used for opioid dependence overlap with the upper end of the dosing range

evaluated for chronic pain (2–16 mg per day vs. 0.4–3.2 mg sublingually per day, respectively). Several studies have shown that buprenorphine treatment for opioid dependence reduces illicit opioid use, retains patients in treatment, has few side effects, and is acceptable to most patients. The combination tablet Suboxone (buprenorphine/naloxone) is the formulation recommended for use in opioid abusers. The inclusion of naloxone in this preparation discourages street diversion for parenteral administration. When taken as directed, the pharmacological effects of the mono- and combination formulations do not differ, so the use of the combination product in opioid abusers with chronic pain is appropriate.

Conclusions

Prescription opioids remain safe and effective pharmacotherapies for chronic nonmalignant pain. Over the last decade, however, a marked increase in abuse of prescription opioids has occurred in the USA. This continuation in nonmedical use of opioid analgesics has resulted in sharp recent rises in morbidity and mortality at the local and national levels. To date, predictive factors for opioid abuse or addiction among opioid-maintained chronic pain patients have not been clearly identified. Moreover, the risk of iatrogenic addiction in patients treated with long-term opioid analgesics has not yet been adequately characterized.

The vast majority of pain patients taking opioids are physically dependent but not addicted; that is, they have developed an expected physiological response to the regular use of opioids and will experience a characteristic withdrawal syndrome, including myalgias and gastrointestinal distress, upon abrupt cessation of the drug. Some opioid-maintained pain patients will also develop addiction, which is a treatable neurobiological disease influenced by genetic, psychosocial, and environmental factors. Addiction is characterized by one or more of the following behaviors: impaired control over drug use, compulsive use, continued use despite harm, and craving.

Aberrant medication-taking behaviors (e.g., unauthorized dose escalation, ‘doctor shopping’ – i.e., visiting multiple physicians concurrently to obtain controlled substance prescriptions) may signal the presence of abuse or addiction. On the other hand, such behaviors sometimes reflect inadequate analgesia (pseudoaddiction) or the misuse of opioid analgesics to treat symptoms of depression, anxiety, or personality disorders. For patients who do develop opioid addiction, opioid substitution with buprenorphine and medical management of addiction through the use of medication agreements and adherence monitoring can be safely and effectively carried out in an office setting. There is a clear imperative to develop analgesics with lower abuse liability, and current research

efforts are also targeting the development of better methods to detect patients at risk for developing addiction.

See also: Brain Imaging and Addiction; Cognitive Control in the Service of Self-Regulation; Cytokine Effects on Neuronal Processes and on Behavior; Drug Addiction; Measuring Stress; Molecular Neurobiology of Addiction; Neurotransmitters and Neuromodulators Regulating Sleep and Wakefulness; Physical and Emotional Pain; Regulation of the HPA Axis by Acute and Chronic Stress; Stress and Drug Craving; Stress and Reward; Transition to Addiction.

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Psychiatric and Substance Use Disorder Comorbidity

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Glossary

Comorbidity – The occurrence of two diagnostic syndromes in one individual. While clinicians typically use this term to refer to disorders that occur simultaneously, epidemiologists often calculate lifetime comorbidity rates for which both disorders may be separated by substantial periods of time. The term ‘co-occurrence’ is more appropriate than ‘comorbidity’ when referring to the simultaneous occurrence of the specific symptoms associated with distinct syndromes.

Genetic epidemiology – A research domain dedicated to the study of genetic factors in determining health and disease in families and in populations, as well as to the investigation of gene–environment interactions. The common paradigms of genetic epidemiology include family studies, twin studies, adoption studies, and linkage or association studies.

Nosology – The branch of medicine that deals with classification of diseases. In psychiatry, the most widely applied systems of nosology include those described in the *Diagnostic and Statistical Manual of Mental Disorders* (DSM; American Psychiatric Association) and the International Classification of Diseases (ICD; World Health Organization). Both systems are based on a categorical approach to diagnosis. The application of diagnostic hierarchies is frequent in psychiatric nosology and may influence the estimations of the magnitude of comorbidity as well as the investigation of the underlying mechanisms.

Prevalence – An estimate of how common a disorder or disease is within a population over a defined period of time. It is calculated based on the total number of cases of the condition divided by the number of individuals in the population. In psychiatric epidemiology, prevalence is most often calculated for disorders occurring over the entire lifetime, over the past 12 months, or at the moment of the diagnostic interview.

that the term comorbidity should only be used to describe the co-occurrence of diseases or disorders, it has also been increasingly applied to describe the co-occurrence of symptoms associated with distinct syndromes. Application of such loose definitions is common and has been an ongoing source of confusion. In this article, the term comorbidity is reserved to describe the occurrence of two diagnostic syndromes in one individual, while the term co-occurrence refers to the simultaneous appearance of specific symptoms associated with distinct syndromes.

A large number of clinical and epidemiologic investigations have demonstrated consistent patterns of association between many mental disorders at the diagnostic level. The most commonly examined forms of comorbidity include associations among anxiety, mood, behavioral, and substance use disorders. Strong correlations between symptom measures of these syndromes also verify this relationship at subclinical levels. However, the assignment of dual diagnoses has often been counter to the prevailing clinical practice, and similarly, the intent of many experimental studies has been to attain symptom measures with the greatest discriminative power. This lack of acknowledgment that mental illnesses naturally co-occur with high frequency has hindered research on this topic, and much remains to be known about the underlying mechanisms of comorbidity for a large number of mental disorders. Nonetheless, it is increasingly clear that psychiatric comorbidity is associated with a worsened course and poorer outcome among affected individuals and that the implications of psychiatric comorbidity for treatment and prevention strategies place it among major public health priorities.

The Comorbidity of Mental Disorders

Logically, two disorders having high prevalence rates are more likely to be found in the same individual than two disorders with low prevalence rates. For this reason, any study of comorbidity must first start with an understanding of the base rates of these disorders in the general population. The random probability of the two disorders occurring together can then be identified, thereby allowing an assessment for the probability of a second disorder occurring if the first is already present.

Several large-scale epidemiologic studies have underscored the frequent overall occurrence of mental

Introduction

The term ‘comorbidity’ was first used to describe any distinct additional clinical entity that has existed during the course of an index disease in any one patient. Although psychopathology researchers have cautioned

disorders in the general population, but with surprising variance in lifetime prevalence rates. These discrepancies are partly attributable to changes in diagnostic criteria, societal or cultural characteristics of the populations studied, and other differences across surveys. However, among the most substantial reasons for the wide range of prevalence rates concerns the application of methodological techniques in the more recent investigations that reduce response biases that may have underestimated lifetime prevalence rates among participants. **Table 1** presents an illustration of this effect across four large-scale psychiatric surveys conducted in the 1980s and 1990s. While some differences are observed among the first three investigations concerning the prevalence of common conditions such as anxiety, mood, or substance use disorders, the overall prevalence rates are generally comparable. By contrast, the National Comorbidity Survey (NCS) reported markedly higher rates not only for most categories of disorder but also for the overall lifetime prevalence of any mental disorder. The prevalence rates in this landmark investigation are higher because they reflect strategies to reduce errors in disorder estimates or data collection, such as adjustments for non-response bias (i.e., depressed individuals are more likely to decline participation in such surveys) and for the tendency of long diagnostic interviews to encourage respondents to progressively deny having particular symptoms in order to finish the interview more quickly. Importantly, this same investigation reported that among all individuals who experience at least one mental disorder over their lifetime, the majority would meet diagnostic criteria for at least one more mental disorders (and nearly one-third would meet the criteria for three or more disorders). Comorbidity rates for mental disorders over a 12-month period were also particularly high, indicating that most affected individuals experience two or more disorders simultaneously or in very close temporal proximity. Investigations such as the NCS thereby confirmed in community samples what clinicians have long observed among patients seeking treatment: psychiatric comorbidity is the norm rather than the exception, and

that a specific and singular diagnosis of individual conditions is often an oversimplification of the true clinical state of these patients.

Two unassociated disorders that each have a prevalence rate of 10% should be expected to be comorbid in approximately 1% of individuals. However, if the true rate of comorbidity is 5%, for example, then their association far exceeds chance expectations. The magnitude of comorbidity between two disorders is generally represented by an odds ratio (OR), a statistic that indicates the degree to which two disorders occur together relative to what would be expected by their base prevalence rates. In general, ORs tend to be greater within than between broad classes of disorders, such as for internalizing (e.g., anxiety or mood) disorders or externalizing (e.g., conduct or oppositional defiant) disorders. The magnitude of comorbidity may frequently appear to be inversely related to the base prevalence of disorders, where more rare or severe disorders seem to be more strongly associated with other forms of psychopathology. For example, commonly reported ORs for the comorbidity of alcohol dependence and prevalent mood disorders such as major depression generally range from 1.5 to 4.0, but are much higher when considering severe but less prevalent mood syndromes such as bipolar I disorder. It is important to note, however, that even a modest OR of 1.5 among highly prevalent disorders translates into substantial portions of the general population being affected by both conditions.

In addition to the basic information concerning the magnitude of comorbidity among mental disorders, their patterns of association provide clues as to the nature and mechanisms of comorbidity. Aside from methodological issues or biases, the most commonly cited explanations for comorbidity involve causal and shared etiologic models (see **Figure 1**). Causal models assume that the presence of an initial disorder increases the risk of a secondary disorder. Basic examples of such mechanisms can be found among individuals who develop alcohol or drug abuse as a means of alleviating anxiety or, conversely, among individuals who become depressed due to the physical, psychological, or social consequences induced by alcohol or drug use. Therefore, causal associations may, at times,

Table 1 Prevalence of mental disorders

	ECA 1984	Edmonton 1988	CPES 1989	NCS 1994
Anxiety	15.5	11.2	10.5	24.9
Mood	7.9	10.2	14.7	19.3
Substance	16.7	20.6	21.0	26.6
At least one disorder (%)	33	34	37	48

CPES, christchurch psychiatric epidemiology study; ECA, epidemiologic catchment area study; NCS, national comorbidity survey.

Causal etiology

Disorder A → Disorder B

Disorder B → Disorder A

Shared etiology

Factor C → Disorder A
Factor C → Disorder B

Figure 1 Explanations for the comorbidity of two disorders.

be bidirectional, although most theoretical and treatment models emphasize unidirectional associations due to their frequent focus on the prevention or remission of a specific disorder.

Shared etiologic models point to a specific factor or mechanism that increases the risk of more than one condition, such as the possibility that a common genetic vulnerability might increase the risk of both depression and panic disorder, or that traumatic life events may increase the risk of both depression and alcohol dependence. The two disorders are not causally linked to each other, but both occur together at a rate greater than chance because both are linked to a common underlying risk factor. These pathways of association may be direct or indirect, and multiple mechanisms of association may be simultaneously active for a single form of comorbidity (both at the population level as well as within any given individual).

This heterogeneity may be illustrated by the case of an individual with panic disorder and alcohol dependence. The self-medication for persistent anxiety using this substance over several years may have led to increased tolerance for alcohol as well as increased time and energy spent to obtain it. The cessation of alcohol use once dependence has occurred, in turn, increases the risk that this individual would experience severe agitation, nervousness, or panic attacks. These symptoms may not be limited only to a period of immediate alcohol withdrawal, but may persist over time due to fear of the consequences of these anxious states and conditioned anxiety upon the experience of such symptoms (leading to panic disorder). Based on their family history, which may reflect social modeling and genetic factors, this same individual may have already been at greater risk for alcohol dependence and panic disorder as independent syndromes. The capacity of these disorders to interact through causal mechanisms simply adds to this shared underlying vulnerability in increasing the overall risk of comorbidity. Causal models of association are often raised to explain comorbidity when at least one of the associated disorders has a strong behavioral component (such as for substance dependence or agoraphobia), while shared etiology is often raised in the context of comorbidity among internalizing disorders. However, the models presented in **Figure 1** are potentially applicable to explain comorbidity both within and between all psychiatric and substance use disorder categories.

In light of their complexity, specific mechanisms of comorbidity can only be fully tested using a diversity of research paradigms. The case of anxiety and depression will serve to illustrate this point, as it is among the most commonly examined forms of psychiatric comorbidity and has been investigated within a number of scientific disciplines. Cross-sectional investigations over the past two decades have repeatedly found that the lifetime

comorbidity of major depression and any anxiety disorder is elevated, but without equal associations between these disorders. That is, anxiety disorders are more often found among individuals with depression than is depression found among individuals with anxiety disorders. The cross-sectional examination of anxiety and depression reveals the additional pattern that most episodes diagnosed as depression also involved significant anxiety symptoms, but that the reverse is less frequent. It is relatively rare to find a person with an isolated (or pure) depressive disorder, but relatively common to find a person with a pure anxiety disorder. Longitudinal data from community cohorts have also demonstrated that patients with anxiety disorders tended to develop depression in conjunction with anxiety at follow-up more often than did subjects with depression. By contrast, diagnoses of depression were found to be relatively stable over time and did not evolve as often into anxiety disorders. Among those subjects who developed both disorders longitudinally, the majority of subjects with a first diagnosis of anxiety later developed depression, compared with a minority of those with depression who later developed anxiety.

Taken together, these findings may be consistent with the possibility that anxiety is a causal risk factor for later depression, or that pure anxiety and mixed anxiety-depression represent distinct syndromes. This latter possibility has been subsequently examined through several paradigms of genetic epidemiology. Family studies of depression and specific anxiety disorders such as panic, for example, are in agreement in indicating that both conditions are familial, as demonstrated by the increased risk of each disorder in families of probands with that disorder. However, while some of these studies indicate that depression and anxiety disorders do not breed true, others provide evidence of specificity of transmission for panic and major depression. Family studies are limited, however, in that they only describe the relationship between anxiety and depression within a given family lineage, and therefore cannot specifically examine the question of genetic transmission separately from shared environmental factors. The analysis of the questions among twins has demonstrated that mixed anxiety-depression is relatively frequent among the monozygotic twin of probands with pure anxiety, pure depression, or mixed states. Such findings seemingly support the hypothesis that mixed anxiety and depressive syndromes (comorbid states) are partly variants of both anxiety and depressive disorders. However, when such data are reanalyzed without using diagnostic exclusion hierarchies for these disorders, the concordance rate of pure depression and mixed anxiety-depression is three times higher in monozygotic twins than in dizygotic twins. Similarly, for monozygotic twins, major depression without anxiety in probands predicts major depression with anxiety in the

co-twins, and the reverse. In contrast, the occurrence of pure anxiety disorders in monozygotic probands predicts only the same disorder in co-twins. Taken together, such results argue for a similar genetic basis of pure major depression and mixed anxiety–depression, and also indicate a different genetic etiology for pure anxiety disorders without major depression. These conclusions are also in accordance with the observations from family studies that when depression is present with an anxiety disorder in probands, families tend to have aggregations similar to families of probands with pure major depression rather than pure anxiety. The identification of transmission patterns, in turn, may provide guidance for additional research paradigms, such as by improving the validity of phenotypic description for linkage and association studies. This illustration for the comorbidity of depression and anxiety disorders demonstrates the manner in which findings from diverse research disciplines are necessary for understanding the underlying mechanisms of association, each providing a complementary source of information.

Barriers to Investigating Comorbidity

Limitations of the Categorical Approach

Studies of comorbidity rely on the benefits and limitations of the nosologies used in categorizing and separating these disorders, and comorbidity rates are inevitably a function of the structure of diagnostic categories and hierarchies. For example, much of the confusion in family studies (such as for the example of anxiety and depression) is due to the fact that most family studies have applied nosological hierarchies in diagnosing comorbid episodes, and, therefore, certain diagnoses have often been excluded. When this is done, studies are unable to test the degree of specificity or cross-transmission of these disorders, and, therefore, it becomes impossible to draw conclusions regarding the etiologic origins. An important outcome of such criticisms has been a critical examination of the current nosological systems used in psychiatry, which many researchers claim has often forced diagnostic labels and boundaries between disorders when no distinction may be warranted.

The majority of critics of the categorical system propose that a dimensional approach be used to prevent inaccurate descriptions of clinical states. The dimensional approach to classification (based on a continuum of features and symptoms) does not systematically ignore certain symptom presentations and does not force a categorical solution. In the most general sense, the description of symptom co-occurrence is the starting point for understanding the more complex associations at the diagnostic level. Diagnostic syndromes are in themselves collections of more basic symptoms, and patients with coexisting

disorders, by definition, show a greater overlap at the symptom level than at the diagnostic level. It has therefore been argued that researchers should first describe the mechanisms of this association at more fundamental levels before increasingly complex (diagnostic) associations can be fully understood.

Barriers of Time and Context

A second major impediment confronting comorbidity researchers concerns the important differences between the natural phenomena under study and the methods used in their investigation. In particular, this discrepancy is most visible with regard to the assessment of temporal relationships among symptoms. In contrast to diagnostic criteria that are based, in part, on the importance of duration over time as a defining feature of mental disorder, the actual pathological mechanisms at the origin of a given syndrome—and of comorbidity—are often highly dynamic. That is, the expression of many forms of psychopathology is characterized by a relatively short life cycle concerning the period of time in which a given vulnerability or risk factor may influence the severity of symptoms or the expression of abnormal behavior. Such phenomena are observable over periods that are typically limited to a matter of minutes or hours, while most standard methodologies apply retrospective, cross-sectional, or longitudinal assessments spanning weeks, months, or years.

An illustration of the importance of the timing of symptom expression can be easily seen in the comorbidity of alcohol dependence with both anxiety and depression. A large body of clinical and epidemiologic research has demonstrated the strong association between these syndromes, with approximately equal ORs for the association of alcoholism with each disorder. Despite our ability to conclude with confidence that there is a general association among these syndromes, however, the actual mechanisms of comorbidity remain a source of debate. One of the most commonly cited potential explanations is that of self-medication, whereby the individual would use or abuse alcohol to assuage pre-existing anxiety or depressive states. The self-medication model would indeed have important treatment implications if it were able to accurately characterize the majority of cases of these forms of comorbidity, as it would clearly indicate that comorbid disorders should be addressed before the treatment of alcohol dependence could be effective. The association between these conditions is illustrated by the results of an example investigation in **Figure 2**, where the severity of anxiety or depressive syndromes is correlated with the quantity of alcohol use. However, the ability for clinicians to exploit the knowledge of these associations depends heavily on the nature of data collected in the

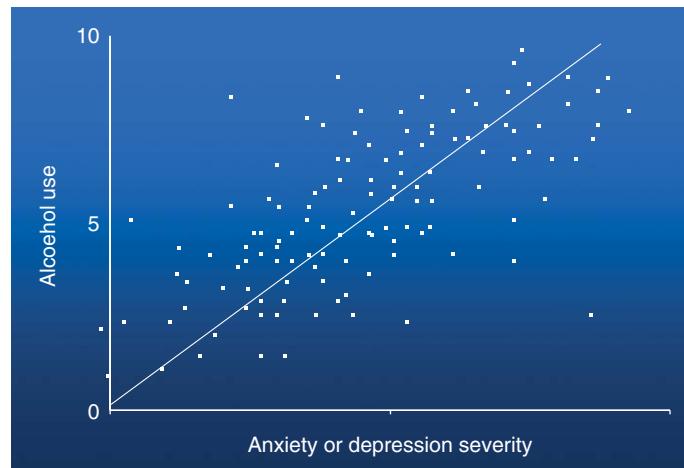


Figure 2 Example of common research demonstrating a correlation between alcohol use and anxiety or depression.

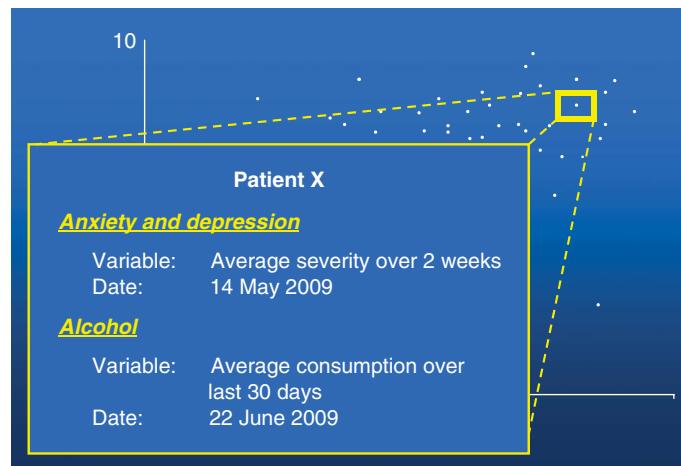


Figure 3 Illustration of why correlations cannot examine causes of comorbidity.

samples studied. Using an example patient, **Figure 3** illustrates to what extent this same study is based on assessments of anxiety or depression averages that show little or no within-subject variation. As a result, at no point did this example investigation demonstrate that Patient X, or anyone else in the sample, was more likely to consume alcohol when anxious or depressed. The phenomenon of interest was therefore never studied directly, rendering it difficult to exploit such investigations on a clinical level. Logically, a person consuming alcohol to alleviate anxiety or depression would be motivated to do so only if they were anxious or depressed at that particular moment, and not because they are an anxious person on average nor because they were anxious the week or month before. Self-medication is therefore an example of phenomena that can only be directly studied over considerably shorter time frames.

A third major impediment to understanding comorbidity expression concerns the ecological validity of the

existing literature. Although it remains possible for laboratory- or clinic-based investigations to overcome some of the temporal constraints described previously, they remain limited to the study of artificially induced states or to assessments conducted within the same context (clinic or hospital). It is therefore difficult or impossible to determine how these variables will express themselves in naturalistic contexts. To again take the example of self-medication, alcohol consumption has been shown to immediately reduce the negative emotional states induced through certain laboratory-based experimental procedures. However, this paradigm cannot determine if subjects would actually choose to use alcohol as a means of assuaging negative affect outside of the laboratory. It is also impossible to determine the contexts in which the phenomenon of self-medication is most likely to express itself (e.g., when the individual is alone vs. accompanied, or when under specific forms of stress). A clearer description of the contexts in which symptoms

express themselves *in vivo* would permit a better understanding of the comorbidity mechanisms that may vary by environmental context.

New Methods for Comorbidity Research

As previously underscored, a dimensional approach to the study of comorbidity would palliate many of the limitations imposed by categorical diagnostic systems. However, overcoming barriers associated with the brief temporal period of symptom expression or with environmental context specificity requires entirely new research strategies. Ambulatory data collection methods, such as the experience sampling method (ESM) or ecological momentary assessment (EMA), hold particular promise in this regard as they permit the examination of a representative sample of mental states in daily life through the use of electronic devices (e.g., personal digital assistants (PDAs) and cellular telephones). Such devices are used to indicate to subjects the moments to provide specific information concerning their behaviors, environment, specific symptoms, and other data at different moments throughout the day. In this way, ESM and EMA provide brief, prospective, and comprehensive assessments of daily life experiences of the individual. Subject compliance in such studies is particularly high (even for severe disorders such as schizophrenia or drug dependence), and the minor fatigue effects observed are controlled for by assuring that the start day is counterbalanced across subjects, therefore allowing for the number of days of participation to be used as a covariate in analyses. These novel methods are increasingly used in psychiatry, and their capacity to overcome barriers of time and context render them particularly interesting for the study of comorbidity. For example, their application to testing the self-medication hypothesis in recent years has offered new insights into the validity of this hypothesis for different clinical populations. Concerning alcohol use disorders, ambulatory monitoring has shown important differences in the association of drinking with anxiety and depressive symptoms. Strong support was found for the self-medication of anxious states in that anxiety led to increases in alcohol use over subsequent hours, and the eventual use of alcohol was indeed associated with reductions in anxiety. In contrast, no self-medication effect was found relative to depressed moods. In addition, alcohol consumption following anxiety was primarily a male phenomenon, and those with a family history of alcoholism required greater quantities of alcohol to achieve the same anxiolytic effects of alcohol than did participants without a family history of alcoholism. These findings provide potentially important insight into commonly cited sex differences in alcohol use, as well as concerning the strongly heritable nature of alcohol use disorders by explaining why certain individuals might be more

susceptible to use alcohol in greater quantities as a means of alleviating anxiety symptoms. Self-medication was also directly tested using ambulatory monitoring techniques relative to cannabis use in psychosis-prone individuals. Contrary to expectations, increases in psychotic-like symptoms did not lead to increases in cannabis use. Rather, cannabis use in vulnerable individuals exacerbated these symptoms, a finding suggestive of a causal relationship in the reverse direction than self-medication. Such findings therefore have important implications for understanding the mechanisms underlying associations observed at the diagnostic level and that are necessary for ameliorating treatment and prevention programs.

General Conclusions

Faced with the high rates of comorbidity between mental disorders, diverse research paradigms are necessary to investigate and ultimately control underlying mechanisms of association. At first glance, the capacity of such research to lead to more efforts in effective treatment and prevention may appear most obvious in the case of causal associations between conditions. However, evidence for common etiologic factors not only provides information concerning specific risk factors or vulnerabilities that can potentially be controlled, but also offers insight into the most valid boundaries or definitions of specific disorders that may alter treatment approaches. The contributions of epidemiology, clinical psychiatry and psychology, and genetic epidemiology to the study of comorbidity will continue to progress as a function of new questions and findings within each discipline. Future research should also benefit from a dimensional approach to the study of symptom co-occurrence as well as from the application of new research strategies designed to overcome longstanding methodological barriers in this domain.

See also: Alcoholism; Antisocial Substance Dependence; Comorbidity – Depression; Emotion–Cognition Interactions.

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Psychostimulants

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Glossary

Dopamine – One of the monoamine neurotransmitters important in processing reinforcing stimuli including natural rewards and drugs of abuse.

Nucleus accumbens – The terminal region where dopamine is released in the ventral striatum from the brain pathway arising from the ventral tegmental area in the midbrain.

Positive reinforcer – Something that engenders repetition of a behavior that precedes it, such as presentation of an appetitive food or administration of an addictive drug.

primarily an agonist at nicotinic acetylcholine receptors. This is hardly a controversy, because caffeine and nicotine can be termed weak, indirect dopamine agonists inasmuch as they increase dopaminergic activity, though not directly via actions at dopamine transporters (reuptake sites) on dopaminergic nerve terminals. Although it remains to be entirely clarified, the stimulant effects of caffeine may be due to the blockade of adenosine at adenosine A_{2A} receptors that normally function in an opposing fashion to dopamine D₂ receptors. Indirect activation of dopaminergic systems via increased activity of dopamine neurons and/or inhibition of the dopamine metabolic enzyme monoamine oxidase probably contributes to the stimulant effects of nicotine.

In contrast to all of those agents, there are many drugs that have some overlapping pharmacology but whose primary mechanisms of action differ sufficiently from the indirect dopamine agonists that their classifications – both pharmacodynamic and behavioral/clinical – fall outside the realm of psychostimulants. Examples include some antidepressants such as bupropion (Wellbutrin) that has a primary noradrenergic component; monoamine oxidase inhibitors used as antidepressants and/or anti-parkinsonian agents including selegiline (l-deprenyl, Eldepryl); substituted phenethylamines with prominent serotonergic activity at 5-HT_{2A} receptors that are sometimes referred to as entactogens – including methylenedioxymethamphetamine (MDMA or ecstasy) and a group of similar drugs beginning with 2C based on chemical structure; and muscarinic cholinergic antagonists, including scopolamine that, in small doses, has been used in the treatment of motion sickness, and benztrapine that has been used for its anti-parkinsonian effects.

Chemical Classes and Pharmacology of Psychostimulants

Many psychostimulant drugs, including, for example, cocaine and ephedrine, exist in nature as alkaloids and are derived from plants. Synthetic drugs that can be chemically classified as substituted phenethylamines include amphetamine, methamphetamine, and methylphenidate. Despite natural versus synthetic sources and differences based on chemical structures, all of these drugs function primarily as indirect dopamine agonists in central nervous system (CNS) synapses, which leads to their stimulant effects in the human and laboratory animals (**Table 1**). Some pharmacologists may define the term ‘psychomotor stimulants’ by a primary biological activity as an indirect dopamine agonist (i.e., causing dopamine-reuptake inhibition or release at dopamine nerve terminals). A historical term for psychostimulants is sympathomimetics, meaning drugs that mimic endogenous chemicals of the sympathetic nervous system, including all three ‘catecholamine’ neurotransmitters dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline). For example, ephedrine most prominently activates peripheral adrenergic systems, at least in part due to poor CNS penetration. Many psychostimulants have additional pharmacological activities, such as the local anesthetic effects of cocaine, and many have indirect agonist activity in serotonin synapses.

Some pharmacologists would include among the psychostimulants the xanthines such as caffeine that are primarily adenosine antagonists, and nicotine, which is

Historical Perspective on Psychostimulants

The coca plant was named by Lamarck in 1786 after the plant family was coined as *Erythroxylaceae* by Linnaeus. The plant thrives at high elevations such as the Andes and can be found both in the wild and cultivated forms from northwestern Argentina to Ecuador. Ancient Colombian legends describe the people’s emergence from the Milky Way Galaxy in a canoe that included a man, a woman, and various plants including coca. Leaves of the plant have been found in burial mounds in Peru estimated to

Table 1 Summary of psychostimulants

<i>Pharmacodynamic classification</i>	<i>Generic name</i>	<i>Therapeutics</i>	<i>Side effects</i>	<i>Animal measures</i>
<i>Indirect dopamine agonists</i>	Amphetamine	Attention-deficit disorders (ADD/ADHD), narcolepsy,	Abuse liability	Motor hyperactivity
	Cocaine*	topical (local) anesthetic*, cold medicines, diet pills, shift-work sleep disorder, wakefulness/alertness	Insomnia	Fully generalizes to stimulant cue in drug discrimination
	Ephedrine		Increased heart rate	Strong positive reinforcer in self-administration
	Methamphetamine			Decreases prepulse inhibition of startle
	Methylphenidate			
	Modafinil			
<i>Adenosine antagonists</i>	Caffeine		Dependence	Motor hyperactivity
	Theobromine		Insomnia	Generalizes to stimulant cue in drug discrimination
	Theophylline			Positive reinforcer in self-administration under some conditions, particularly dependence
<i>Nicotinic acetylcholine agonist</i>	Nicotine	Nicotine replacement therapy for smoking cessation	Dependence	Weak motor hyperactivity Generalizes to stimulant cue in drug discrimination under some conditions Positive reinforcer in self-administration under some conditions

* Characteristics apply to all drugs in that pharmacodynamic category, with the exception of cocaine that is the only local anesthetic listed and that is its only accepted therapeutic application.

be nearly 5000 years old. When the Inca conquerors arrived in the tenth century, the leaf was consumed primarily by priests and nobility and was considered a gift of the sun god. The plant was accorded such virtues as satisfying the hungry, providing the weak with new vigor, and causing the unhappy to forget their misery.

Shortly after cocaine's purification by Albert Niemann in the mid-nineteenth century, Sigmund Freud investigated the drug's powerful psychoactive effects. Freud came to many of the same conclusions as the Incas, in self-reports that the drug warded off hunger, sleep, and fatigue, enhanced intellectual efforts, and induced cocaine euphoria. Having known about the chewing of coca leaves by Peruvian Indians in order to prolong physical exertion, Freud was interested in the potential of cocaine hydrochloride as a treatment for nervous exhaustion. An additional clinical error was his prescription of the drug for morphine dependence. However, Freud accurately recognized certain properties of cocaine that "suggests its occasional use as a local anesthetic." Freud's associate, Karl Koller, is credited with introducing cocaine's only current medical use, as a local anesthetic for ophthalmic surgery and other specialized applications. However, more commonly used local anesthetics are analogs, such as procaine and lidocaine, that have limited abuse liability relative to cocaine.

The abuse of coca throughout Europe and then the United States of America (USA) came about via an oral preparation patented by a Corsican chemist named Angelo Mariani. Vin Mariani – a wine-containing coca extract – is rumored to have been Europe's most popular beverage in the mid-nineteenth century. For developing coca-containing wine, lozenges, and tea, Mariani was presented with a special medal of appreciation by the Pope. Shortly thereafter, an American chemist from Georgia, John Pemberton, registered a trademark in the USA for Coca-Cola, a refreshment that also included wine and coca extracts. Pemberton later substituted the wine component of the drink with a kola nut extract that contained caffeine, and also incorporated soda water into the beverage. Another pharmacist, Asa Candler, founded the Coca-Cola Company in 1892. As a result of the Pure Food and Drug Act of 1906, coca was removed from the recipe for Coca-Cola. Shortly thereafter, cocaine was included with morphine and opium in the Harrison Narcotic act of 1914, which banned the use of these drugs in the USA and drove cocaine use to the underground.

Although historically the predominant recreational use of cocaine was via oral administration (e.g., chewing coca leaves, drinking beverages containing coca extracts), common routes in modern society include insufflation (snorting) and intravenous administration (injecting) of cocaine hydrochloride, and inhalation of cocaine base (smoking crack). Compared with oral

administration, effective concentrations of cocaine in the blood and brain can be achieved more rapidly by these routes, particularly for intravenous injection and vapor inhalation. After insufflation, peak blood levels of cocaine are achieved in about 10 min, and after injection or inhalation, peak blood levels are achieved within the first several minutes. Cocaine abusers may report experiencing a rush during the rapid onset of the drug's effects, and animal studies confirm that the reinforcing effects of the drug are more easily established when blood and brain levels are achieved rapidly. The more rapid consumption of quantities of drug and the shorter duration of action associated with inhalation and injection probably contribute to the cycle of increasing frequency of administration and resultant addiction.

The devastating public health consequences of cocaine abuse are related not only to the myriad sociopathic aspects of drug addiction, but also to various physiological conditions and comorbid diseases. For example, cocaine self-administration can lead to cardiovascular, cerebrovascular, and respiratory problems, including disturbances in heart rhythm, heart attacks, chest pain, and respiratory failure. Endocrine and liver dysfunctions, gastrointestinal problems, and various neurological disorders including strokes, seizures, and headaches have also been linked to cocaine use. Cocaine abuse and dependence can lead to cycles of anxiety, depression, and even delirium. Perhaps the most recent, insidious, and serious public health consequence of cocaine abuse has been referred to as a twin epidemic comprising of intravenous drug use and infection with human immunodeficiency virus (HIV).

The advent of the amphetamines is much more recent. Ephedrine from *Ephedra vulgaris* has been used as an herbal remedy for congestion in preparations such as *ma huang* tea for at least 5000 years. However, that phenethylamine is more potent in its activation of peripheral adrenergic systems than central dopaminergic synapses. Shortly after ephedrine's isolation and use for the treatment of asthma in the late nineteenth and early twentieth century, the substituted phenethylamine amphetamine was developed as an alternative. By the 1930s, the American Medical Association (AMA) sanctioned the use of amphetamine (Benzedrine) for mild depression and to promote wakefulness as in the treatment of narcolepsy. The latter remains a clinical use of the substituted phenethylamines to this day (e.g., d-amphetamine, methylphenidate). The amphetamines have also been used as appetite suppressants and for general alertness, but the most important clinical utility in modern society is in the management of attention-deficit disorders (e.g., attention-deficit disorder (ADD) and attention-deficit hyperactivity disorder (ADHD)).

Unfortunately, the amphetamines – like cocaine – have significant abuse potential. They also are snorted,

injected intravenously, or smoked (e.g., crystal meth) recreationally. However, they are much more orally active and long-acting, with a half-life, in human blood, of 14 h for d-amphetamine compared with 40 min for cocaine. The longer half-life may contribute to more frequent reports of psychoses with high doses of amphetamines relative to cocaine. Finally, the drug methamphetamine has produced neurotoxicity in laboratory studies, and that may be relevant in the high-dose range and/or chronic recreational use in some of the humans that abuse the drug.

Use of Animal Behavioral Procedures to Study Psychostimulants

There are many unconditioned, overt behaviors that can be used to study the behavioral effects of psychomotor stimulants in laboratory animals. The most common by far is the locomotor activity test. Psychostimulants dose-dependently increase motor activities, and ambulations or distance traveled can be efficiently measured using a photobeam apparatus or other tracking system. There are several important considerations in optimizing such measures for the evaluation of the potency, efficacy, and time course with which a drug produces a change in this behavior, as described below.

First, because psychostimulants can be relatively potent and effective in increasing this behavior, the most sensitive measures occur when ongoing rates of behavior in the absence of the drug are low. For this reason, the drug should be administered after the animals have had a behavioral history of ample habituations to the test chamber, so that spontaneous exploratory activity has been largely extinguished. More generally, drug effects on most rates of behavior are, in part, determined by the ongoing rate of activity. For many behaviors including locomotor activity, psychostimulants often increase low rates of behavior and decrease high rates of ongoing behavior.

Second, the dose or doses of the drug that are studied are a critical determinant of the behavioral effect observed. Specifically, low-to-intermediate doses of psychostimulants dose-dependently increase locomotor activity. But higher doses can produce repetitive, more restricted motor behaviors (such as sniffing, grooming, licking, and biting), that are often collectively termed motor stereotypies, that compete with ambulatory activity, and reduce overall distance traveled. Thus, locomotor hyperactivity decreases as motor stereotypies increase with higher doses of psychostimulants. By studying a full range of doses of a drug, relative potencies for locomotor hyperactivity and stereotypies can be obtained.

Third, the time course of the psychostimulant drug is a critical factor in making assessments. For example,

studying total ambulations over a 12-h period for equivalent doses of cocaine and amphetamine in terms of their peak effects at any given moment would yield much larger values for amphetamine due to its longer duration of action. But measuring the behavior for 1 h during the peak effects of each drug's time course at equivalent doses would yield more comparable effectiveness.

Finally, the subjects, in terms of drug history, behavioral history (see above), species, and strain are all critical determinants. For example, locomotor activity has been widely used to evaluate a phenomenon termed behavioral sensitization. In that case, prior exposure of outbred rats or mice to several daily injections of a psychomotor stimulant sensitizes the animals to locomotor hyperactivity, particularly in a novel test environment in which they received a psychostimulant on prior occasions. The species and strain of the animal can also markedly influence drug effects. For example, most rats that have been studied exhibit locomotor hyperactivity in response to drugs that produce either dopamine D₁ or D₂ receptor stimulation. In contrast, most strains of mice exhibit locomotor hyperactivity only in response to dopamine D₁ receptor stimulation.

In summary, the locomotor activity test in rodents can be a sensitive assay for evaluating the potency, efficacy, and time course of psychostimulants, when behavioral and drug history, dose and time course, and species and strain are all taken into account. This test has been used to screen drugs for psychostimulant activity that may have clinical implications (see above).

Another test of unconditioned behavior used widely in behavioral neuroscience that is useful for evaluating psychostimulants is the 'prepulse inhibition of startle' (PPI). In this test, when a startling stimulus, such as a brief, 120-dB acoustic noise burst, called a pulse is presented to a subject, a motoric startle response can be measured, such as the jump of a rodent positioned atop a pressure-sensitive platform. Presentation of a weaker brief stimulus called a prepulse (e.g., 75 dB, just 10 dB above a constant 65 dB background noise), that by itself does not alter the behavior, approximately 100 ms prior to the pulse reduces the startle response to the pulse. This behavioral inhibition produced by the prepulse is thought to be an operational measure of sensory gating. Psychostimulants that are indirect dopamine agonists (see Table 1) dose-dependently decrease PPI. This test has been very useful for measuring dose- and time-dependent activation of dopaminergic pathways by psychostimulants. Perhaps more importantly, reversal of psychostimulant effects on PPI has been broadly used as a preclinical screen for drugs that may have therapeutic activity as antipsychotics. For example, most drugs that are effective in treating schizophrenia are also effective in reversing psychostimulant-induced decreases in PPI. As with the locomotor test described above, PPI is also dependent upon critical

variables, such as dose and time course of the psychostimulants tested, and species and strain of the subjects. Of course, this test is extremely sensitive to manipulations of the intensity and duration of the startling stimulus and the prepulse, as well as factors such as background noise and interstimulus intervals.

Conditioned behaviors including operant conditioning can also be used to evaluate the behavioral effects of psychostimulants. Drug-discrimination procedures are used to evaluate the interoceptive effects of drugs in laboratory animals, sometimes termed ‘subjective’ effects in the human. In this procedure, animals are trained to press one of two levers in order to obtain a food reward if they detect a training drug in their body, and to press the other of the two levers to obtain food reward if they do not detect the training drug in their body. Typically, training is required for at least 30 or 40 daily sessions in which conditions are randomized on different days for one lever to be reinforced with food after cocaine was administered to the animal, and the other lever to be reinforced with food after saline was administered to the animal. Then tests can be conducted in which the animal’s lever selection essentially reports whether the drug discriminative stimulus or cue was detected by the animal. The indirect dopamine agonists all strongly generalize to each other, and not nearly as much to drugs in other pharmacological classes. In other words, if an animal is trained to a cocaine cue, substitution with d-amphetamine produces identical responding to cocaine, and vice versa. Xanthines generalize to indirect dopamine-agonist cues under most conditions, and nicotine under some conditions. Psychoactive drugs that are not psychostimulants, such as sedative hypnotics or κ -opioid agonists, may engender very little or no generalization to psychostimulants. This procedure has been very useful for classifying the interoceptive effects of novel drugs as stimulant-like or not. It also has been extensively used to study the pharmacological mechanisms of stimulant drugs. For example, various types of dopamine agonists often generalize to stimulant drugs, and conversely, dopamine antagonists (e.g., haloperidol) when given in combination with psychostimulants can block their discriminative stimulus effects. By contrast, noradrenergic and serotonergic agonists and antagonists are less effective than dopamine agents in this regard.

Drug self-administration procedures are a type of operant conditioning in which the positive reinforcing effects of psychostimulants can be measured, and they can be predictive of abuse liability in the human. Animals are trained to associate a behavioral response, typically a lever press or nose-poke, with rapid administration of a drug, often by automated intravenous infusions immediately following each appropriate behavioral response. Drugs that maintain lever-pressing behavior above levels maintained by saline infusions are

termed positive reinforcers. Over 20 drugs that are abused by the human function as positive reinforcers in the rat self-administration test. Psychostimulants such as cocaine and amphetamine are probably the most effective drugs ever observed to produce reinforcing effects in this procedure. This procedure has been used for evaluating the abuse liability of new chemical entities, including psychostimulants, that may have therapeutic applications. It has also been used for studying the pharmacological and neurobiological mechanisms underlying drug abuse and addiction.

Neuropharmacology and Brain Pathways Underlying Psychostimulant Effects

The use of psychostimulants to study the synaptic mechanisms of action and brain pathways underlying the behavioral effects of stimulant drugs, and, in particular, their abuse-related effects, represents an enormous body of literature and will only be summarized briefly.

Most of the psychostimulants that are indirect dopamine agonists (**Table 1**) are either inhibitors of dopamine reuptake into dopamine nerve terminals such as cocaine, or else are releasers of dopamine from dopamine nerve terminals such as amphetamine. All of those drugs act, at least partly, by binding to the dopamine transporter (reuptake site). In contrast to the indirect dopamine agonists, indirect agonists for other neurotransmitters – and even for other monoamine neurotransmitters, such as norepinephrine and serotonin – fail to produce psychostimulant effects in assays of locomotor activity, prepulse inhibition of startle, drug discrimination, and drug self-administration. Generally, dopamine receptor antagonists (e.g., haloperidol) produce rightward shifts in dose–effect functions for psychostimulants in all of those assays, indicating competitive antagonism of the dopamine receptor antagonists with the indirect dopamine agonist effects of the psychostimulants. Finally, removal of dopamine in the brain pathway arising from the ventral tegmental area (VTA, located in the midbrain) and terminating in the nucleus accumbens (ventral striatum located in the basal ganglia) attenuates behavioral effects of psychostimulants in all of those assays. Collectively, these experimental observations suggest that enhanced dopaminergic activity in the mesolimbic pathway (from the VTA to nucleus accumbens) is the principal mechanism underlying most of the important behavioral effects of psychostimulants that are indirect dopamine agonists. In contrast, enhanced dopaminergic activity in the brain pathway arising from the substantia nigra and terminating in the dorsal striatum has been associated with motor stereotypies produced by psychostimulants. It should be noted that psychomotor stimulant drugs that have a principal mechanism of action different from the indirect dopamine agonists appear to be

able to produce stimulant effects independently of dopamine. For example, the locomotor hyperactivity produced by the adenosine receptor antagonist caffeine, or by the muscarinic receptor antagonist scopolamine, are not blocked by depletions of dopamine in the mesolimbic pathway.

Psychostimulant Addiction: Abuse, Dependence, and Withdrawal

The abuse of psychostimulants arises from the drugs' powerful reinforcing effects that can be measured using the self-administration test in a variety of laboratory animals and in the human under controlled conditions. The development of psychostimulant addiction begins with abuse and probably includes the development of dependence on the drug as well as some form of withdrawal in the absence of the drug. However, evidence of marked tolerance to the drug (i.e., the need for higher doses to achieve a given effect), and a physical withdrawal syndrome, are nearly absent for this drug class compared with addiction to opioid drugs, for example.

Activation of the mesolimbic dopamine pathway occurs naturally, in the absence of psychostimulant drugs, under a variety of conditions, and particularly when unexpected rewarding stimuli are presented. Psychostimulant drugs hijack this pathway and drive it unnaturally. One prominent hypothesis is that dopamine systems are compromised during the addiction cycle and this contributes to withdrawal, which may lead to decreased motivation for nondrug-related stimuli and increased sensitivity to the abused drug.

Psychostimulant withdrawal in the human is associated with fatigue, depressive symptoms, anxiety, and psychomotor retardation. In animals, psychostimulant withdrawal is associated with decreased reinforcing effects of natural rewards and decreased exploratory activity, measured using operant conditioning and the locomotor activity test, respectively. Those behavioral effects probably involve decreased dopaminergic function. Decreases in the activity of the mesolimbic

dopamine system and decreases in serotonergic neurotransmission in the nucleus accumbens occur during drug withdrawal in animal studies. Decreases in the firing of dopamine neurons in the VTA have been observed during withdrawal from other drugs including opioids, nicotine, and ethanol. Imaging studies in the drug-addicted human have consistently shown long-lasting decreases in the numbers of dopamine D₂ receptors in drug abusers. In addition, cocaine abusers have reduced dopamine release in response to a pharmacological challenge with a stimulant drug. Decreases in the number of dopamine D₂ receptors, coupled with the decrease in dopamine neuron firing activity in cocaine, nicotine, and alcohol abusers, probably results in decreased sensitivity of reward circuits to stimulation by natural reinforcers. These findings suggest an overall reduction in the sensitivity of the dopamine synapses and dopamine brain pathways to natural reinforcers and other drugs in drug-addicted individuals.

See also: Basal Ganglia; Drug Addiction; Drug Sensitization and Drug Abuse; Neural Basis of Attention-Deficit/Hyperactivity Disorder; Neural Systems of Motivation; Value of Animal Models for Predicting CNS Therapeutic Action.

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Rewarding Brain Stimulation

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Glossary

Deep brain stimulation – Electrical activation of neurons provided by passing current through an electrode that is insulated along its length, but has an uninsulated tip, so that neurons near the tip are excited.

Myelinated axon – The part of the neuron that carries action potentials from the soma to the terminals (i.e., the axon) will conduct these action potentials faster and more efficiently when the axon is insulated by a fatty surrounding sheath (i.e., myelin) provided by surrounding cells.

Neurotransmitter – A chemical released from neurons at synapses that acts on other neurons to increase or decrease their activity or excitability.

Priming stimulus – A stimulus given before testing to facilitate performance, such as brain stimulation given by the experimenter to facilitate bar pressing for that stimulation by the animal.

Receptor – A protein that chemicals combine with to change cell functions.

Reinforcer – A stimulus, such as food or brain stimulation, whose presentation leads to increased responding to obtain that stimulus, for example, by bar pressing.

Theory of drive reduction – The proposal by Hull that all primary reinforcers, such as food and water, have their reinforcing effects on behavior by reducing specific motivated states, such as hunger and thirst, respectively.

these discoveries showed the value of behavioral methods and analysis (especially operant methods) to the understanding of brain functions.

Predecessors

In 1949, the Swiss physiologist Walter Rudolf Hess was awarded the Nobel prize for developing methods for chronic stimulation of the brain in freely behaving cats. These methods led Hess to discover several autonomic and motor functions of the midbrain and forebrain. Hess found that stimulation of the hypothalamus led to visceral changes (including salivation, feeding, and pupillary responses) and behavioral changes (including aggressive responses). Stimulation of the dorsal midbrain led to head and eye turns, which he called ‘visual grasp reflexes.’ Although electrical brain stimulation had been used since the 1860s, especially in mapping cortical areas for movement, Hess’s method of implanting deep brain electrodes permanently was essential for long-term studies of deep brain functions.

In the early 1950s, Jose Delgado at Yale University used Hess’ methods to study motivational changes in cats. He found that food intake increased with sustained electrical stimulation of the lateral hypothalamus, and that the cats will work to remove sustained electrical stimulation of some deep brain sites. He later moved to Spain where he studied electrical stimulation of the limbic system in monkeys, humans, bulls, and rats, using radio transmitters to control behavior and emotions.

Olds was trained at Harvard University, where he learned operant techniques developed by B.F. Skinner. Milner was an electrical engineer from England, conducting graduate studies in psychology at McGill University. Together, they implanted electrodes using the methods of Hess and Delgado in the deep brain of rats. Their rats were placed in a Skinner box, and were observed to respond to electrical brain stimulation by returning to the place in the box where the stimulation was delivered, a phenomenon now called ‘conditioned place preference’. Using Skinnerian shaping methods, Olds and Milner were able to train the rats to press a lever for deep brain stimulation at high rates (up to 70 presses per minute) for hours. Many rewarding sites were tested, from the septum in the limbic part of the forebrain, to the tegmentum of the midbrain.

Discovery

In 1954, James Olds and Peter Milner at McGill University reported that rats bar-press vigorously to electrically stimulate their own brains, in many deep brain sites. This phenomenon (which was soon called intracranial self-stimulation, and is now most commonly called brain-stimulation reward) quickly led to a re-evaluation of psychological theories of learning, reinforcement, motivation, and emotions. Over the next two decades, maps of brain regions and neural systems supporting brain-stimulation reward led to the discovery of several brain systems needed for natural rewards, drug rewards, and motivated behaviors of many sorts. Furthermore,

Psychological Theories

Olds and Milner showed that deep brain stimulation is positive reinforcement by definition, since it leads to increased probability of responding. They inferred that the stimulation was rewarding to the rats based on the vigorous approach behaviors of the rats toward the lever, and the lack of signs of pain after stimulation.

The finding that rats bar-press at stable rates for hours (i.e., the lack of satiation) challenged the leading theory of Clark Hull and his Yale University colleagues that reinforcement occurs due to drive reduction. Reinforcers, such as food and water reduce the motivation to eat and drink, so Hull proposed that all reinforcers work by the principle of drive reduction. Brain-stimulation reinforcement, however, was at least as powerful a reinforcer as food or water, yet seemed to increase motivation when delivered. Unlike food and water, this motivation was not decreased by repeated delivery of the reinforcer. So reinforcement does not require drive reduction.

Olds' assertion that brain-stimulation reward identifies 'Pleasure Centers in the Brain' (the title of his 1956 *Scientific American* review) was challenged by behaviorists who, like Skinner himself, were often philosophically opposed to subjective inferences about animal emotions. Studies of deep-brain stimulation in human patients by Robert Heath and Jose Delgado, however, showed that self-stimulation of amygdala and hypothalamic sites often leads to verbal reports of arousal, interest, as well as motivational or sexual feelings in some cases. Intracranial self-stimulation has since been described in dozens of different species, including goldfish, mice, rats, hamsters, monkeys, and humans.

In the following decades, studies of acquisition, extinction, and recovery of intracranial self-stimulation in many brain sites in rats led to competing psychological theories. First, continuous stimulation of lateral hypothalamic rewarding sites for seconds without interruption becomes aversive to the rats, who will then learn to turn the stimulation off by bar pressing. Second, rewarding hypothalamic stimulation in many sites has an arousing effect on the rat, leading to vigorous locomotor exploration, including sniffing, biting, and rearing. These exploration and approach responses facilitate learning of responses associated with the stimulation. One short stimulation provided by the experimenter quickly reinstates lever pressing in rats that have been previously trained, a powerful phenomenon called the priming effect. Different theorists have emphasized the arousing effects, the priming effects, or the aversive effects of sustained brain-stimulation reward.

Brain Maps

Many maps showed that hundreds of brain regions, including well over 20% of the volume of the brain, support brain-stimulation reward (Figure 1). These include the limbic frontal cortex, hippocampus, olfactory bulb, parts of the cerebellum, and regions as far back as the motor nuclei of the trigeminal and vagus nerve of the hindbrain. In many of these sites, rats bar-press at low rates (below 10 presses per minute), and required extensive training to press reliably. The most reliable sites were found in ventral brain regions stretching continuously from the basal forebrain to the tegmentum of the midbrain and rostral pons. These sites followed the anatomical

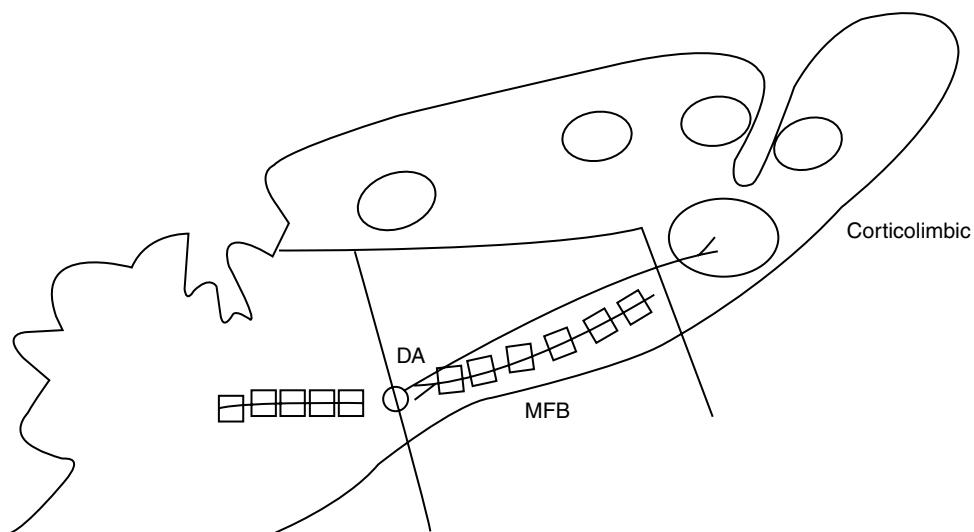


Figure 1 Brain-stimulation reward sites. Reproduced with permission from Yeomans JS (1990) *Principles of Brain Stimulation*. Oxford: Oxford University Press.

course of the medial forebrain bundle (MFB), the major pathway that connects forebrain limbic systems with the brain stem. The MFB sites with the lowest current thresholds and highest bar-pressing rates were located in the lateral and posterior hypothalamus, and ventral tegmental area (VTA), where MFB axons are most concentrated. This observation led to a search for the critical neurons and transmitters responsible for the rewarding effects of brain stimulation in these regions.

Catecholamine Theories

In the late 1960s, it was discovered that rats also bar-press at low, but steady, rates to inject drugs such as morphine, amphetamine, and cocaine into their veins by way of indwelling catheters (i.e., intravenous self-administration). The practical value of understanding brain mechanisms for drug abuse has remained a major motivation for understanding brain-stimulation reward.

About the same time, Swedish anatomists discovered several small clusters of neurons that use the catecholamine neurotransmitters, norepinephrine and dopamine. These catecholamine neurons are most concentrated in the pontine and midbrain tegmentum, respectively, and send their ascending axons to the forebrain via the MFB. The dopamine neurons in the VTA project to the nucleus accumbens and frontal limbic cortex by way of mesolimbic and mesocortical axons. The more lateral dopamine neurons of the substantia nigra project to the dorsal striatum by way of nigrostriatal axons.

Remarkably, the reinforcing drugs amphetamine and cocaine were found to act specifically on these neurons, increasing release of both norepinephrine and dopamine from their axon terminals in the forebrain. Arvid Carlsson won the Nobel prize in 2000 for showing the importance of catecholamine neurons and pharmacology to diseases. In particular, the motor disorder Parkinson's disease is caused by the death of dopamine neurons of the substantia nigra and is treated effectively by giving patients L-dopa, the chemical precursor of dopamine, to restore dopamine function. Schizophrenia is treated effectively by drugs that block dopamine receptors. Therefore, understanding how catecholamine neurons and drugs work in behaviors, such as brain-stimulation reward, provided a second major motivation for studies of brain-stimulation reward circuits.

In the late 1960s and early 1970s, both norepinephrine and dopamine neurons were proposed to be critical for brain-stimulation reward. Studies using amphetamine, or specific blockers of norepinephrine or dopamine functions, or their receptors, soon proved that dopamine neurons and receptors, and not norepinephrine neurons and receptors, were most critical for the activating and the rewarding effects of brain stimulation. Local brain

injections of dopamine-specific drugs and blockers, or of the specific catecholamine neurotoxin, 6-hydroxydopamine, showed that dopamine neurons are especially critical for brain-stimulation reward, or for cocaine, or amphetamine self-administration via dopamine axon terminals of the mesolimbic pathway in the nucleus accumbens.

NonDopamine Axons

The success of the dopamine hypothesis of reward, however, led to two problems in the 1980s. First, mesolimbic dopamine neurons that send their axons from the ventral tegmentum to the nucleus accumbens include less than half of the brain regions where brain-stimulation reward is obtained. Second, dopamine axons are tiny and lack a myelin sheath, and so are insensitive to brain stimulation at the lowest currents used to support brain-stimulation reward. Therefore, unlike cocaine or amphetamine, which acts directly on dopamine neurons, brain-stimulation reward stimulates dopamine neurons indirectly in most cases.

Detailed studies showed that thicker myelinated axons are the directly stimulated neurons in the MFB. To define these neurons, better stimulation methods, using trains of short pulses rather than 60-cycle sine waves, were needed. In particular, the refractory periods and conduction velocities of the MFB axons mediating brain-stimulation reward were measured by delivering trains of twin pulses, and then varying the interval between the twin pulses. If the interval between the two pulses is shorter than the refractory period of the axons, the second pulse will fail to produce any action potentials. The effectiveness of the second pulse in increasing reward was found to increase as the interval between the twin pulses increased from 0.4 to 2.0 ms. This indicates that the critical neurons for reward have refractory periods mainly from 0.4 to 1.2 ms, with smaller contributions from 1.2 to 2.0 ms. Dopamine axons in MFB have refractory periods from 1.2 to 2.5 ms, however.

When stimulating with two electrodes in MFB, say 3 mm apart, trains of pulses can be delivered via both electrodes at the same time. If the trains are slightly out of phase (say by 0–5 ms), the action potentials from the second train can be blocked by the action potentials from the first train, due to a well-studied phenomenon called 'collision' by which the action potentials collide and erase one another. Just as in the refractory period experiment, the effectiveness of the second train of pulses is blocked by the loss of action potentials. For MFB self-stimulation, the effectiveness of the second train was found to increase as the delay of the second train increased from 1 to 3 ms, indicating an added conduction time of 0.5–2.0 ms between the MFB electrodes. The conduction velocity

of the critical MFB axons was determined to be $1\text{--}8 \text{ m s}^{-1}$. Dopamine axons in MFB have conduction velocities of $0.3\text{--}1.0 \text{ ms}$, however.

These results indicate that small myelinated axons (diameters $0.5\text{--}2.0 \mu\text{m}$) mediate most of brain-stimulation reward, with smaller contributions from dopamine-like unmyelinated axons (diameters $0.1\text{--}0.5 \mu\text{m}$). Higher currents than are normally used are needed to stimulate these higher threshold dopamine-like axons. The more-sensitive myelinated axons pass down through the MFB to indirectly activate dopamine neurons in the ventral tegmentum (**Figure 1**). Similar myelinated axons also mediate feeding elicited by MFB stimulation, or the aversive effects of sustained MFB stimulation.

It is still uncertain exactly which neurons send their myelinated descending axons through the MFB to mediate brain-stimulation reward. Lesion studies using excitotoxins suggest that intrinsic neurons of the lateral hypothalamus provide a substantial contribution to brain-stimulation reward. For example, lateral hypothalamic neurons which use the peptide neurotransmitter orexin/hypocretin are known to excite dopamine neurons via descending MFB axons. Other clusters of neurons in the basal forebrain regions known as the substantia innomina-ta, bed nucleus of the stria terminalis, and ventral pallidum provide small contributions. Many neuropeptides found in the basal forebrain can activate or inhibit dopamine neurons, so these are of interest for brain-stimulation reward.

Acetylcholine and Nicotine

Acetylcholine is an important neurotransmitter at skeletal muscles where it excites muscles by way of nicotinic receptors. In the autonomic nervous system, acetylcholine can activate or inhibit visceral muscles via muscarinic receptors. Both nicotinic and muscarinic acetylcholine receptors are found widely in the brain.

Brain-stimulation reward is strongly inhibited by injecting cholinergic receptor blockers near dopamine neurons in the VTA. By contrast, brain-stimulation reward is facilitated by injections of cholinergic agonists, such as acetylcholine, nicotine, or carbachol applied to the same sites. Especially, carbachol in these VTA sites is highly rewarding, since carbachol works via both nicotinic and muscarinic acetylcholine receptors.

Nicotine is important as the addictive agent in tobacco that leads to smoking. Rats can be trained to self-administer nicotine, although it is much easier to train the rats using cocaine or amphetamine as the reinforcer. Nicotine is weakly rewarding in tests of conditioned place preference. Nicotine induces its rewarding effects mainly through activation of dopamine neurons in the VTA via nicotinic receptors.

VTA dopamine neurons are directly activated by way of cholinergic nerve terminals that release acetylcholine which then acts via nicotinic and muscarinic receptors near dopamine neurons. Nicotinic receptors excite dopamine neurons for seconds, while muscarinic receptors activate dopamine neurons more strongly, and over many minutes. Similarly, the rewarding effects of MFB stimulation or carbachol are due mainly to VTA muscarinic receptors, with smaller contributions from VTA nicotinic receptors.

The cholinergic activation of substantia nigra and VTA dopamine neurons comes from two cholinergic cell groups located in the pedunculopontine tegmental nucleus (PPT) and laterodorsal tegmental nucleus. The cholinergic neurons most important for brain-stimulation reward are in the PPT at the midbrain–hindbrain border. In particular, carbachol inhibits these PPT cholinergic neurons, and strongly inhibits brain-stimulation reward or locomotor arousal. PPT cholinergic neurons are themselves activated by water, food, or brain stimulation in rats, leading to release of the transmitter acetylcholine in VTA. This indicates an important role for these PPT neurons in natural rewards as well as brain-stimulation reward. It is not yet clear how PPT cholinergic neurons are activated by MFB or hindbrain stimulation, or by natural reward signals coming from the viscera or higher sensory systems. PPT receives strong, direct anatomical inputs from the lateral hypothalamus, and nucleus of the solitary tract (receiving visceral information from the mouth and gastrointestinal tract), however.

Opiate Reward and Brain-Stimulation Reward

Due to the widespread distribution of opiate receptors and endorphins in the brain, it is not yet clear how opiates induce rewarding effects in the brain. First, opiates can produce rewarding effects in the forebrain (such as the nucleus accumbens, or limbic frontal cortex) by acting independently of dopamine neurons. Lesions of dopamine neurons with 6-hydroxydopamine, therefore, have much weaker effects on opiate reward than on amphetamine, cocaine, or nicotine reward, or even on MFB brain-stimulation reward.

Second, opiates in the VTA are highly rewarding, which initially suggested that dopamine neurons may be sufficient for opiate rewards. High levels of opiate receptors (and endorphins) are found in the ventral tegmentum, but these opiate receptors are found on the nondopamine γ -aminobutyric acid (GABA) neurons, not on dopamine neurons. Several studies showed that the μ -type opiate receptors in the ventral tegmentum are important for a large part of the opiate reward.

PPT Lesions, Opiates and Brain-Stimulation Reward

PPT lesions have been made by infusing excitotoxins that kill cells in the nucleus. These lesions prevent rats from learning to bar-press for brain-stimulation reward or for heroin. Furthermore, van der Kooy and colleagues found that PPT lesions block the ability of morphine or food to induce conditioned place preference. This suggests that cholinergic or noncholinergic PPT neurons are important for opiate rewards, food rewards, and brain-stimulation reward.

PPT lesions, however, do not block the maintenance of brain-stimulation reward, or heroin self-administration in previously trained rats, and do not block the acquisition of conditioned place preference in rats previously exposed to multiple doses of morphine. Therefore, opiates can work by mechanisms independent of those for brain-stimulation reward.

Most theories now propose that opiates work by inhibiting tegmental GABA neurons that, in turn, inhibit PPT and dopamine neurons. Even when dopamine neurons are blocked, VTA opiates are rewarding via PPT.

Addiction and Glutamate

Dopamine neural changes are more important for opiate addiction (i.e., after repeated high doses of morphine or heroin) than for the initial rewarding effect of opiates. How repeated drug exposures and addictive behaviors increase the sensitivity of dopamine neurons is an active area for research. Descending excitatory inputs to dopamine neurons from limbic forebrain seem to be especially important for drug-induced plasticity and for the learned aspects of drug rewards. In particular, glutamate is the major excitatory input to dopamine neurons from the forebrain. The α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) types of glutamate receptor in VTA are most important for morphine-induced plasticity in sensitization and drug self-administration.

However, brain-stimulation reward is not dependent on addiction-like changes in dopamine neurons. Brain-stimulation reward thresholds are stable over months of bar pressing, unlike self-administration of addictive drugs. This indicates that brain-stimulation reward does not work through highly plastic brain mechanisms (such as those for glutamate AMPA and NMDA receptors), but maintains its rewarding qualities more like those for food and water over the lifetime of the animal.

Natural Rewards and Brain-Stimulation Reward

The stable thresholds for food, water, and brain-stimulation reward over months of testing indicate that stable inputs to dopamine neurons from the MFB and from PPT cholinergic neurons are more critical for these rewards than the highly plastic inputs from the forebrain via glutamate receptors. Many electrode sites in the lateral hypothalamus near the fornix also elicit feeding, as well as brain-stimulation reward. The stimulation-elicited feeding sites are a smaller subset of the more medial MFB sites. Thresholds for brain-stimulation reward in these medial MFB sites are strongly influenced by food-related signals, including food deprivation, and the feeding-inhibiting neuropeptides leptin and cholecystokinin. These results suggest that brain-stimulation reward can activate pathways important for food sensitivity, for food seeking, and for food-related rewards; besides, MFB stimulation activates many different groups of axons, and consequently involves many different motivational signals in the activation of rewarding effects, in all likelihood.

Genes and Brain-Stimulation Reward

Brain-stimulation is useful to map rewarding inputs to dopamine systems, especially from MFB reward sites. In particular, brain-stimulation reward has been used to study hypothalamic and mesopontine systems and transmitters needed for dopamine-neuron and dopamine-receptor activation.

Dopamine Receptor Genes

Of the dopamine receptors, both D1 and D2 dopamine receptor families have been found to contribute to brain-stimulation reward. Selective blockers of these two main types of dopamine receptor reduce bar pressing (an effect on motor performance) and, in some cases, reduce the sensitivity of rats to brain-stimulation reward (an effect of reward sensitivity that is independent of performance).

Of the five dopamine receptor genes, two are of the D1 type (D1 and D5 genes) and three are of the D2 type (D2, D3, and D4) with all subtypes expressed in varying amounts in the VTA, substantia nigra, nucleus accumbens, and other dopamine terminal regions. In particular, the D2 dopamine receptor knock-out mouse shows excellent bar-pressing performance, but decreased reward sensitivity, requiring about 2 times more MFB stimulation to achieve similar response rates as wild-type control mice. These D2 knock-out mice show normal potentiation of brain-stimulation reward by amphetamine, but greatly reduced or reversed potentiation in response to

morphine. These findings indicate that the D2 dopamine receptor subtype is important for brain-stimulation and morphine-activated rewarding inputs to dopamine functions, but not to amphetamine-activated dopamine outputs. By contrast, the D1 dopamine receptor knockout mouse shows impaired bar-pressing performance in a brain-stimulation reward task.

Acetylcholine Receptor Genes

Many cholinergic receptor genes have been cloned and mapped in the brain. In particular, five muscarinic and nine nicotinic receptor genes have been identified (excluding four nicotinic subunits found on muscles and two in the cochlea that have not been found in the mammalian brain). Four muscarinic receptor subtypes and nine nicotinic receptor subtypes have been found in the ventral tegmental region. Of the four muscarinic subtypes, however, only the one for the M5 muscarinic receptor gene is expressed by dopamine neurons (as indicated by M5 messenger RNA), with messenger RNA for the M3 muscarinic receptor associated with nondopamine neurons. M2 and M4 muscarinic receptors are also found in VTA/SN, without mRNA for these two receptor subtypes, suggesting that these receptors are expressed on axon terminals of neurons projecting from other brain regions.

The M5 muscarinic receptor in VTA has been found most critical for sensitivity to brain-stimulation reward in rats. Specifically, messenger RNA for the M5 receptor was gradually removed by infusing a complementary DNA (called an ‘antisense oligonucleotide’) into the VTA for 6 days, leading to a reduction in the M5 receptor. As the M5 mRNA was removed from the VTA, the sensitivity of the rat to brain-stimulation reward was gradually reduced. Removing the M5 receptor in this manner reduced sensitivity in a way similar to nonselective muscarinic receptor blockers in VTA. When the antisense oligonucleotide infusion was stopped, allowing production of more M5 receptors, the sensitivity of the rat to brain-stimulation reward quickly returned.

When the M5 gene was removed completely in a genetically engineered M5 knock-out mouse, the mouse was less sensitive to morphine tested by dopamine activation, by locomotor activation, or by conditioned place preference. Furthermore, the M5 muscarinic receptor was needed for sustained activation of dopamine neurons by muscarinic receptors in VTA. Therefore, the M5 receptor subtype appears to be most critical for rewarding inputs to dopamine neurons from the brain stem. Of the nicotinic receptor genes, the beta-2 and alpha-4 subtypes have been shown to be most important for nicotine self-administration and conditioned place preference in knockout mice.

Brain-Stimulation Reward and Social Behaviors

Rats and mice communicate by way of high-frequency calls (termed ‘ultrasonic vocalizations’) that humans cannot hear. Mice make calls in the 30–110 kHz range in social situations where rewards, such as food in female mice, or sex, or amphetamine in male mice, are anticipated. M5 knockout mice show many fewer 30–110 kHz ultrasonic calls than wild-type mice in response to female mice, to amphetamine, or to palatable food odors. These findings suggest that the M5 receptor is needed for reward-induced social communications.

Brain-stimulation reward has recently been shown to activate similar 40–70 kHz ultrasonic vocalizations in male rats. These calls occur most often before the bar pressing begins, when the rat anticipates the entry of the bar, as signaled by a conditioned stimulus. These ultrasonic calls are similar to the calls evoked by amphetamine, by sex, by social play, or by tickling in rats, indicating that the calls are activated by rewards, and depend in part on dopamine, and in part on social communication.

In conclusion, the discovery of rewarding brain stimulation provided a major impetus to understanding neural substrates of emotion and learning. Presently, brain-stimulation reward is used to distinguish neural systems, transmitters, and receptors mediating several categories of drug reward, natural reward, and social behavior.

See also: Cellular Plasticity in Cocaine and Alcohol Addiction; Drug Addiction; Ethanol and Nicotine Interactions; Nicotine; Psychostimulants.

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Stress and Drug Craving

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Glossary

Autonomic nervous system – The autonomic nervous system is the part of the peripheral nervous system that acts as a control system, maintaining homeostasis in the body. These activities are generally performed without conscious control or sensation and affect heart rate, digestion, respiration, salivation, perspiration, the diameter of the pupils, urination, and sexual arousal. Although most of the actions of the autonomic nervous system are involuntary, some, such as breathing, work in tandem with the conscious mind.

HPA axis – The hypothalamo-pituitary-adrenal (HPA) axis is a complex set of direct and feedback interactions among the hypothalamus, the pituitary gland, and the adrenal glands. The interactions among these organs constitute the HPA axis, a major part of the neuroendocrine system that controls reactions to stress and regulates a variety of physiological processes, including digestion, the immune system, mood and emotions, sexuality, and energy storage and expenditure.

Mesocorticolimbic dopaminergic system – The mesocorticolimbic dopaminergic system is one of the neural pathways in the brain that links the ventral tegmental area (VTA) in the midbrain to the nucleus accumbens and amygdala (in the limbic system) and the medial prefrontal cortex. This pathway is thought to be involved in producing pleasurable feelings and is often associated with reward and desire. Therefore, this pathway has also been implicated in many neurobiological theories of addiction.

Reinforcement – Reinforcement is an increase in the strength of a response following a change in the environment (e.g., the delivery of a food pellet to a laboratory rat following the pressing of a response lever). The environment change contingent upon the response is called a reinforcer (e.g., food in the proceeding example). It is the strength of the response that is reinforced, not the organism.

Stress – Stress is an emotional and physical response to threats from the outside world. According to the pioneering neuroendocrinologist Hans Selye, stress is defined as the nonspecific response of the body to any demand placed upon it to adapt, whether that demand produces pleasure or pain.

Introduction

The relationship between stress and drug craving and relapse is not a novel concept; this phenomenon has been known to the lay public for many years. An excellent example can be found in the 1980 movie *Airplane* (Paramount Pictures, Hollywood, CA). Air traffic controller Steve McCroskey (played by Lloyd Bridges) is responsible for the safe landing of a pilotless passenger airliner in this comedy feature. As more mishaps befall the stricken aircraft, McCroskey is heard to say, "Looks like I picked the wrong week to quit drinking." This was subsequently followed by smoking, amphetamines, and sniffing glue, which was an undeniable reference to the stress of the situation increasing McCroskey's desire to use these substances. In general, addictive drugs (including alcohol, cigarettes, and psychomotor stimulants) tend to alter activity within the primary mediator of stress in the body, the hypothalamo-pituitary-adrenal (HPA) axis. Thus, it should come as no surprise that scientists and clinicians alike have reported a connection between substance abuse and the HPA axis. Recovering addicts often claim that their addiction was under control until they were faced with what they perceived as a stressful life situation. Obviously, drug addiction is a much more complex physical and psychological phenomenon than a simple cause and effect based on HPA axis activation. However, increasing evidence suggests that an addict's belief that his or her drug of choice provides relief from stress or control over life's stressors has a biological basis. This article reviews the evidence for a physiological basis underlying the role of stress and the HPA axis in drug craving and relapse.

Stress and the HPA Axis

Stress is perceived differently by different individuals, and individual responses to stressors are just as varied. However, people generally tend to focus on the negative ramifications associated with exposure to stress. For example, some individuals might complain about stress on the job, stress from dealing with family or friends, or stress related to a traumatic event, and in each case they would likely describe the unpleasant impact the stressor produced, which could include problems such as sleeping, increased anxiety, ulcers, heart disease, depression, or other more serious psychiatric disorders. However,

despite popular belief, stress does not have to be exclusively associated with negative events. The modern definition of stress and its implication for disease was developed by the pioneering neuroendocrinologist Hans Selye, who defined stress as the nonspecific response of the body to any demand placed upon it to adapt, whether that demand produces pleasure or pain. Therefore, positive events can be just as stressful to the body as negative events. Accordingly, stress can result from the loss of a loved one or from a marriage or birth of a child, a job promotion or the loss of a job, moving into a new house or losing one's home, or any number of events that impact upon an individual's daily life.

Stressors produce an activation of two functionally related biological systems, the sympathetic nervous system and the HPA axis. The activation of these systems makes it possible for an individual to cope with or adapt to an environmental event through the production of a stress response or the stress cascade. The stress-induced activation of the sympathetic nervous system, mediated through the neurotransmitter norepinephrine, results in an increase in heart rate, a rise in blood pressure, a shift in blood flow to skeletal muscles, an increase in blood glucose, a dilation of the pupils, and an increase in respiration. This automatic response, also called the 'fight or flight' response, makes it possible for the individual to face the stressor or attempt to escape from it and occurs below the level of consciousness. In reality, however, people cannot run away from many of life's stressors but instead must learn to adapt to both external and internal environmental changes. During positive events, an individual may believe that his or her increased heart rate represents feelings of happiness and joy, while an increased heart rate resulting from a negative event may be associated with anger or fear. Many abused substances also produce changes in the activity of the sympathetic nervous system, and these effects are felt differently by different individuals. One person may relish the increased heart rate (or rush) produced by cocaine while the same autonomic response may cause another individual to experience feelings of panic.

The HPA axis consists of a complex, well-regulated interaction between the brain, the anterior pituitary gland, and the adrenal cortex. The initial step in the activation of the HPA axis is the neuronal-regulated secretion of the peptide corticotropin-releasing factor (CRF). Although CRF is distributed in a number of brain regions, it is those CRF-containing neurons localized in the medial parvocellular subdivision of the paraventricular nucleus (PVN) of the hypothalamus projecting to the external zone of the median eminence that initiate HPA axis activity. These neurons release the peptide into the adenohypophyseal portal circulation in a circadian manner or in response to neuronal stimulation. The interaction of CRF with CRF₁ receptors located on

anterior pituitary corticotrophs results in the synthesis of proopiomelanocortin (POMC), a large precursor protein which is proteolytically cleaved to produce several smaller biologically active peptides, including β -endorphin and adrenocorticotropin hormone (ACTH). Vasopressin is also released from the parvocellular neurons of the PVN and produces synergistic effects on ACTH release. POMC-derived ACTH diffuses through the general circulation until it reaches the adrenal glands. There it stimulates the biosynthesis of adrenocorticosteroids, most notably the glucocorticoids, cortisol (in humans), and corticosterone (in rats), which results in their secretion from the adrenal cortex. Two types of adrenocorticosteroid receptors have been identified, both of which bind corticosterone. The type I mineralocorticoid receptor has a higher affinity for corticosterone and is usually fully occupied at basal concentrations of the hormone. This receptor also displays a high affinity for the mineralocorticoid, aldosterone. In contrast, the type II glucocorticoid receptor has a lower affinity for corticosterone and is more likely to be occupied when plasma corticosterone is elevated (e.g., during stress). This receptor also has a high affinity for the synthetic glucocorticoid, dexamethasone. The HPA axis is finely tuned through negative feedback processes from circulating cortisol at the level of the anterior pituitary and the PVN.

However, glucocorticoid receptors are also localized in brain regions above the hypothalamus, including the hippocampus, the limbic system (e.g., amygdala and bed nucleus of the stria terminalis), and the prefrontal cortex, suggesting that these higher brain centers are involved in the psychological stress response and may alter HPA axis responding. It is relevant to point out that these same higher brain centers have also been implicated in drug reward, and that both stress and addictive drugs produce a similar excitation of dopamine in these brain regions. As stated above, addictive drugs also tend to alter activity within the HPA axis. Cocaine and other psychomotor stimulants increase HPA axis activity, as does cigarette smoking and alcohol intoxication and withdrawal. Heroin users typically exhibit a hyporesponsive HPA axis with decreased plasma cortisol.

Stress and Vulnerability to Drug Addiction

There is a growing clinical literature describing the link between stress and addiction. One group of individuals who appear to be at greater risk for substance abuse are combat veterans, especially those suffering from post traumatic stress disorder (PTSD), and a number of studies have identified individuals with the dual diagnosis of combat-related PTSD and substance abuse. Veterans with PTSD typically report a higher lifetime use of nicotine, alcohol, cocaine, and heroin than veterans screening

negative for PTSD. However, people exposed to stressors other than combat, such as an unhappy marriage, dissatisfaction with employment, or harassment also report increased rates of addiction. Sexual abuse, trauma, and sexual harassment are also more likely to produce symptoms of PTSD and alcoholism and/or other addictions in women than in men. Adolescents are especially susceptible to social stressors and traumatic life events, and exposure to these stressors can significantly impact their substance use. Examples of such events range from childhood sexual abuse or other childhood trauma to the inability to effectively cope with the demands produced by everyday family or social stressors.

Prevalence estimates suggest that rates of substance abuse among individuals with PTSD may be as high as 60–80%, while the rates of PTSD among substance abusers are between 40% and 60%, indicating that there is a clear relationship between PTSD and increased substance use. One explanation for the high concordance between PTSD (and related disorders) and drug addiction (i.e., dual diagnosis) is the self-medication hypothesis. According to this hypothesis, a dually diagnosed person uses the abused substance to cope with tension associated with life stressors or to relieve or suppress symptoms of anxiety, irritability, and depression resulting from a traumatic event. Others may also engage in substance abuse to manage symptoms of anxiety and/or depression that may be unrelated to a specific life event.

Drug Relapse

Exposure to external cues or triggers previously associated with drug use can induce craving and relapse in recovering addicts. A better understanding of how these cues contribute to the precipitation of relapse may lead to the development of more effective and efficient strategies for the treatment of addiction. Accordingly, the development of animal models that reflect many of the salient features of relapse in humans has received considerable attention. One well-studied animal model of relapse involves a reinstatement procedure. Using this model, rats are trained to self-administer a given drug. Once stable self-administration is observed, the rats are subjected to repeated extinction whereby responding is no longer reinforced by the delivery of the drug or to a period of abstinence from drug availability. Once extinction has been successful or the period of abstinence has been reached, the rats are exposed to various events in an attempt to reinstate drug-seeking behavior.

In humans and nonhumans, the acute re-exposure to the addictive drug itself (i.e., drug priming) is a potent event for provoking relapse to drug seeking. This drug-induced reinstatement has been observed for stimulant and opiate self-administration. Exposure to stress, or

simply the presentation of stress-related imagery, is another event that induces craving and relapse in humans. In rats, exposure to stress in the form of intermittent electric footshock has been reported to reinstate heroin- and cocaine-seeking behavior without affecting food-seeking behavior. Pretreatment with the corticosterone synthesis inhibitor ketoconazole can block the stress-induced reinstatement of cocaine seeking and the plasma corticosterone response to electric footshock, suggesting an important role for corticosterone in the ability of this stressor to reinstate cocaine-seeking behavior in rats.

The ability of cues previously paired with cocaine self-administration to reinstate extinguished cocaine-seeking behavior has also been investigated. In these experiments, responding during reinstatement testing results in the contingent presentation of a tone and house light cue that had been paired with cocaine delivery during self-administration training, which reliably reinstates extinguished cocaine-seeking behavior. Conditioned increases in plasma corticosterone are evident during cocaine extinction as well as during reinstatement. However, while plasma corticosterone returns to basal levels by the end of the session during extinction, it remains elevated through the end of the 2-h session during reinstatement, suggesting a potential relationship between HPA axis activation and cue-induced cocaine seeking. In humans, exposure to drug-related cues also increases plasma cortisol and ACTH and activates the sympathetic nervous system, suggesting an important involvement of the HPA axis and the sympathetic nervous system during drug cue-induced cocaine craving. In animal studies, pretreatment with ketoconazole reverses the conditioned cue-induced reinstatement of extinguished cocaine-seeking behavior and also blocks the conditioned increases in plasma corticosterone observed during reinstatement, which further underscores a role for the HPA axis in this behavior. In other studies, pretreatment with oxazepam or alprazolam also prevents the cue-induced reinstatement of extinguished cocaine seeking, suggesting that benzodiazepines may be useful in combating craving and relapse induced by environmental triggers associated with cocaine use.

CRF₁ receptor antagonists have been reported to decrease the stress- and cocaine-induced reinstatement of extinguished cocaine-seeking behavior in rats. Pretreatment with the CRF₁ receptor antagonist CP-154,526 also reduces the ability of conditioned cues to reinstate extinguished cocaine seeking. Another model of cue-induced cocaine seeking is based on the observation that animals will continue to respond for days during extinction when presented with cues that had been previously paired with cocaine. In such studies, rats are trained to self-administer cocaine, and, when responding stabilizes, saline is substituted for cocaine and the animals are tested for extinction. Other rats are allowed to

self-administer cocaine for an additional 30 days, and extinction is tested once again. CP-154, 526-treated animals respond significantly less than vehicle-treated animals during extinction on the first day of testing and also after 30 days of self-administration training, demonstrating a reduction in cocaine seeking. Interestingly, CP-154,526 does not suppress plasma corticosterone, suggesting that the effects of this compound are acting, in part, independently of the HPA axis and are likely mediated at sites located outside of the hypothalamus. However, these data do underscore an important role for CRF in the ability of environmental cues to stimulate cocaine-seeking behavior in rats.

The HPA Axis, Prefrontal Cortex, and Addiction

Cocaine stimulates HPA axis activity, which indicates that this system has the potential to influence many of the neurochemical and behavioral effects of the drug. The acute administration of cocaine dose-dependently increases plasma concentrations of corticosterone, ACTH, and β -endorphin in rats. Cocaine also stimulates the release of ACTH and cortisol in humans and nonhuman primates by increasing the peak amplitude of secretory pulses of these hormones without altering pulse frequency, suggesting that these increases are driven by hypothalamic CRF. However, although the HPA axis is involved in addiction, there are data that suggest that many of the effects of the so-called HPA axis drugs are mediated through sites located outside of the hypothalamus. For example, pretreatment with a CRF receptor antagonist does not produce reliable, reproducible effects on plasma corticosterone, and there is evidence that the prefrontal cortex, or more specifically the medial prefrontal cortex, may function as an interface between the HPA axis and the central nervous system. One series of experiments that supports this hypothesis involves the effects of cocaine on benzodiazepine receptor binding. Chronic, daily injections of cocaine result in differential effects on central benzodiazepine receptor binding in various regions of the rat brain. In general, cocaine decreases binding in terminal fields for the mesocorticolimbic dopaminergic system, while increasing labeling in terminal fields for the nigrostriatal system. Statistically significant decreases in binding in the medial prefrontal cortex and increases in the ventral tegmental area (VTA) are still observed for up to 2 weeks following the final injection of cocaine, suggesting that benzodiazepine receptors in mesocorticolimbic dopaminergic system may be especially sensitive to the effects of the drug. These cocaine-induced changes in binding appear to be mediated, at least in part, through the effects of the drug on dopaminergic neuronal activity since intraventricular

injections of 6-hydroxydopamine (6-OHDA) attenuate or reverse these effects. Continuous exposure to cocaine can also alter benzodiazepine receptor binding in various structures of the rat brain.

Some of the major symptoms associated with cocaine withdrawal often include severe anxiety, restlessness, and agitation, further suggesting that benzodiazepines may be useful for alleviating these negative symptoms during the early stages of withdrawal. These drugs are also useful in the emergency room for the treatment of some of the medical complications associated with cocaine intoxication since convulsions are often apparent following an acute overdose. These seizures can be treated with intravenous diazepam. Interestingly, the number of benzodiazepine receptors in platelets from chronic cocaine users is augmented when compared to those obtained from alcoholics or normal controls. In addition, peripheral benzodiazepine receptors labeled with [³H]PK11195 are decreased in neutrophil membranes from the blood of male inpatients following 3 weeks of cocaine abstinence. These data indicate that benzodiazepine receptors may mediate some of the biological effects of cocaine in humans. Since benzodiazepines are effective in reducing cocaine self-administration and the cue-induced reinstatement of extinguished cocaine seeking, benzodiazepines may also be useful in the treatment of cocaine addiction.

In rats, cocaine-induced increases in ACTH and corticosterone can be blocked by pretreatment with the CRF antagonist α -helical CRF9-41, by immunoneutralization of CRF with an anti-CRF antibody, or by bilateral electrolytic lesions of the PVN, indicating that these increases are also mediated by the cocaine-induced release of CRF from parvocellular neurons in the PVN. It has been reported that cocaine administration also increases hypothalamic CRF mRNA and alters CRF receptor binding measured autoradiographically in various regions of the rat brain. These data suggest that the complex relationship between cocaine reinforcement and corticosterone secretion may ultimately result from actions on extra-hypothalamic CRF.

Ketoconazole-induced changes in cocaine self-administration and reinstatement do not always correspond with decreases in plasma corticosterone either, which suggests that other mechanisms must be underlying these behavioral effects. Interestingly, significant increases in CRF content in the median eminence are observed after the acute administration of ketoconazole. Acute, repeated, and chronic treatment with ketoconazole also significantly increase CRF content in the medial prefrontal cortex, but do not consistently affect the peptide in other brain regions. Since the medial prefrontal cortex and CRF have been implicated in the neurobiology of cocaine addiction, CRF-induced alterations in dopaminergic neurotransmission may play an important role in

this peptide's effects on cocaine responsiveness. Accordingly, the effects of the CRF₁ receptor antagonist CP-154,526 on cocaine-induced dopamine overflow in the nucleus accumbens and medial prefrontal cortex have been measured using *in vivo* microdialysis. Rather than decreasing dopamine content as initially expected, CP-154,526 actually enhances the cocaine-induced increases in dopamine in the medial prefrontal cortex and the rostral part of the nucleus accumbens without altering these increases in dopamine in the rest of the nucleus accumbens. Ketoconazole produces a similar augmentation of cocaine-induced increases in dopamine overflow in the medial prefrontal cortex. These data implicate prefrontal cortex dopamine in the ability of CRF-receptor antagonists and the corticosterone synthesis inhibitor ketoconazole to attenuate cocaine seeking in rats. Taken together, these data suggest that ketoconazole, as well as CRF receptor antagonists and benzodiazepines, may affect cocaine reward, at least in part, through interactions with dopamine and CRF within the medial prefrontal cortex.

The neurochemical and neuroendocrine responses to cocaine have been measured during response-contingent and response-independent cocaine administration in rats. In these studies, male rats are divided into triads of three rats each: one rat is selected as the self-administration rat, while the other two rats are designated as the yoked-cocaine and yoked-saline rats, respectively. Self-administration rats are trained to self-administer cocaine, and each infusion is accompanied by an identical simultaneous cocaine infusion to the yoked-cocaine rat and saline to the yoked-saline rat. Baseline corticosterone in the medial prefrontal cortex was low and stable in all groups prior to the start of the self-administration session and remained stable throughout the entire experiment in the yoked-saline rats, suggesting that the procedure itself was not unduly stressful. Medial prefrontal cortex corticosterone was significantly elevated in microdialysates from the self-administration rats during cocaine self-administration, while corticosterone was increased significantly more in the medial prefrontal cortex of the yoked-cocaine rats. These data suggest that corticosterone in the medial prefrontal cortex may mediate the differences between cocaine reward (in the self-administration rats) and the more aversive aspects of cocaine (in the yoked-cocaine rats), further highlighting the role for this brain region in addiction and suggesting that the medial prefrontal cortex may also serve as an interface between the central nervous system and the HPA axis.

In humans, exposure to cues associated with cocaine induce craving and result in changes in blood flow and metabolic activity in the orbitofrontal cortex and the dorsolateral and anterior cingulate cortices, which loosely correspond to the prefrontal cortex in rats. Increases in glucose metabolism using ¹⁸F fluorodeoxyglucose were

observed in the dorsolateral cortex and medial orbitofrontal cortex after the presentation of cues associated with cocaine when compared to neutral cues. In a more recent study, increases in glucose metabolism were found in the right superior frontal gyrus in the dorsolateral prefrontal cortex and the left lateral orbitofrontal cortex. In contrast, glucose metabolism in the left medial prefrontal cortex and the ventromedial frontal pole decreased after the presentation of cocaine-associated cues, and interviews with the subjects about cocaine use produced increases in metabolism in the orbitofrontal gyrus, but not in the frontal cortex or cingulate gyrus. Both the anterior cingulate cortex and the dorsolateral prefrontal cortex were activated after the subjects watched a cocaine-related video as measured by blood oxygenation using functional magnetic resonance imaging (fMRI). In a more extensive study, the activation of the left dorsolateral cortex as well as the left anterior cingulate cortex and the left posterior cingulate cortex were also identified using fMRI. Finally, cerebral blood flow, measured using ¹⁵O-labeled water, increased in the anterior cingulate cortex, but not in the orbitofrontal cortex, in research subjects after internally generated craving or following the presentation of a cocaine-related video. Taken together, these data highlight the importance of the frontal cortex in cocaine craving in humans, and taken with the preclinical data reviewed above, these data further suggest that the prefrontal cortex may mediate the induction of craving resulting from the cue-induced activation of the HPA axis.

Conclusion

Drug craving is a complex phenomenon. The cues or triggers that stimulate craving and the relapse to drug use do so, at least in part, through an activation of the HPA axis and prefrontal cortex using CRF, cortisol, norepinephrine, dopamine, gamma aminobutyric acid (GABA), and likely a host of other mediators. The continued investigation into how stress and the subsequent activation of the HPA axis impact addiction will likely result in the identification of more effective and efficient treatment for substance abuse in humans. Stress reduction and coping strategies, either alone or in combination with pharmacotherapies targeting the HPA axis, may prove beneficial in reducing cravings and promoting abstinence in individuals seeking treatment for addiction.

Acknowledgment

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See also: Animal Tests for Anxiety; Brain Imaging and Addiction; Drug Addiction; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Incentive Motivation and Incentive Salience; Molecular Neurobiology of Addiction; Neurotransmitters and Neuromodulators Regulating Sleep and Wakefulness; Psychoneuroendocrinology of Stress; Psychostimulants; Regulation of the HPA Axis by Acute and Chronic Stress; Stress and Emotionality; Stress and Reward.

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- <http://www.cnn.com> – Cable News Network.
- <http://www.hbo.com> – Home Box Office.
- <http://www.nida.nih.gov> – National Institute on Drug Abuse (NIDA); stress; stress and drugabuse.
- <http://health.nih.gov> – National Institutes of Health (NIH).
- <http://www.pbs.org> – Public Broadcasting Service.

Stress and Reward

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Glossary

Drug addiction – The compulsive drug-taking behavior that is maintained due to physical and/or psychological dependence as a consequence of long-term changes in the brain.

Drug craving – The desire for a drug that persists long after dependence has subsided.

Reinforcer – Any natural reward or conditioned stimulus that increases the probability that a particular behavior will be repeated, when the reinforcer is associated with that behavior.

Reward – Something that an animal or individual will work to receive. Some things are naturally rewarding, which means that these items or events have some intrinsic value (e.g., food or sexual activity); other things become rewarding through repeated pairing with natural reinforcers (e.g., money).

Self-administration – In laboratory animals, it is a task where they have a chronic intravenous catheter and they learn to inject a dose of a drug into their veins by pressing a bar or poking their nose into a hole in the chamber wall. The rewarding value of a drug can be determined by the extent that it is self-administered.

Sensitization – The progressive increase in the effect of a drug with repeated exposure to the same dose of the drug.

This article begins with brief descriptions of the reward system, followed by how the stress system and reward system interact. Finally, the article concludes with a brief discussion of sex differences in stress and reward.

Reward

A ‘reward’ is defined as something that an animal or individual will work to receive. Some things are naturally rewarding, which means that these items or events have some intrinsic value (e.g., food or sexual activity); other things become rewarding through repeated pairing with natural reinforcers (e.g., money). A ‘reinforcer’ is any natural reward or conditioned stimulus that increases the probability that a particular behavior will be repeated, when the reinforcer is associated with that behavior. With all rewards, an animal must first learn that something is

rewarding. Food preferences are learned by eating the food, or in some species through interaction with an individual that has eaten a novel food. Sexual experience results in changes in motivational and consummatory aspects of sexual behavior in both males and females. Other stimuli acquire rewarding properties by association with external or internal primary reinforcers.

Our understanding of the neural basis of reward began over 50 years ago when Olds and Milner reported that rats would press a bar to receive electrical stimulation in certain areas of the brain. From these pioneering studies, it was concluded that there are ‘rewarding’ areas in the brain or ‘pleasure centers’ of the brain. With subsequent research it became generally accepted that the reinforcing properties of intracranial self-stimulation, drugs of abuse, and natural rewards are mediated by activation of the ascending dopamine (DA) pathway, and in the absence of DA these things are not rewarding. Nevertheless, it is clear that DA is not the only mediator of reward. As summarized in the 2007 work of Becker and Meisel, “In general, activation of dopamine release in the nucleus accumbens is an internal cue that tells an animal that something desired is available (or is soon to be available). We conclude, as others have, that this dopamine signal is associated with enhanced incentive salience ... of the desired stimulus. Glutamate and GABA neurons modulate dopamine release, providing input related to context and motivational state. GABA and endogenous opioids also provide information about the hedonic value of the stimulus.” This is generally true for food reward and drugs of abuse, although there are subtle differences.

Stress Hormones Regulate Reward Systems

As discussed previously, exposure to stimuli that threaten homeostasis (i.e., stressors) activates autonomic, behavioral, and neuroendocrine systems that permit the organism to respond to the threat. The hypothalamic–pituitary–adrenal (HPA) axis is exquisitely responsive to stress exposure and its activation is critical for the response to the current threat. Stressors induce activation of neural systems that result in the release of corticotropin-releasing hormone (CRH) and other stress-related peptides, which result in the release of corticosterone (CORT) from the adrenal cortex. In the brain, CORT and CRH influence anxiety-related behaviors, memory

and other cognitive functions, and the effect of drugs of abuse on subsequent drug-taking behavior.

Of particular interest to the reward system, stress can activate or inhibit cocaine- and amphetamine-regulated transcript (CART) and orexin (ORX). These peptides act in the amygdala, ventral tegmental area (VTA), and nucleus accumbens (NAcc), among other brain regions, where they modulate the response of the stress system to subsequent challenges (i.e., habituation and/or sensitization) and interact with the reward system. Conversely, drugs of abuse are stressful and induce an increase in the secretion of stress hormones and related neuropeptides. A schematic diagram of how the stress system and reward system interact is presented in **Figure 1**.

As mentioned above, when stressful stimuli activate the HPA axis a number of behaviors are affected, including anxiety-related behaviors, memory and other cognitive functions, drug-taking behavior, and feeding behavior. For the purposes of this review, we focus on drug-taking behavior as a model behavior for discussing interactions among stress and reward.

Stress and Drug Abuse

In humans and nonhumans, it is widely accepted that the rewarding aspect of drugs of abuse are due to the ability of these drugs to activate the neural system characterized in **Figure 1** as the ‘reward’ system. The ascending DA system and its targets are part of a motivational system that regulates responses to natural reinforcers (e.g., food, drink, and sex) and drugs of abuse. Importantly, activity in the ascending DA system is also modulated by the neural responses to stressors, and the effect of drugs of abuse and the neural stress response interact in a way that can influence the initiation of drug-taking behavior.

Animals and many humans will rapidly learn to self-administer psychomotor stimulant drugs (e.g., cocaine, amphetamine (AMPH), and methylphenidate) and opiates, at least in part due to their ability to increase DA in the NAcc. It is also apparent from animal models of drug abuse that the neural stress response influences the reinforcing effects of these drugs of abuse. This is due to both acute and long-term effects of drugs of abuse on the stress axis. First, drugs of abuse induce activation of the HPA axis to induce an increase in CORT release. CORT, in

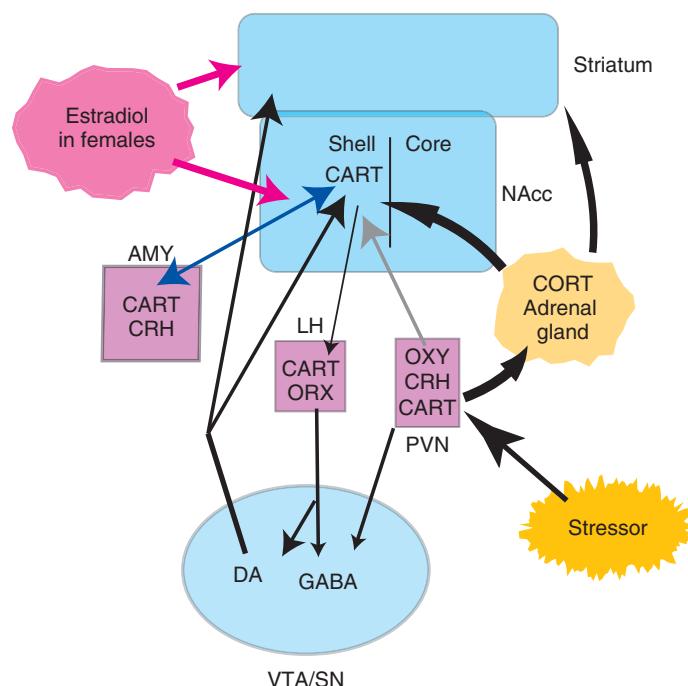


Figure 1 Interactions between the reward system and the stress system. The stress system interacts with the mesolimbic DA systems through input from the PVN to the NAcc shell and from the AMY to the NAcc shell, as well as through the release of CORT that feeds back to the NAcc where it enhances CART and DA release, and to the striatum where DA release is augmented. CRH and OXY also act in the VTA to enhance DA release. CART-containing neuronal terminals are found in the VTA and to a lesser extent in the substantia nigra (SN), and many CART-containing cells and processes are found in the NAcc shell. Most CART input to the VTA/SN derive from neurons in LH where CART may play a role in integrating food and drug reward through stimulation of DA neurons directly or indirectly through inhibition of GABA neurons. In the NAcc, DA terminals are found on CART neurons (GABAergic medium-spiny output neurons), suggesting that DA also influences CART. CART synapses in the NAcc indicating that CART can influence NAcc output. In addition, estradiol in females acts in the striatum and NAcc to enhance stimulated DA release. There are also sex differences in the stress system as described in the text, resulting in greater CORT in females under basal conditions and in response to stress.

turn, acts in the NAcc and the striatum at glucocorticoid receptors to influence neuronal activity and drug-taking behavior. With repeated exposure to the drug, there are long-term changes both in the neural system mediating the reinforcing effects of the drug and in the stress system.

The animal models used as behavioral indices of the neural changes associated with drug abuse that are used most frequently are ‘sensitization’ and drug ‘self-administration.’ Sensitization is the progressive increase in the effect of a drug with repeated exposure to the same dose of the drug. In other words, the more doses of a drug an individual is exposed to, the greater is the behavioral response to the drug – the individual has become ‘sensitized’ to the drug. The neural processes associated with sensitization are thought to be related to the neural basis of drug craving, or the desire for a drug that persists long after dependence has subsided. Drug self-administration in animals is a task where animals have a chronic intravenous catheter and they can learn to inject a dose of a drug into their veins by pressing a bar or poking their nose into a hole in the chamber wall. Both, self-administration of drugs of abuse and sensitization induced by repeated drug treatment, are modulated by the increase in glucocorticoid levels induced by drug treatment.

Animals that cannot mount a stress response due to removal of the adrenal gland neither learn to self-administer drugs nor do they show behavioral sensitization; conversely, CORT enhances acquisition of cocaine self-administration. If endogenous CORT is elevated in male rats or mice due to repeated stressful encounters with a dominant male prior to self-administration (i.e., social defeat), they will acquire self-administration of cocaine more rapidly and take more cocaine. Animals will also exhibit greater behavioral sensitization to psychomotor stimulants following the stress of social defeat. On the other hand, administration of a glucocorticoid receptor antagonist attenuates the motivation to self-administer cocaine. These findings, and others, support the idea that one way in which drugs of abuse induce neural changes that result in addiction, in susceptible individuals, is through activation of the stress axis and the glucocorticoid receptors in particular. Drug-taking behavior also results in long-term changes in the stress response. For example, prior cocaine self-administration enhances the effect of restraint stress on increases in plasma CORT. Thus, exposure to drugs of abuse enhances the HPA response to stressful stimuli, and prior stress enhances drug-taking behavior.

The response to stress may also impact individual differences in the response to drugs of abuse. When experimentally naive rats are exposed to the mild stress of a novel environment, some rats exhibit high rates of exploratory locomotion, and these animals are known as high responders (HRs); other rats, known as low responders (LRs), exhibit low rates of locomotor activity in a

novel environment. In addition, HR rats will seek out a novel and varied environment over an environment to which the rats have become habituated. Importantly, the rate of novelty-stress-induced locomotion predicts the subsequent behavioral responses of these animals to drugs such as AMPH and cocaine. HR rats exhibit higher rates of AMPH- and cocaine-induced locomotor activity, and will self-administer these drugs at lower doses than will LR rats.

HR rats have a prolonged CORT response after exposure to a novel context compared with LR rats. On the other hand, administration of exogenous glucocorticoid induces LR animals to self-administer AMPH, but reduces self-administration in HR animals. Similarly, social defeat enhances acquisition of self-administration of cocaine in LR rats, but reduces drug taking in HR rats. These results suggest that there is an optimal amount of CORT that will enhance drug-taking behavior. Since HR rats have a greater endogenous increase in CORT than do LR rats, when CORT is enhanced through either investigator administration or social stress, the HR rats are exposed to excessive CORT and this inhibits drug taking. The LR rats, with a more ‘optimal’ increase in CORT after prior stress or exogenous CORT, experience enhanced drug taking. This effect may be mediated by a direct effect of glucocorticoids on the striatum and NAcc, or through an enhanced effect of the drugs to induce an increase in DA, or both. Thus, these and other findings support the idea that one way in which drugs of abuse induce neural changes that result in drug abuse, in susceptible individuals, is through activation of the stress axis.

Stress and Drug Addiction

Maintenance of compulsive drug-taking behavior, or ‘addiction,’ in humans and nonhuman animals is thought to be due to long-term changes in the brain that are induced as a consequence of exposure to drugs of abuse. Induction of these long-term changes is another way in which the stress axis and reward system interact. In the paraventricular nucleus (PVN), CRH-containing neurons are known to project to the NAcc and VTA where they regulate the activity of dopaminergic and γ -aminobutyric acid- (GABA-)ergic neurons, and this CRH activity is thought to impact drug-taking behavior in addicted individuals. In animal models, as a consequence of repeated drug taking on an extended access schedule, a pattern of drug taking that might be seen with a cocaine addict, the central CRH system (CRH neurons projecting to the VTA and NAcc shell) is hypothesized to be overactive. This overactivity is thought to contribute to compulsive drug-taking behavior and addiction; hence, when a CRH-1 receptor antagonist is given to an animal that is already self-administering cocaine, the intake of cocaine is

reduced in a dose-dependent manner. This can be seen with a number of schedules of reinforcement, but is seen most dramatically with animals that are avidly taking drugs in over a period of 3–6 h day⁻¹ or animals on a progressive ratio schedule (where each dose of the drug requires the animal to work harder for the next drug dose).

CART, co-localized with glutamate in a subpopulation of lateral hypothalamus (LH) neurons, is another potential regulator of both the stress response and drug-taking behavior. CART protein is increased by cocaine and AMPH in the shell of the NAcc, the PVN, the LH, and the central nucleus of the amygdala (ceAMY) as well as in a number of other brain regions and the sympathetic nervous system. With chronic activation, CART in the NAcc (and perhaps in other brain regions) is thought to serve as a compensatory agent to downregulate changes in the NAcc associated with drug taking. In the VTA, CART stimulates DA neurons and inhibits GABA neuronal activity. Thus, activation of CART may help to decrease susceptibility to addiction.

Finally, ORX co-localized with glutamate in neurons in the LH, which project to the VTA, also plays a role in mediating the interface between stress and drug abuse and, in particular, the neuro-adaptations associated with compulsive drug-taking behavior. CRH stimulates the release of ORX, and this neural circuit is thought to be critical for the induction of sensitization to cocaine, activation of the reward circuitry, and drug seeking during withdrawal. Thus, convergence of stress and reward interactions occurs at the mesolimbic DA neurons at the level of the cell bodies and terminals, for acute and long-term adaptation to repeated drug exposure.

Sex Differences in Stress and Reward

Sex Differences in Drug Abuse in Humans

Adult men are 2–3 times more likely than women to have a drug abuse/dependence disorder; however, this current gender difference is thought to reflect historical differences in opportunity, rather than sex differences in vulnerability to drug abuse. The male predominance in drug use may be waning, as data collected in the past 5 years in the USA indicate that among males and females of high school age, there is no longer a sex difference in drug-taking behavior. Among individuals who use drugs, more females are using cocaine and other psychomotor stimulants than males. There are also sex differences in the rate of escalation of drug use, with women tending to increase their rate of consumption of alcohol, marijuana, opioids, and cocaine more rapidly than do men. The sex differences in the psychomotor stimulants are the best studied, and women begin using cocaine or AMPH earlier in life and enter treatment at earlier ages than men.

Women also have more severe cocaine use at intake and more severe dependence than men. Furthermore, once addicted to a drug, women can find it more difficult to quit than men do.

Animal Models of Sex Differences in Drug Use

Basic research on the role of sex and ovarian hormones in the neurochemical and behavioral responses to acute and repeated exposure to drugs of abuse also finds sex differences and may provide insight into the biological causes of sex differences in drug abuse. The acute behavioral response to psychomotor stimulants in rodents can reflect both the sex differences and the modulation role of gonadal hormones in males and females. Research on rodents and humans indicates that the behavioral effects of drugs of abuse and, the psychomotor stimulants, in particular, are both sexually dimorphic and modulated by the gonadal steroid hormones.

Sex differences have been reported during all phases of the addiction process as assessed using various self-administration paradigms. When a low dose of drug is used, intact or ovariectomized (OVX) female rats acquire cocaine self-administration at a faster rate than do intact or castrated (CAST) males. Estradiol treatment enhances acquisition of cocaine self-administration in OVX female rats, but not in males; and the estradiol antagonist, tamoxifen, when given to intact females, inhibits acquisition. Therefore, there are inherent sex differences independent of circulating gonadal hormones in the acquisition of cocaine self-administration, with females being more vulnerable than males. Furthermore, estradiol enhances acquisition in females, but not in males.

Once animals are reliably taking cocaine, if female rats are given a choice between two doses of cocaine, females in estrus prefer higher doses of cocaine compared with females in other phases of the estrous cycle or male rats. If rats are housed in self-administration chambers 24 h a day and then given access to drug in limited times during the day, female rats 'binge' for a longer initial period of time, take more cocaine over a 7-day access period, and show a greater loss of diurnal control over cocaine intake than do males. Furthermore, estradiol treatment increases the initial binge length and the total amount of cocaine self-administered. These results show that estradiol influences both cocaine self-administration under high access conditions and the motivation to take cocaine.

The effects of estradiol on the motivation to take cocaine has also been demonstrated when animals are responding to low doses of cocaine under a schedule in which the number of responses required to obtain a cocaine infusion progressively increases. Under this 'progressive ratio schedule,' intact female rats reach much higher final ratios than do males, indicating that females are more motivated to obtain cocaine. Females also

worked harder for access to cocaine during the phase of the estrous cycle when estradiol was elevated. When estradiol treatment is given to OVX rats, it enhances responding on a progressive ratio schedule at some doses of cocaine. Thus, there are sex differences in the motivation to take cocaine, and 'estradiol enhances the motivation' to take cocaine. Sex differences have also been observed under reinstatement testing conditions designed to parallel relapse in humans, with females exhibiting a greater susceptibility to reinstatement of responding for cocaine after prolonged abstinence.

Sex Differences in Stress and Drug Abuse

In rodents and humans, there are sex differences in activation of the HPA axis that can play a role in behavior and physiology. For example, in female rats, both basal and stress-induced CORT secretions are more pronounced than in males. Furthermore, HPA secretions vary across the estrous cycle in the rat. Females in proestrus or estrus show elevated basal and stress-induced CORT concentrations relative to diestrous animals. Similarly, women show greater adrenocorticotropic hormone (ACTH) and cortisol responses in the luteal phase (when estradiol and progesterone are elevated) compared with the follicular phase (when estradiol is slowly rising). Females are also less sensitive to suppression of the HPA axis by the synthetic glucocorticoid, dexamethasone, than are males. Sex differences and ovarian hormones also affect the central neural circuits regulating neuroendocrine and behavioral stress responses, as indicated by stress-induced c-fos messenger RNA (mRNA) expression. Proestrous and estrous females show attenuated cortical and hippocampal induction of c-fos relative to males or diestrous females, while luteal-phase women demonstrate impaired glucocorticoid feedback relative to the follicular phase. In males, testosterone attenuates stress-induced CORT and ACTH secretion, and inhibits CRH gene expression in PVN neurons controlling the HPA-axis response. If the HPA axis is enhancing the response to drugs of abuse, establishment of drug-taking behavior, and subsequent neural changes resulting in addiction, sex differences in the HPA axis may play a role in the enhanced susceptibility of females to drug abuse.

Finally, there is greater stress-induced activation of CART in the PVN of female rats, compared to males. CART mRNA expression in the NAcc is sexually dimorphic, with males having higher basal expression than estradiol-treated females. On the other hand, females have higher basal expression of ORX precursor expression in the hypothalamus than male rats. Therefore, if CART decreases drug-taking behavior and ORX enhances drug-seeking behavior, as previously discussed, these hormones may also play a role in sex differences in drug abuse. One could postulate that females exhibit less

suppression of drug-associated behavior during initial drug exposure due to lower CART activity. If females also have greater basal ORX activity, the lower CART response coupled with enhanced ORX may underlie the more rapid escalation of drug-taking behavior in females and lead to the more rapid onset of addiction in females compared to males. This intriguing possibility needs to be explored further.

Summary

This brief discussion has shown how the neural systems mediating stress and reward are intimately intertwined. Agents that activate the reward system, such as drugs of abuse, also activate the neural stress systems. Activation of the stress system can be necessary for some of the reinforcing effects of drugs of abuse. Conversely, the stress system can also inhibit the reward system. Sex differences and individual differences in the stress system and the reward system affect how they interact. For example, females have a greater stress response and are more likely to acquire self-administration of drugs of abuse, with more rapid escalation of drug-taking behavior. In addition, long-term changes in both the stress and reward systems that occur during repeated exposure to drugs of abuse result in sensitization of each neural system and alter how the two systems interact in a sexually dimorphic way.

See also: Cellular Plasticity in Cocaine and Alcohol Addiction; Drug Addiction; Drug Sensitization and Drug Abuse; Drug Withdrawal – Motivational View; Molecular Neurobiology of Addiction; Motivation; Psychostimulants; Stress and Drug Craving; Transition to Addiction; Vulnerability Factors in Addiction Disorders.

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Relevant Websites

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Δ 9-THC

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Glossary

Catalepsy – Nervous condition characterized by muscular rigidity and fixity of posture irrespective of external stimuli, as well as decreased sensitivity to pain.

Endocannabinoids – Endogenous agonists of cannabinoid receptors. Endocannabinoids are derived from long-chain polyunsaturated fatty acids. The family of endogenous agonists for CB₁ receptors is larger than initially thought. The first-discovered and best-studied endocannabinoids are anandamide (*N*-arachidonoyl-ethanolamine) and 2-arachidonoyl-glycerol (2-AG). Newly proposed endocannabinoids are 2-arachidonylglyceral ether (noladin, 2-AGE), O-arachidonoyl-ethanolamine (virodhamine), and *N*-arachidonoyldopamine (NADA). The physiological functions for these compounds have not yet been established.

Hemopressin was recently identified as an endogenous CB₁ receptor antagonist.

G-protein-coupled receptors (GPCRs) – A large protein family of transmembrane receptors that sense molecules outside the cell and activate intracellular signal-transduction pathways and, ultimately, cellular responses. Most processes of signal transduction involve ordered sequences of biochemical reactions inside the cell, which are carried out by enzymes, activated by second messengers, resulting in a signal-transduction pathway. Such processes are usually rapid, lasting on the order of milliseconds in the case of ion flux, or minutes for the activation of protein- and lipid-mediated kinase cascades.

GTPgammaS (GTP γ S, guanosine 5'-O-[gamma-thio]triphosphate) – Nonhydrolyzable G-protein-activating analog of guanosine triphosphate (GTP). Its 35-S-labeled radioligand [³⁵S]GTP γ S is used to evaluate the functional activity of G-protein-coupled receptors.

Immunohistochemistry – The process of localizing proteins in cells using the principle of antibody binding to specific antigens in biological tissues. For immunohistochemical evaluation of receptor localization, an antibody is developed that selectively binds to the C- or N-terminal regions of the receptor. The antibody is typically tagged with a fluorophore and, thus, the antibody distribution in tissue can be visualized by fluorescence analysis.

Nociception – The neural processes of encoding and processing noxious stimuli and is produced in the

peripheral and central nervous system by stimuli that have the potential to damage tissue. This activity is initiated by nociceptors (also called pain receptors) that can detect mechanical, thermal, or chemical stimuli. Once stimulated, a nociceptor transmits a signal along the spinal cord, to the brain. Nociception triggers a variety of autonomic responses and may also result in the experience of pain.

Orexigenic – Increasing or stimulating the appetite.

Psychoactive (or psychotropic) drugs – Chemical substances that act primarily upon the central nervous system to alter brain function, resulting in temporary changes in perception, mood, consciousness, and behavior. These drugs may be used recreationally – to purposefully alter one's consciousness – or therapeutically as medication. Because psychoactive substances bring about subjective changes in consciousness and mood that the user may find pleasant (e.g., euphoria) or advantageous (e.g., increased alertness), many psychoactive substances are abused, that is, used excessively, despite risks or negative consequences. With sustained use of some substances, physical dependence may develop, making the cycle of abuse even more difficult to interrupt.

Radioligand binding – Evaluates the distribution of a selective receptor ligand (agonist or antagonist) that has been tagged with a radioactive moiety such as tritium [³H]. Radioligand distribution is typically evaluated by apposing prepared brain slices with X-ray film that, thus, produces a density image of the radiotracer presence throughout the tissue.

Cannabis Δ9-THC Content, Use, and Pharmacokinetics

Among the 66 different types of cannabinoids found in extracts of the plant *Cannabis sativa*, three are known to be psychoactive: Δ9-tetrahydrocannabinol (Δ9-THC), Δ8-THC, and Δ9-tetrahydrocannabidiol. Of these, Δ9-THC is the principal psychoactive component with Δ8-THC and Δ9-tetrahydrocannabidiol being 50–70% and 25% as potent. Cannabis also contains non-psychotropic cannabinoid acids that are decarboxylated to Δ9-THC. Other constituents of the plant include

cannabidiol (CBD) and cannabinol that are nonpsychoactive and do not bind to CB₁ or CB₂ receptors.

The highest concentration of Δ9-THC in the cannabis plant is in the oil obtained from the leaves and flowers, with decreasing amounts found in the stems, roots, and seeds. Marijuana prepared from dried flowers and leaves has Δ9-THC concentrations ranging from 0.5% to 5%. Hashish, a dried sticky resin of the flowers from the female plant, can contain 15–20% Δ9-THC, while chemically extracted hash oil has the highest Δ9-THC content of any marijuana preparation (50–60%).

Cannabis leaves are typically smoked like a cigarette or in a pipe (often a water pipe, or ‘bong’), though it also produces psychotropic effects after oral administration (e.g., prepared in foods or as a tea). Humans very rarely inject cannabis extracts intravenously. Δ9-THC can be detected in plasma within seconds after inhalation of cannabis smoke with 10–40% bioavailability depending on the experience of the smoker. Peak plasma concentrations are reached 3–10 min after inhalation and are typically in the range of 70–160 ng ml⁻¹. Due to its lipophilic nature Δ9-THC and related compounds are rapidly cleared from blood and deposited in fatty tissues, with plasma levels falling <20 ng ml⁻¹ within 30–60 min after smoking. Bioavailability is lower following oral consumption (6–7%), and the rise and fall of plasma Δ9-THC levels are delayed with this route of administration. Δ9-THC accumulates in fatty tissues and accordingly the elimination half-life for Δ9-THC is estimated to be in the range of 20–60 h, with metabolites present for longer times (elimination half-life of 5–6 days). Elimination is faster in heavy long-term users as compared with non-users, though acid metabolites of Δ9-THC can be detected in urine for up to 27 days in long-term users.

Cannabinoid Receptors

Cannabinoid research has experienced major breakthroughs over the last 25 years since the discovery of receptors for Δ9-THC and related cannabinoid substances. The cannabinoid receptor type 1 (CB₁ receptor) was the initial cannabinoid receptor to be characterized. CB₁ receptor distribution was first mapped in rat brain through autoradiographic imaging of the radioligand [³H]CP-55,940 that binds with high affinity to CB₁ sites. Subsequently, the CB₁ receptor was cloned from rat cerebral cortex, then from the human brain and testis, and finally from the mouse brain. Based on this knowledge, antibodies were developed that target the C- or N-terminal regions of the receptor and these were used for immunohistochemical mapping studies. The overall pattern of CB₁ receptor distribution revealed by radioligand binding and immunohistochemistry is remarkably similar.

CB₁ receptors are G-protein-linked and couple to the inhibitory Gi/o class of G proteins. CB₁ receptors are among the most abundant G-protein-coupled receptors in the brain and are mainly localized to axons and nerve terminals and are largely absent from neuronal soma or dendrites. The predominant presynaptic localization is consistent with the CB₁ receptor influence on neurotransmitter release. Functional CB₁ receptors are also found in intracellular vesicles.

In both animals and humans CB₁ receptors are regionally expressed in brain with a pattern consistent with the behavioral effects produced by cannabis. CB₁ receptors are present in very high density in areas involved in motor coordination and function (cerebellum and basal ganglia) with dense expression also in areas involved in learning and memory (hippocampus), emotionality (amygdala), cognitive information processing (cerebral cortex, particularly frontal areas, etc.), reward and motivation (mesolimbic and mesostriatal areas), and food intake (hypothalamus and limbic system). CB₁ receptors in the brainstem and spinal cord may participate in nociception, though the relatively low expression of CB₁ receptors in these regions may account for the low levels of lethality with cannabinoids. The distribution of cannabinoid-activated G proteins (as measured by [³⁵S]GTPγS autoradiography) generally parallels CB₁ receptor-expression density, although evidence suggests receptor density does not necessarily predict receptor signaling efficiency. CB₁ receptor expression has also been detected in astrocytes and oligodendrocytes, and glial cells have been shown to produce and release endogenous cannabinoid compounds.

CB₂ receptors are expressed in the spleen, tonsils, and immune cells (B cells, monocytes, and T cells) indicating a role in immune function. The extent of CB₂ expression in the brain has been controversial as initial studies failed to find appreciable levels of CB₂ receptor expression in the brain tissue. However, CB₂ receptor mRNA expression and immunoreactivity have been shown in glial and neuronal cells in a number of rat brain regions, and CB₂ receptor mRNA and protein has been found in rat, ferret, and mouse brainstem neurons. CB₂ receptors are also expressed in perivascular microglial cells of the human cerebellum. The functional influence of CB₂ receptors in the brain is presently unknown.

Studies on CB₁ and CB₂ receptor knock-out mice suggest there may be several additional cannabinoid receptors. For example, a variety of plant, synthetic, and endogenous cannabinoids bind to and activate the orphan G-protein-coupled receptor 55 (GPR55), which is expressed in the brain and various peripheral tissues in humans and rodents, leading to recent suggestions that GPR55 is a cannabinoid receptor. Endogenous cannabinoids such as anandamide can also produce effects through modulation of ion channels, an example of

which is the transient receptor potential vanilloid type 1 (TRPV1) receptor. However, these ion channels are generally not considered cannabinoid receptors, *per se*.

Endogenous Cannabinoids

In the early 1990s, two derivatives of arachidonic acid were identified and characterized as being endogenous ligands for cannabinoid receptors. These substances are *N*-arachidonylethanolamide (AEA; anandamide) and 2-arachidonoyl glycerol (2-AG). Subsequently, other possible endocannabinoids have been proposed such as noladin ether (arachidonyl glycerol ether), viroldhamine, and *N*-arachidonoyl-dopamine (NADA), although the natural occurrence and functional influence of these other compounds remains unclear. AEA and 2-AG are characterized by different structures, different biosynthetic and degradation pathways, and somewhat distinct pharmacological effects (although similar to Δ9-THC, both AEA and 2-AG exert agonist effects at CB₁ receptors). Endocannabinoids are lipophilic neuromodulators and are not stored in synaptic vesicles, but rather appear to be synthesized primarily on an 'as needed' basis through increased neuronal activity. The pharmacology of these endogenous ligands is similar to Δ9-THC, though clear differences in the behavioral effects of endocannabinoids and synthetic or plant-derived cannabinoids have been documented.

The endogenous cannabinoid system exerts prominent modulatory effects on a variety of behaviors and may be involved in a number of movement disorders, psychiatric disorders, problem eating and obesity, and addictive disorders. Despite the obvious overlap in between plant-derived, synthetic, and endogenous cannabinoid substances the present article concentrates on the behavioral effects produced by Δ9-THC.

Behavioral Effects of Δ9-THC

In view of the widespread presence of cannabinoid receptors and their ligands, it is not surprising that cannabinoid signaling can modify virtually every mode of behavior. Indeed, no single behavioral parameter is specifically influenced by cannabinoids. The effects of cannabis and cannabinoid receptor ligands on behavior and cognition in animals and humans are reviewed below and a summary of these findings can be found in Table 1.

In the early 1990s, Billy Martin and colleagues developed a battery of behavioral tests as an *in vivo* assay for evaluating cannabimimetic effects. Collectively, these procedures were shown to provide a sensitive index of a drug affinity and efficacy at centrally located cannabinoid receptors. The full battery of behavioral tests includes a

Table 1 Similar effects of cannabis/Δ9-THC in rodents and humans

Rodents	Humans
Analgesia	Analgesia
Hyperphasia	Increased appetite
Distorted sense of time	Distorted sense of time
Reward/aversion	Euphoria/dysphoria
- Voluntary self-administration	- Voluntary self-administration
- Anxiolysis (low dose)	- Anxiolysis (low dose)
- Anxiogenesis (high dose)	- Anxiogenesis (high dose)
- Place preference (low dose)	
- Place aversion (high dose)	
- Increased sensitivity to brain stimulation reward	
Withdrawal	Withdrawal
- Decreased sensitivity to brain stimulation reward	- Depression
- Somatic symptoms (scratching, ataxia, and ptosis)	- Irritability
	- Anxiety
	- Decreased appetite
	- Sleep disturbances

fourfold behavioral evaluation in mice (the 'tetrad'), drug discrimination and catalepsy test in rats, static ataxia tests in dogs, and an evaluation of operant suppression in monkeys. For practical reasons, however, most evaluations use only parts of the battery, and many studies simply employ the mouse tetrad.

The Mouse Tetrad Test

The mouse tetrad consists of four simple evaluations, which may be measured in sequence in the same animal. As discussed below, the four evaluations are of spontaneous motor activity, catalepsy, hypothermia, and analgesia. Δ9-THC and synthetic cannabinoid agonists such as WIN 55,212-2 and CP55,940 produce characteristic disruptions in each of these measures and the effects of each of these ligands are reversed by the CB₁ antagonist Rimonabant (SR141716A), providing good evidence of CB₁-related mechanisms. Endogenous cannabinoids such as AEA and 2-AG induce effects similar to Δ9-THC though some differences have been documented. The most notable difference is that AEA-induced hypothermia, analgesia, and catalepsy are not reversed by SR141716A administration.

The effects of Δ9-THC and related cannabinoids on 'spontaneous activity' (or hypomotility) are determined in an open field. Behavior is typically monitored for a 10-min period, and is performed either by manual observation or by digital scanning.

'Catalepsy' is assessed either through the ring-immobility test or the bar test. The ring-immobility test was developed by Pertwee and colleagues, and measures the amount of time in which the mouse is immobile after placement on a 5.5 cm metal ring fixed 16 cm above a table top. The criterion of immobility is defined as the absence of all voluntary movements, including whisker movement (but excluding respiration). In the bar test, a metal bar is fixed horizontally above a cage floor. The animal's forepaws are placed on the bar and the length of time during which the mouse maintains the initial position is measured.

Hypothermia is typically measured by a telemeter.

The fourth assay in the tetrad is analgesia. Systemically administered Δ9-THC and synthetic cannabinoids have antinociceptive and antihyperalgesic effects in a variety of animal models of acute and inflammatory pain. Δ9-THC produces antinociceptive effects both in phasic (e.g., tail-flick and hot-plate tests) and tonic (e.g., stretching) nociceptive tests. Substantial evidence suggests that the antinociceptive effects of Δ9-THC are mediated, at least in part, through activation of CB₁ receptors which are present in central nervous system (CNS) sites associated with the transmission and processing of nociceptive information (spinal cord, thalamus, periaqueductal gray, rostroventromedial medulla, dorsal-root ganglia, afferent-fiber terminals, etc.). For example, the analgesic effects of Δ9-THC and many synthetic CB₁ agonists are prevented by the CB₁ receptor antagonist SR141716A. In addition, the antinociceptive response to Δ9-THC is largely reduced in CB₁ receptor knock-out mice. However, some studies have found that Δ9-THC continues to exert some antinociceptive actions in CB₁ knock-out mice tested in the hot-plate and formalin tests. This suggests that Δ9-THC-induced analgesia is not mediated exclusively by CB₁ receptors.

There is evidence for an interaction between cannabinoid and opioid mechanisms in analgesia. Δ9-THC and morphine have been found to act synergistically in tests of acute and chronic inflammatory pain, and this synergism can be blocked by either CB₁ (SR141716A) or opioid (naloxone) antagonists. Intrathecal Δ9-THC administration can induce the release of endogenous opioids (most likely dynorphin) that stimulate both δ- and κ-opioid receptors. Moreover, subchronic Δ9-THC administration increases both prodynorphin and proenkephalin gene expression in rat spinal cord, and Δ9-THC-induced analgesia is attenuated in prodynorphin knock-out mice. Interestingly, co-administration of low-dose Δ9-THC has been reported to prevent tolerance to opioid-induced analgesia, suggesting that low-dose Δ9-THC combination therapy may reduce the need to escalate opioid doses for maintenance of analgesia.

Feeding and Appetite

Cannabis is well known to stimulate appetite even in previously sated human subjects, and laboratory studies since the 1970s have demonstrated that Δ9-THC stimulates feeding in a variety of animal models. The hyperphagic effect of Δ9-THC in rats is remarkably potent and can induce overconsumption even in animals that are sated. The effect of cannabinoids appears to be selective for high-fat or sweet-high-fat diets, and in laboratory animals cannabinoid-induced increases in food intake are not observed in animals given only standard rat chow. The orexigenic effects of Δ9-THC and synthetic and endogenous cannabinoids are mediated through CB₁ receptors as demonstrated by the ability of the CB₁ receptor antagonist Rimonabant (SR141716A) to block cannabinoid-induced food consumption. Under certain circumstances, administration of the antagonist itself can reduce appetite and body weight. A commonality among hypotheses of cannabinoid-induced feeding is that CB₁ receptor stimulation increases sensitivity to the sensory properties of foods, thereby increasing the salience or incentive value of food.

Cannabinoid effects on feeding behavior are likely mediated through mechanisms in both the central and peripheral nervous systems. In the CNS, the hypothalamus has long been recognized to play a key role in feeding and weight regulation. Intra-hypothalamic cannabinoid-agonist administration induces eating, and cannabinoid activity in the hypothalamus varies as a function of changes in nutritional status and the expression of feeding behaviors. Further, rodents genetically prone toward obesity (e.g., Zucker rats, ob/ob and db/db mice) are characterized by defective leptin signaling (leptin is an appetite-suppressing hormone) and increased endocannabinoid levels in the hypothalamus, and treatment of normal rodents with leptin decreases hypothalamic endocannabinoid levels. CB₁ receptors are colocalized with appetite-regulating hormones such as cocaine–amphetamine-regulated transcript (CART), melanin-concentrating hormone (MCH), and corticotropin-releasing factor (CRF) in the paraventricular nucleus (PVN) of the hypothalamus. There is also evidence of functional interactions between cannabinoids and the orexigenic peptide orexin A, most likely through interactions between the hypothalamic CB₁ and orexin OX₁ receptors. Elements of the limbic forebrain also play an important role in the motivational effects of food consumption, and the shell subregion of the nucleus accumbens is another highly sensitive site for cannabinoid-induced feeding behavior. Finally, recent evidence suggests an important functional interaction between the endogenous cannabinoid and opioid systems in the modulation of the rewarding properties of food. For example, the hyperphagic effects of Δ9-THC are reversed by the nonselective opioid-receptor antagonist

naloxone. Moreover, behaviorally inactive subthreshold doses of naloxone and Rimonabant produce a profound synergistic anorectic action. CB₁ and opioid receptors are colocalized in a number of regions involved in the regulation of feeding, most notably the PVN of the hypothalamus, and increased feeding induced by intra-PVN morphine administration is reversed by Rimonabant.

Cannabis Intoxication, Reward, and Dependence

In humans, Δ9-THC produces behavioral effects characteristic of both sedative-hypnotic and psychedelic drugs. Subjective accounts of cannabis intoxication in humans vary considerably, depending on the dose of the drug, environment, history of use, and expectations of the user. The high is characterized by enhanced mental associations, a sharpened sense of humor, a distorted sense of time, increased appetite, and a relaxed, calm, dreamlike state. Users typically note anxiolytic effects of marijuana, although increased anxiety is not uncommon. Psychedelic-like perceptual effects are sometimes noted following high-dose administration, along with decreased motivation and impaired cognitive functioning. This collection of acute behavioral effects produces a complex state most often characterized as euphoric, and this is believed to be a primary motivating factor for repeated drug use. Evidence for positive reinforcing effects of Δ9-THC in laboratory animals has been produced using a variety of behavioral paradigms including drug discrimination, brain-stimulation reward, conditioned place preference, and intravenous self-administration.

Discriminative stimulus effects

Drug discrimination procedures in animals evaluate drug-induced interoceptive stimulus conditions (or subjective effects) perceived by the subject in the context of interoceptive stimulus effects produced by other drugs in similar or different drug classes. Animals are typically trained to lever press for food reinforcement using a two-lever operant chamber. During training, food reinforcements are obtained by responding at one of the levers in sessions following drug administration, while reinforcers are earned by responding at the other lever in sessions following vehicle administration. In subsequent tests, the animals receive either the training drug at a different dose or a different drug, and lever selection is monitored as an index of whether the pretreatment produces a discriminative stimulus effect similar to the training drug.

Cannabinoid drugs show a pharmacological specificity in the drug-discrimination procedure. Only drugs that activate CB₁ receptors fully generalize to the THC-training stimulus in animals trained to discriminate Δ9-THC from saline. Similarly, training-drug-appropriate responding is produced

by Δ9-THC administration in animals trained to discriminate synthetic CB₁ agonists (such as WIN 55,212-2 and CP 55,940) from saline. The discriminative stimulus effects of Δ9-THC and synthetic CB₁ agonists can be competitively blocked by pretreatment with the CB₁ receptor antagonist SR141716A (Rimonabant), further demonstrating that cannabinoid discrimination is mediated by CB₁ receptors. No cross-generalization has been observed with a wide variety of drugs from different classes including opioids, psychostimulants, psychedelics, antipsychotics, and anticonvulsants. Partial generalization to Δ9-THC has been observed with diazepam, phencyclidine (PCP), and methylenedioxymethamphetamine (MDMA). However, the partial generalization of diazepam to Δ9-THC is not blocked by SR141716A and from this it was suggested that the generalization is mediated by non-CB₁ modulation of γ-aminobutyric acid (GABA-ergic) signaling. In general, the observations made in the drug-discrimination paradigm indicate that the interoceptive effects of Δ9-THC are mediated through CB₁ receptor activation.

Brain-stimulation reward

In 1954, Olds and Milner reported that rats will repeatedly lever-press to receive an electrical stimulation of the medial forebrain bundle, which is a component of the brain reward circuit. Subsequent work has shown that the sensitivity to brain stimulation (as indexed by the threshold level of stimulation frequency or current delivery that produces positive reinforcing effects) can be used to evaluate the function of the reward circuit. For example, most major drugs of abuse dose-dependently decrease the threshold for brain-stimulation reward (e.g., enhance the function of the reward circuit). A limited number of studies have evaluated cannabinoid-induced alterations in brain-stimulation-reward thresholds, and the results have not been entirely consistent. Low Δ9-THC doses (1–1.5 mg kg⁻¹) decrease brain-stimulation-reward thresholds in Sprague Dawley (SD) and Lewis rats, though this effect is not observed in Fisher rats. Moreover, in Lewis rats the synthetic CB₁ receptor agonist CP 55,940 does not alter stimulation-reward thresholds at doses comparable to Δ9-THC doses that reduce thresholds. Interestingly, brain-stimulation-reward thresholds are elevated during withdrawal from a single Δ9-THC administration suggesting a withdrawal-associated deficit in the function of the reward circuit.

Conditioned place preference

The conditioned place-preference paradigm is a classical conditioning procedure that evaluates drug-related reward/aversion effects. In the conditioning procedure, animals are confined to a distinct environment following drug administration or are confined to an adjacent compartment that differs in color, size, or texture following vehicle administration. Conditioning is produced through a number of treatment/environment-pairing sessions. On

the final test day, no injections are administered and the animal is allowed to freely explore both compartments. The relative time spent in the drug-paired versus saline-paired compartments is used as an index of the rewarding or aversive effects of the drug treatment.

Most drugs abused by humans increase the time spent in the drug-paired environment (i.e., produce a place preference) in a manner dependent on drug dose and the postadministration environmental conditioning period. Δ9-THC produces both place preference and place aversion in rats and mice, depending on the dose and experience of the animals. Place aversions are more commonly reported, and have been observed with acute administration of moderate to high Δ9-THC doses. Similar profiles have been observed with synthetic cannabinoid agonists such as CP 55,940, WIN 55,212-2, and HU 210. In general, place preferences are observed following low-dose Δ9-THC administration or following pre-exposure to the drug prior to environmental conditioning. Interestingly, two studies demonstrate that the CB₁ receptor antagonist SR141716A (Rimonabant) produces place preference, though an equal number of reports have found that this compound induces no significant place preference or aversion.

Self-administration

Nearly every drug that is abused by humans also maintains operant self-administration behavior by nonhuman subjects under controlled laboratory conditions. This has been repeatedly demonstrated for psychostimulants, opioids, nicotine, ethanol, and other abused substances. However, cannabinoids have long been considered an exception to the correspondence between drugs abused by humans and those that maintain operant self-administration in animals. Several different research groups have made unsuccessful attempts to demonstrate reliable, persistent, dose-related, and drug-specific self-administration of Δ9-THC or synthetic cannabinoid agonists by laboratory animals. Although early studies reported intravenous Δ9-THC self-administration by food-restricted rats, drug intake decreased to placebo levels when the food restriction was discontinued. Several reports have demonstrated the self-administration of synthetic CB₁ agonists such as WIN 55,212-2, CP 55,940, and HU-210 both in rats and mice, although in some cases chronic diet restriction was shown to be a necessary condition for these effects. Moreover, most of these rodent studies did not evaluate the persistent maintenance of drug-taking behavior over time.

Several studies from the Goldberg laboratory have demonstrated long-term stable operant Δ9-THC self-administration by squirrel monkeys that were not diet restricted. Initial experiments were performed in monkeys with a prior history of cocaine self-administration, though subsequent work demonstrated the initiation and

maintenance of intravenous Δ9-THC self-administration behavior in experimentally naive monkeys. Clinically relevant Δ9-THC doses were employed in these studies, and steps were taken to ensure complete solubilization of the drug in the self-administered solution. This is of import as early animal studies employed relatively high drug doses that were not fully solubilized in the self-administered solution. These drug suspensions were of low concentration and thus required large injection volumes delivered at slow infusion speeds, conditions that have been shown to adversely affect the reinforcing effects of a drug by intravenous administration.

Other factors may also underlie the difficulty in establishing operant cannabinoid self-administration in laboratory animals. It is well established that response-reward associations necessary for operant drug-taking behavior are facilitated by a rapid onset and dissipation of the drug's pharmacological effects. However, Δ9-THC and other cannabinoid agonists produce prolonged pharmacological and behavioral effects whose onset is delayed from initial administration, and this may preclude the necessary association between the operant task required for drug infusion and the resultant psychoactive effects of the drug. In addition, the apparent aversive effects of cannabinoids (as demonstrated in place-conditioning paradigms) and motor-depressant effects of these drugs may also confound the acquisition and maintenance of operant self-administration.

During the past 30 years, a substantial number of clinical studies have evaluated Δ9-THC self-administration by humans. When given a choice, experienced marijuana smokers prefer marijuana cigarettes with greater Δ9-THC content over those with lesser Δ9-THC content. Moreover, several (but not all) studies have provided evidence that experienced marijuana users titrate their smoking behavior according to the Δ9-THC content, and a Δ9-THC concentration-effect in the subjective ratings of intoxication has been shown in several studies.

Mechanisms for the rewarding effects of cannabinoids

The neuronal circuits known to be involved in mediating the rewarding aspects of most abused drugs originate with a subgroup of dopamine (DA) neurons located in the ventral tegmental area (VTA). These DA neurons possess axons that target GABAergic medium spiny neurons located in the nucleus accumbens (NAcc) that send reward-relevant information to the ventral pallidum (VP), back to the VTA and to other structures. The VTA, NAcc, and VP are interconnected via reciprocal axon collaterals that are critical for the performance of reward-relevant behaviors. These brain reward nuclei also receive glutamatergic and GABAergic inputs,

whose functional integrity is necessary to observe drug-related reward behavior.

Δ9-THC and several synthetic CB₁ receptor agonists increase the firing rate and burst activity of DA cell bodies in the VTA, likely through a CB₁ receptor-dependent inhibition of GABA release in the VTA (thereby diminishing inhibitory control over DA cell firing) and this is thought to contribute to the rewarding effects of Δ9-THC and other cannabinoid agonists. Increased NAcc DA levels are also believed to partially mediate the rewarding effects of several other classes of abused drugs.

It has become apparent that many abused drugs, including cannabinoids, also exert reward-relevant neurochemical effects that are not reliant on dopaminergic neurotransmission. For example, the CB₁ agonist WIN55,212-2 reduces GABA release onto NAcc medium spiny neurons, resulting in the disinhibition of GABAergic output to the VTA, VP, and other target structures. However, cannabinoids also inhibit glutamate release onto NAcc medium spiny neurons, which likely results in reduced activation of these GABAergic output neurons. The relative contribution of the CB₁ receptor modulation of GABAergic and glutamatergic synaptic transmission to the output of the NAcc is presently poorly understood and likely relies on the behavioral circumstances under which each system predominates.

Dependence and withdrawal

For many years, cannabis was not considered to be an addictive drug. This was based on difficulty in establishing cannabinoid self-administration by laboratory animals and the fact that abstinence from cannabis use was not associated with obvious physical withdrawal symptoms either in humans or animals. However, views on this subject have changed dramatically in recent years and it is now accepted that cannabis dependence is a clinically observable phenomenon, and that a substantial percentage of users meet the *Diagnostic and Statistical Manual*, Version-IV (DSM-IV) criteria for substance abuse and substance dependence. Marijuana appears to produce dependence less readily than most other illicit drugs and recent studies indicate that 9% of those who try marijuana eventually develop dependence on the drug, as compared with 15% of people who try cocaine and 24% of those who try heroin. Because cannabis use is much more common than the use of other illegal drugs, cannabis dependence is estimated to be twice as prevalent as is cocaine or heroin dependence.

Recent studies in humans have shown that a reliable and clinically significant withdrawal syndrome occurs in cannabis users during drug abstinence. Withdrawal symptoms are observed in a substantial percentage of heavy users (16%) and include craving for cannabis, decreased appetite, weight loss, sleep disturbances, restlessness, irritability, anxiety, anger, and aggression. The onset of

these symptoms typically occurs within 1–3 days of drug abstinence, with peak effects observed at 2–6 days' abstinence with most symptoms abating after 2 weeks of abstinence. However, some clinicians suggest that a protracted withdrawal period exists for up to 18 months that is characterized by intermittent cognitive deficits and sleep disturbances.

Cannabinoid withdrawal has also been characterized in rats and mice. In these studies, a withdrawal state is typically precipitated by the administration of a CB₁ receptor antagonist, which induces a behavioral syndrome that includes wet-dog shakes, scratching, face rubbing, arched back, ptosis, ataxia, and hunched posture. This withdrawal syndrome has been observed following repeated administration of not only Δ9-THC, but also synthetic CB₁ receptor agonists such as WIN 55,212-2 and CP 55,940. Although mild somatic withdrawal symptoms have been observed during abstinence from WIN 55,212-2, spontaneous somatic signs of withdrawal have not been observed during Δ9-THC abstinence. However, some motivational indications of Δ9-THC withdrawal have been reported such as elevations in brain-stimulation-reward thresholds.

Cognitive Function

Long-standing clinical evidence suggests that cannabis use can compromise cognitive or 'executive' functions. Executive functioning comprises cognitive processes such as attention, behavioral flexibility, decision making, inhibitory control, planning, time estimation, and working memory. High levels of CB₁ receptor expression are found in the human cerebral cortex, hippocampus, and basal ganglia. Heavy cannabis use by humans is associated with abnormal frontal cortical function and neurocognitive deficits including impairments in attention, inhibitory control, certain forms of impulsive behavior, decision making, and/or cognitive flexibility that are thought to be associated with altered prefrontal cortical function. Neuroimaging studies have found cannabis-induced alterations in the function of prefrontal (orbitofrontal) cortex, hippocampus, and components of the basal ganglia, and it has been proposed that neural dysfunction in these regions contributes to a loss of inhibitory control that propels continued cannabis use.

Attention

It has long been recognized that acute challenge or prolonged cannabis use impairs attentional processing in humans. Although there have not been many examinations of this issue using laboratory animals, a few recent preclinical studies are consistent with these long-standing observations in humans. For example, chronic intermittent treatment of laboratory rats with Δ9-THC impairs visuospatial attention in a lateralized-reaction-time task

and similar effects have been observed in this task following acute challenges with the CB₁ receptor agonist WIN 55,212-2. Effects of cannabinoids on attentional capacity have also been evaluated using the 5-choice serial-reaction-time task (5-CSRTT), which is a procedure that requires subjects to scan a horizontal array of five spatial apertures for the location of a very brief visual target stimulus. Animals are tested in a large number of discrete trials, thereby taxing attentional capacity. Decreased accuracy in the 5-CSRTT has been observed following a 2-week period of high-dose Δ9-THC administration, though performance on this task was not altered following acute administration of the synthetic CB₁ agonist WIN 55,212-2. The difficulty of the 5-CSRTT can be varied by altering the brightness, duration, or frequency of the target visual stimulus; the stimuli can be made temporally and spatially unpredictable (similar to a nonpaced version of the continuous-performance task); and distracting stimuli (e.g., bursts of white noise) can be introduced. These manipulations can increase the sensitivity of this task for detecting attentional deficits and this may be a useful approach for future work characterizing the effects of repeated Δ9-THC administration on attentional capacity.

Inhibitory control

Impulsivity and compulsive stereotyped behavior have emerged as potentially major contributing factors in drug dependence. Heavy cannabis use by humans is associated with abnormal frontal cortical function and certain forms of impulsive behavior thought to be associated with altered prefrontal cortical function. Acute challenges with Δ9-THC and marijuana have been shown to increase impulsive responding in the stop-signal task (e.g., impaired ability to inhibit ongoing behavior) and heavy cannabis users have been found to have specific deficits in the ability to balance rewards and punishments, as evidenced by their preference for immediate rewards despite the incurrence of costly losses in a gambling task. From these observations, it has been argued that cannabis users may choose immediate gratification in the form of continued drug use without careful consideration of negative consequences, thereby perpetuating abuse and dependence. In rodent tests, acute administration of the CB₁ agonist WIN 55,212-2 mildly impairs response inhibition in the stop-signal task although alterations in performance of the delayed-discounting task have not been observed following acute WIN 55,212-2 administration.

Time estimation

In humans, marijuana or Δ9-THC use reportedly makes time appear to pass more slowly. In laboratory tests, subjects typically overestimate the amount of elapsed time, or produce shorter-than-appropriate intervals when asked to signal a period of elapsed time. In laboratory animals, Δ9-THC and WIN 55,212-2 shortens the

response interval in rats trained to respond for food reward using a fixed-interval schedule and similar underestimations of time passage are observed in the time-discrimination and time-estimation procedures.

Working memory

It is well established in humans that acute and chronic intoxication with cannabis produces an impairment of short-term memory, particularly as assessed in tests that depend heavily on attention. Animal studies have also found that Δ9-THC and synthetic cannabinoids induce deficits in short-term memory as determined in delayed-matching and -nonmatching tests, and radial arm maze performance. It is likely that these effects are produced through cannabinoid-induced alterations in hippocampal function. CB₁ receptors are expressed in high densities in the hippocampus, and are positioned in a manner that allows a potent influence both on GABA and glutamate release in hippocampal circuits. Memory formation is thought to involve the processes of long-term potentiation (LTP) and long-term depression (LTD) at glutamatergic synapses in the hippocampus. Cannabinoids appear to inhibit the induction of both LTP and LTD by reducing glutamate release to below the level needed to activate N-methyl-D-aspartic acid (NMDA) receptors (a requirement for LTP and LTD). The CB₁ modulation of the function of hippocampal GABAergic interneurons may be to control oscillatory electrical activity at frequencies important for synchronizing pyramidal cell activity.

Behavioral flexibility

Adaptive behavior (e.g., flexibility) relies heavily on the ability to modify behavior in response to transient changes in the predictive relevance of environmental stimuli. This ability – variously termed strategy-shifting, behavioral flexibility, and attentional set-shifting – requires both the learning of new stimulus-response relationships and the suppression of responses that have been appropriate previously but are no longer so. In humans, set-shifting ability is commonly measured with the Wisconsin Card Sorting Task (WCST). Tests of this nature have revealed that set-shifting ability is impaired in certain disease states, such as schizophrenia, and in individuals with frontal lobe damage providing evidence of deficits in cognitive flexibility which limits behaviors in these individuals to constrained, inflexible repertoires. In a similar manner, heavy cannabis use by humans is associated with decreased mental flexibility, increased perseveration, and a reduced ability to shift attention. In a laboratory task requiring subjects to alternate between two response options in order to maximize monetary gains, marijuana dose-dependently impaired the ability to switch behavioral responses, thus resulting in loss of monetary gains. Deficits in the performance of the WCST have also been observed in adults and adolescents who are

heavy cannabis users. Few studies have evaluated the effects of cannabinoids on behavioral flexibility in rodents, and the literature in this regard is inconsistent. Acute Δ9-THC has been found to impair intradimensional, but not extradimensional, learning in rats. In contrast, the synthetic CB₁ agonist HU-210 reportedly impairs extradimensional learning and the CB₁ antagonist SR141716A improves extradimensional learning. The onset of cognitive deficits as a function of the amount and duration of cannabis use has not been characterized and it is not known how long deficits persist following cessation of cannabis use. In addition, it is not known whether the deleterious effects of cannabis are more or less pronounced in adolescent or adult users.

Neural mechanisms for the cognitive effects of cannabinoids

Although the neurochemical mechanisms through which cannabis affects cognitive function have not been fully characterized, some insight into the neural processes underlying this effect of cannabis use can be gleaned from the distribution of brain CB₁ receptors and human neuroimaging studies. High levels of CB₁ receptor expression are found in the human cerebral cortex, hippocampus, and basal ganglia. Heavy cannabis use by humans is associated with abnormal frontal cortical function and neurocognitive deficits, including impairments in attention, inhibitory control, certain forms of impulsive behavior, decision making, and/or cognitive flexibility that are thought to be associated with altered prefrontal cortical function. Neuroimaging studies have also revealed cannabis-induced alterations in the function of prefrontal (orbitofrontal) cortex, hippocampus, and components of the basal ganglia, and it has been proposed that neural dysfunction in these regions contributes to a loss of inhibitory control that propels continued cannabis use.

As reviewed above, cannabinoids have clear, though generally mild, effects on a variety of cognitive functions. However, it has been less clear whether more severe deficits in cognitive function develop over the course of years of heavy cannabis use by humans, or in animals treated for prolonged periods of time with the drug. Some studies in long-term very heavy cannabis users (10–20 joints per day for more than 10 years) failed to show any significant difference between users versus nonusers using a battery of tests of cognitive function. Several other reports have shown deficits in the performance of complex cognitive tasks in long-term cannabis users, although the available evidence does not suggest that these deficits are more severe than those observed after initial drug use. Thus, there is presently little evidence suggesting a sensitization of the cognitive impairing effects of Δ9-THC with long-term continuous cannabis use. It is likely that neural adaptations develop in response to long-term frequent cannabis use, such as a

downregulation of cannabinoid receptor expression or function. These adaptations may serve to maintain relatively normal function in the presence of relatively high Δ9-THC levels maintained during ongoing cannabis use. It is possible such neural adaptations contribute to cognitive impairments when brain Δ9-THC levels decline during cannabis abstinence. However, cognitive function during acute or prolonged abstinence from cannabis use has not been well characterized in either human or animal studies. Finally, few studies in humans have sought to determine whether abnormalities in cognitive function observed in long-term marijuana smokers result from long-term cannabis exposure or were preexisting factors that may have contributed to drug use.

Psychiatric and Cognitive Disorders

Schizophrenia and psychosis

Following the ingestion of large doses of cannabis (typically in food or drink), a form of drug-induced psychosis can occur. This condition is characterized by delusions of being controlled by an outside force, grandiose identity, persecution, thought insertion, auditory hallucinations, altered perceptions, and emotional blunting. Because of the overlap between these symptoms and those present in paranoid schizophrenia, this has led to the suggestion that cannabis use can precipitate schizophrenia. Many studies have evaluated a potential relationship between cannabis use and psychosis. For example, in a prospective study of nearly 2500 German individuals in the age range of 14–24 years, it was found that cannabis use at baseline testing was associated with an increased incidence of psychotic symptoms at follow-up. A 14-year study in a similarly aged Dutch population concluded that cannabis use predicts future psychotic symptoms in individuals who did not have such symptoms before they began using cannabis. Probably the strongest evidence for a linkage comes from a Swedish study in which detailed records obtained on entry into the Swedish army were used for following up on subsequent medical history over a 15-year period. In this study, the relative risk of schizophrenia in those who had used cannabis was 2.4 times greater than in the nonusers, while in 'heavy users' (more than 50 episodes of cannabis use) the relative risk increased to 6.0. While these and related results provide support for a linkage between cannabis use and schizophrenia, a causal relationship between cannabis use and long-term psychotic illness remains unproven.

Depression and anxiety

Most studies evaluating the effects of cannabis use on psychiatric conditions have focused on psychosis, and far fewer have investigated a potential link with more common affective disorders such as bipolar disorder (BD), depression, and anxiety. However, various studies

have shown that a range of pathological conditions – including depressive, bipolar, and anxiety disorders – and the degree of their comorbidity are significantly associated with cannabis use and the progression to cannabis-use disorder.

Longitudinal studies suggest a modest association between early-onset regular or problematic cannabis use and later BD or depression. For example, a recent evaluation of nearly 5000 subjects found that cannabis use at the initial evaluation was significantly correlated with the incidence of manic symptoms during follow-up evaluations over the course of 3 years. This association between cannabis use and mania was independent of the incidence of psychotic symptoms, and manic symptoms at baseline did not predict the onset of cannabis use during follow-up. Similar studies have reported that rates of major depressive disorder are increased in individuals seeking treatment for cannabis dependence and that heavy cannabis use is associated with two- to fourfold greater likelihood of depressive symptoms and suicidal ideation as compared with individuals who do not use cannabis. A number of studies have found a modest association between early-onset regular cannabis use and later depression. Of the studies that examined a linear trend across cannabis-use frequencies, four have reported a consistency in the ‘dose–response’ effect of cannabis use and depression outcomes and one report observed that lifetime DSM, Version III, revised (DSM-III-R) cannabis dependence was associated with a 3.4 times increased risk of major depression. Further, in monozygotic twin studies, a cannabis-dependent twin was found to be more likely to have major depression disorder, suicidal ideation, and attempted suicide than is their non-cannabis-dependent co-twin. At least two hypotheses have been forwarded to explain the possible association between cannabis use and depression. For example, long-term heavy cannabis use may induce neuroadaptations in neurotransmitter signaling that results in an increased likelihood of depressive symptoms. Alternately, evidence suggests that regular and early-onset cannabis use is associated with social difficulties that are themselves risk factors for the development of depression (e.g., reduced educational success, increased unemployment, and crime).

It has long been recognized that acute cannabinoid administration can produce biphasic and bidirectional effects on affective state, depending on the mode of administration, dose, tolerance, and a variety of environmental and individual factors. Thus, under certain circumstances, cannabis can exert anxiolytic, antidepressant, and hypnotic effects while, at higher doses, it can produce dysphoric reactions, anxiety, panic, paranoia, and psychosis. Similar biphasic effects of natural and synthetic cannabinoid agonists on anxiety-like behaviors have been reported in rats and mice. Increased anxiety has been

observed in most, but not all, prospective studies of cannabis use in healthy volunteers, and cannabis use is associated with increased anxiety in young adults independent of the use of additional illicit drugs.

CB_1 receptors are expressed in high density in the amygdala, hippocampus, anterior cingulate cortex, and prefrontal cortex that are key regions in the regulation of anxiety. The mechanisms contributing to the dose-related differences in cannabinoid-induced anxiety-like behavior have not been characterized. It is possible that varied behavioral effects are produced by the activation of CB_1 receptors versus novel non- CB_1 cannabinoid-sensitive receptors, or that there is a region-specific difference in CB_1 receptor sensitivity or coupling to G_s and G_i proteins. In this regard, it is interesting that one of the constituents in cannabis –CBD – possesses anxiolytic and potentially anti-psychotic properties that are not mediated through CB_1 receptors. Moreover, the anxiolytic effects of CBD are not biphasic or bidirectional even at high doses.

See also: Drug Addiction; Drug Sensitization and Drug Abuse; Hallucinogens; Pain and Addiction; Schizophrenia; Stress and Drug Craving.

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Transition to Addiction

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Glossary

Intravenous self-administration – An operant behavior measured in experimental animals. A behavioral response, a nose-poke or a lever press, is followed by a drug injection (the reinforcer) through a chronic indwelling catheter. Maintained self-administration has been reported for most of the drugs that humans abuse.

Progressive ratio schedule – A schedule whose goal is to measure the efficacy of a reinforcer to maintain an operant behavior. It is a measure of the strength of the operant behavior. It is also commonly interpreted as a measure of the motivation of the subject for the reinforcer. In the case of self-administration, the method consists in increasing response requirement (fixed ratio) after each drug injection. The maximum fixed ratio (FR) a subject will complete to receive a drug injection is called the break point and is the variable that is classically used to estimate the subject's motivation for the drug.

Reinforcing effect – In the context of operant behavior, a reinforcer is a consequence that causes a behavior to occur with greater frequency. A positive reinforcing effect occurs when a behavior (response) is followed by a favorable stimulus (positive reinforcer) that increases the frequency of that behavior. Negative reinforcing effect occurs when a behavior (response) is followed by the removal of an aversive stimulus (negative reinforcer) thereby increasing that behavior's frequency.

The DSM-IV describes five possible behavioral alterations characterizing loss of control (**Table 1**) that can be grouped into three main categories: (1) a difficulty to limit drug use and drug seeking (items 3 and 4), (2) an apparent strong motivation for the drug (items 5 and 6), and (3) maintenance of drug use despite consciousness of negative consequences. In conclusion, while in the DSM-III transition to addiction was considered as the appearance of changes in drug effects (tolerance and withdrawal), in the DSM-IV, it is characterized by changes in the modality of drug taking (loss of control over drug intake).

It took over 10 years for the scientific community to integrate and adopt this drastic conceptual change in the clinical vision of addiction. Today, drug addiction or dependence is consensually defined as a chronic relapsing disorder characterized by compulsive drug use. Drug addiction is, indeed, an absence of control over drug taking and drug seeking, which is associated with a high probability to relapse even after prolonged periods of withdrawal. Transition to addiction consequently corresponds to the shift from controlled, nonpathological drug use to compulsive drug intake.

Two Theoretical Frameworks for Explaining Addiction

The voluntary intake of drugs of abuse is a behavior largely conserved throughout phylogeny. Thus, the preference for drug-associated environments or learning of tasks reinforced by drugs has been found in several species, including worms, honeybees, rodents, and nonhuman primates. Voluntary intake of drugs has been studied in animals since 1962, thanks to the development of the intravenous self-administration model.

Based on this model in animals and on clinical observations in humans, two main theoretical frameworks have been proposed to explain the transition to addiction: the drug-centered and the individual-centered theories. These two theories have been grounded, respectively, on two commonly admitted clinical observations. First, transition to addiction occurs only after prolonged drug use. Second, addiction is observed only in a limited number of drug users.

Drug-centered theories of addiction are the most followed by experimental researchers. They have guided research and therapy design over the past 40 years. According to these theories, transition to addiction results from the psychopharmacological changes in drug effects

Introduction

Definition and Characteristics of Addiction

The clinical definition of drug addiction (or drug dependence) considerably evolved from the early 1980s to the mid-1990s as testified by profound revisions of the reference manual of psychiatry, the *Diagnostic and Statistical Manual of Mental Disorders* (see **Table 1**). In the DSM-III in 1980, addiction was defined as tolerance to the drug and/or withdrawal symptoms when drug use was stopped. By contrast, with the DSM-IV in 1994, tolerance and withdrawal can be present but are no longer necessary criteria. In the DSM-IV, behavioral symptoms related to an absence of control over drug taking and drug seeking must be present in order to make a diagnosis of addiction.

Table 1 Evolution of diagnostic criteria for drug addiction

<i>DSM III diagnosis for drug addiction (1980)</i>	<i>DSM IV diagnosis for drug addiction (1994)</i>	<i>Main symptoms (DSM IV 1994)</i>
<i>At least one of these two criteria</i>	<i>Three out of these seven criteria</i>	
1. Tolerance	1. Tolerance	Alterations in drug effects (no longer exclusive)
2. Withdrawal	2. Withdrawal	
	3. Substance is used more or for longer periods than intended	1. Difficulty to limit drug use and drug seeking
	4. Persistent desire to or unsuccessful efforts to cut down	
	5. Considerable time spent in obtaining the substance or using, or recovering from its effects	Behavioral symptoms: absence of control over drug use and drug seeking
	6. Important social, work, or recreational activities given up because of use	2. An apparent strong motivation for the drug
	7. Continued use despite knowledge of problems caused by or aggravated by use	3. Continued use despite knowledge of problems caused by or aggravated by use

that result from chronic drug use, such as tolerance, sensitization, conditioning, and withdrawal. These visions of addiction have been reinforced by behavioral observations showing that the longer the exposure to a drug, the greater the amount of drugs taken by animals. The discovery of very significant neurobiological alterations at molecular, cellular, synaptic, or network levels following chronic drug intake have also strengthened these theories.

The individual-centered theory posited 20 years ago has received less attention. This theory was initially based on the clinical observation that transition to addiction only occurs in 15–35% of drug users. According to this theory, transition to addiction results from a pathological response to the drug, which, in turn, depends on the biological phenotype of the drug user. Several experimental observations have strengthened the hypothesis of the existence of a drug-vulnerable phenotype. Indeed, in rodents, large differences in vulnerability to developing drug use have been observed between individuals of outbred rodent strains as well as by comparing different inbred mice strains.

Since individual vulnerability to developing drug intake is observed in both humans and animals, an interesting question is how drug-centered theories have been and still are the leading theories of addiction. This has been achieved by a series of criticisms and alternative

explanations of the individual differences observed in humans and in animals. Concerning human studies, it has often been hypothesized that individual risk in vulnerability to addiction is underpinned by contingent factors, such as drug availability or peer pressure, irrespective of a particular biologically drug-vulnerable phenotype. In other words, some individuals have a greater risk of developing addiction simply because they are more likely to be exposed to the drug. Concerning individual differences observed in animals, it has also been proposed that they are due to uncontrolled contingent factors such as experimental error and/or to specific experimental conditions, such as very low doses of accessible drug. For this reason, it has been proposed that individual differences are irrelevant in real-world conditions, where drug doses can be freely titrated by the individual. This idea has also been reinforced by statements from leading figures in the field that even in animal studies using the right experimental conditions, for example, high unitary doses of drugs, individual differences do not exist.

Research in animals during the past 20 years has finally set all these criticisms to rest and demonstrated that individual vulnerability to drugs does exist and is due to an underlying biological phenotype that increases the reinforcing effects of drugs in certain individuals. First,

individual differences are not due to uncontrolled experimental contingencies. In fact, they can be predicted by relevant drug-independent behaviors, such as behavioral response to stress and anxiety- and impulsivity-related behaviors. Second, individual differences in drug use also exist when animals have exactly the same access to drugs and are maintained over the entire range of doses that can be explored in animals. Importantly, dose-response studies have shown that individual differences in drug intake are due to differences in drug efficacy and not to differences in drug sensitivity. Differences in sensitivity could be irrelevant in the real world owing to the absence of limitation in drug dose. In contrast, differences in efficacy might also predict a higher vulnerability in the real-world conditions since they imply that, whatever be the dose, some individuals will take a larger quantity of drugs and will be more prone to drug use. Finally, research on the biological substrates of these differences has now revealed some of the biological alterations determining the higher vulnerability to take drugs in certain individuals, and that this biological predisposition can be genetically determined or stress induced.

In conclusion, we now know that certain individuals are more prone to develop and maintain intake of large amounts of drugs because of specific biological phenotypes. It is also refreshing to observe that leading groups in the field, which have denied the relevance of individual differences and discarded them as irrelevant or not existing, have started finding them and studying them.

Experimental Models of Transition to Addiction

Most preclinical research has tended and still tends to assimilate increased drug use to drug addiction. However, as first argued by Andrea Heyne and Jochen Wolffgramm in the early 1990s and as clearly described in the DSM, taking drugs is not the landmark of the clinical diagnosis of addiction. Indeed, for clinicians, even an increased or escalating drug use does not prove addiction. The drug user has to express specific behavioral deficits attesting loss of control over drug use, such as maintenance of drug intake despite negative consequences.

For these reasons, one of the major challenges of addiction research in recent years has been to develop models for studying not only changes in drug intake but also the transition between controlled and compulsive drug use.

To model human addiction, a reasonable approach seems to adequately model symptoms of addiction, evaluate these symptoms repeatedly over an extended period of drug use, and consider results at the individual level so as not to discard potential individual differences. In other words, building a model that has true face validity with

the clinical reality and that consequently targets the drug-directed behaviors considered as addiction in humans. Over the past 10 years, a few teams have investigated addiction-like behaviors in rodents. Our research group developed a multisymptomatic model for investigating the transition to addiction, taking the abovementioned constraints into account. Four monosymptomatic models were also developed considering specific addiction-like behaviors. Both types of models are discussed below.

The Multisymptomatic Model

Intravenous cocaine self-administration was used as a model of drug use. Adult male 10-week-old Sprague-Dawley rats were used as subjects. The choice of an outbred strain was deliberate to mimic the human situation and to assess potential individual differences. The rats are allowed to self-administer cocaine intravenously at a relatively high dose (0.8 mg kg^{-1} per infusion) for 2 h on a daily basis.

Assessing addiction-like symptoms and transition to addiction

It clearly appears from the clinical definition that behavioral differences between drug users and addicts might be best revealed in challenging conditions; to obtain drugs, addicts are willing to overcome barriers that users are not prepared to overcome. Therefore, besides the daily training protocol of cocaine self-administration, rats are episodically challenged to assess addiction. Cocaine use severity is assessed by using three behaviors resembling one or more of the seven DSM-IV criteria for addiction: (1) high motivation for the drug, measured by a progressive ratio schedule of reinforcement; (2) inability to refrain from drug seeking even if the drug is not available, measured by active responding during periods of signaled drug nonavailability; and (3) drug use despite negative consequences, measured by resistance to footshock-induced punishment during cocaine self-administration.

To detect an eventual transition from controlled use to addiction, the rats are trained for intravenous cocaine self-administration over a much longer period of time (over 80 sessions) than that classically used in the monosymptomatic models (no more than 40 days). After early, intermediate, and late training (20, 40, and 70 sessions), cocaine addiction severity is evaluated by testing the rats for the three addiction-like symptoms.

Performing an individual diagnosis

In humans, the diagnosis of addiction is performed by counting the number of DSM diagnostic criteria met by a drug user; a positive diagnosis is made when at least three out of the seven criteria are present. Addicts can also be assigned a score on a scale of severity (addiction

severity scale or ASI), which depends on the number of diagnostic criteria met by the patient, comorbidities, and addiction-induced social impairments. To take into account the possible individual dimension of addiction, we used a qualitative DSM-like diagnosis and a quantitative ASI-like diagnosis as in humans.

For the qualitative diagnosis, animals are ranked for each addiction-like behavior independently. When a rat has a score in the 35th highest percentile of the population, it is considered positive for this addiction-like criterion. This allows us to separate our sample of rats into four groups according to the number of positive criteria met (zero to three) and to identify two very different subpopulations, 0crit and 3crit rats, which model nonaddicts and addict drug users, respectively. The 1crit and 2crit rats could either represent specific stable cocaine-use-related troubles or intermediate steps toward addiction.

For the quantitative diagnosis, the three scores are computed into an addiction score for each rat after normalization. This score quantifies cocaine addiction severity and can be useful to establish potential correlations with neurobiological or other behavioral variables.

Transition to addiction depends on individuals, not on drug intake

After short or intermediate exposure to cocaine (up to 40 days), qualitative diagnosis of addiction did not make it possible to identify addicted (3crit) rats. After prolonged cocaine use, however, transition to addiction occurred in some rats. Indeed, after late training, some rats were positive for the three addiction-like criteria, while their behavior after early training was similar to that of the other rats and, in particular, to that of nonaddicted (0crit) rats. Furthermore, like human addicts, 3crit rats had a high propensity to relapse even after a long period of withdrawal. Finally, the percentage of 3crit animals (17%) was very similar to the percentage (15–20%) of human cocaine users diagnosed as addicts. Strikingly, these individual differences were not dependent on the amount of drug intake, since animals that did or did not develop addiction-like behavior had an identical intake of cocaine during the baseline training protocol. However, when having extended access to the drug (5 h in a row), the drug intake of addicted rats rapidly became higher than that of nonaddicted (0crit) rats, further showing the difficulty of these individuals to limit their drug use.

Similar results were obtained using the quantitative ASI-like diagnosis. The calculated addiction score proved to be linearly related to the number of addiction-like criteria met using the DSM-like qualitative diagnosis. The 0crit rats were the only group with a negative addiction score, confirming their resistance to addiction. The 3crit rats were the only group with an addiction score above one standard deviation, confirming their

vulnerability to addiction, while the 1crit and 2crit rats showed intermediate addiction scores.

These findings confirmed that addiction results from an interaction between a prolonged exposure to drug and a vulnerable phenotype, which, importantly, is present also in laboratory animals. This observation opens up new perspectives for the understanding of the dynamics of the addiction process and may be helpful for understanding the different factors involved in the maintenance of controlled drug use or the development of addiction, depending on the individual.

Monosymptomatic Models of Transition to Addiction

Other models studying only one addiction-related behavior have been proposed as a model of transition to addiction. Like the multisymptomatic one, two of these models are based on the DSM-IV diagnostic criteria of addiction focusing, respectively, on adverse consequences or motivation for the drug.

The other two models, on the contrary, do not directly derive from the diagnostic criteria of addiction. One of these models focuses on relapse and, in particular, on the development of craving. Although relapse is a consequence and not a diagnostic criterion of addiction, this model is extremely interesting. It specifically allows modeling of a counterintuitive clinical observation, that is, the fact that the probability to relapse does not decrease but increases after drug discontinuation is initiated.

The last model focuses on the escalation of drug intake that is observed when animals are given longer daily access to drugs. This model is the most far from the clinical diagnosis of addiction since escalation in drug intake is not a clinical sign of addiction in the DSM-IV but a phenomenon often but not obligatorily associated with addiction. Studies on escalation in drug intake have, however, the merit to be the first to attempt to model addiction and to put forward the idea that one should study more than just standard intravenous self-administration.

In conclusion, monosymptomatic models are very heterogeneous, being more or less close to the clinical definition of addiction. These models present the advantage to demand a lower workload than the multisymptomatic model and, consequently, allow for a large range of neurobiological investigations. However, they also have a few drawbacks: (1) they explore only one dimension of the clinical diagnosis of loss of control on drug intake; (2) they are usually studied using shorter periods of drug exposure than those needed for addiction-like behavior to strongly appear; (3) they poorly integrate the individual dimension, although this seems to have been corrected in recent studies from some of these groups.

Transition to Addiction: Psychobiological Basis

In the search for etiological factors of addiction, clinical, and epidemiological studies have focused on psychological dimensions, such as impulsivity or reactivity to stress, and on addiction-associated pathologies such as anxiety or depression. Although variable results have been obtained, associations between addiction and/or sustained drug use and these traits or pathologies have been found. However, in human studies, it is difficult to assess whether a certain behavioral trait is a consequence or a potential cause of the development of addiction. Animal models have now somewhat clarified the relationships between addiction and other behavioral traits.

Response to Stress

Exposure to stressful life events has been consistently evoked in epidemiological studies as a risk factor for human drug addiction. Experimental studies support this hypothesis. Twenty years ago, our research group demonstrated that in rodents, as in humans, certain individuals (high responders, HRs) show a higher vulnerability to develop voluntary drug intake than other (low responders, LRs) more resistant subjects. Vulnerable subjects display enhanced sensitivity to the reinforcing effects of drugs of abuse, self-administering larger amounts of drugs over a wide range of doses. This enhanced vulnerability can be predicted by a greater behavioral reactivity to a stress (locomotor activity in a novel environment) and can be induced by life events and, in particular, by stressful experiences occurring during adulthood or at an early stage of development.

Locomotor response to novelty, however, has been shown to be unrelated to the vulnerability to develop addiction-like behavior. Transition to addiction has the same incidence in both HR and LR rats and once addiction has developed, nonaddict (0crit) and addict (3crit) rats do not differ for locomotor response to novelty. Whether the development of addiction-like behavior in rodents can be influenced by stressful life events remains an open question.

Impulsivity

Poorly conceived, disadvantageous decisions and behaviors characterize impulsivity. This personality trait, however, could be composed of multiple components, including behavioral disinhibition, intolerance for delayed reward, and impaired ability to consider the consequences of behaviors. In humans, incidence of high impulsivity is much higher in drug addicts than in the normal population. There is also a high comorbidity

between drug addiction and disorders characterized by impulsive behavior, such as attention-deficit/hyperactivity disorder (ADHD). In animals, impulsivity might also predict vulnerability to drug use. However, only some components of this complex trait seem related to drug sensitivity. For example, intolerance for delayed reward could predict vulnerability to cocaine use, whilst behavioral disinhibition as measured by the inability to wait and sample predictive stimuli before responding might not. Thus, HR and LR rats, which are, respectively, vulnerable and resistant to psychostimulant use, differ in a DRL task, but do not in the task measuring the inability to wait and sample predictive stimuli before responding.

In the context of transition to addiction, impulsivity, in particular, behavioral disinhibition, is not associated with cocaine addiction but could predict its development. The inability to wait and sample predictive stimuli before responding predicts the vulnerability to shift from controlled to compulsive cocaine use. Transition to addiction mostly occurs in rats showing the highest level of impulsivity prior to cocaine use. It is noteworthy that this specific test, as stated above, fails to predict vulnerability to develop drug use. However, once addiction is developed, addict (0crit) and nonaddict (3crit) rats do not differ for behavioral disinhibition as measured in extinction conditions. Continued responding under extinction conditions is also considered a measure of behavioral disinhibition and impulsivity. Extinction allows for the measurement of persistence of responding to the drug. During this procedure the drug is withdrawn, but is still signaled as available.

Anxiety

Anxiety and drug addiction is a frequently described comorbidity, and whether anxiety precedes or follows addiction remains a question of debate. In rodents, cocaine use and withdrawal from drug use might produce anxiety. Concerning anxiety as a predictive factor for cocaine use, conflicting results have been obtained, that is, increased or reduced anxiety predicting vulnerability to cocaine use. Concerning addiction *per se*, anxiety is likely not a consequence of addiction in rats; addict (3crit) and nonaddict (0crit) rats do not differ with regard to anxiety as measured in an elevated plus maze. Whether anxiety in drug-naïve rats could precede transition to addiction remains to be investigated.

Pattern of Drug Use

Cocaine addiction often emerges with new patterns of use that accelerate drug delivery to the brain. These new patterns include increases in intranasal dosage or switch to new routes of administration, such as smoking or intravenous injections. Experimental studies have shown that

the speed of drug infusion might influence cocaine-induced neurobiological adaptations as well as motivation to drug use. What about transition to addiction?

Despite identical opportunity to self-administer cocaine in all rats, transition to addiction is specifically preceded by the early development of a burst-like pattern of use. Early after the rats start to self-administer cocaine (less than 20 sessions) and although future addicts (3crit) and nonaddicts (0crit) take the same amount of drug, future 3crit rats spontaneously self-administer cocaine faster than future 0crit rats. In summary, a fast pattern of cocaine use appears as an early symptom of the transition to addiction. Whether this behavior is a mere early symptom of the development of addiction or is causally related to it remains to be determined.

In conclusion, different behavioral traits seem to be associated with the vulnerability to develop and maintain drug intake and with the development of addiction. Higher behavioral response to stress, anxiety-like behaviors, and certain dimensions of impulsivity have been shown to be associated with increased drug intake or to predict vulnerability to drug use. By contrast, none of these behaviors seems to predict the development of addiction-like behaviors. Only the pattern of drug intake and certain forms of impulsivity seem to predict the development of addiction.

A Unified Vision of Addiction

Using pertinent animal models, it now appears possible to reconcile the two major theories of addiction and to integrate the experimental and clinical perspectives. Thus, the major hypotheses driving experimental research consider the degree of exposure to drugs as the key factor leading to addiction. On the contrary, clinical visions of drug abuse have progressively shifted from the role of drug exposure to that of the higher vulnerability to drugs of certain individuals. Our unified vision of addiction sees this pathology as the result of drug/individual interactions along a two-step process.

Addiction results from the interaction between (1) the degree of exposure to drugs, because addiction-like behavior appears only after extended access to drug and (2) the degree of vulnerability in the exposed individual, because addiction-like behaviors appear only in a few subjects, despite a similar drug intake in all subjects. It is, therefore, the interaction between the exposure to drug and a vulnerable phenotype, not one or the other factor in itself, which seems to determine the development of addiction.

Addiction is a two-step process in which each step is subsumed by a different type of vulnerability. The first step is the one that brings an individual to acquire and maintain a sustained drug intake for a prolonged period of time. This is a necessary step because addiction-like

behavior develops only after prolonged drug intake. Phenotypes such as a higher vulnerability to stress, anxiety, and certain forms of impulsivity predispose to sustained drug use probably by increasing the appetitive effects of drugs. In other words, drugs have higher reinforcing effects in certain individuals that will then be more prone to acquire and maintain drug intake. However, to shift from sustained drug use to addiction, an individual also needs a second addiction-prone phenotype. The psychobiological basis of this phenotype, which could include a certain dimension of impulsivity, still remains uncertain and needs much more research effort.

Transition to Addiction: Neurobiological Basis

Little is known to date of the biological basis underlying the development of addiction. Thus, as we have seen, theories of addictions have evolved and pertinent behavioral models have become available only recently. For this reason, most of the knowledge available today concerns the neurobiological effects of repeated exposure to drugs and the biological basis of vulnerability to developing sustained drug use.

The relevance of knowledge on the neurobiological effects of repeated exposure to drugs to our understanding of addiction is uncertain for two main reasons. First, most of this research has been performed using short-term drug exposure, that is, drug-induced modifications have been studied well before addiction-like behavior appears. Second, most of these studies have been performed on small groups of animals without taking individual differences into account. Since most individuals exposed to drugs, even for prolonged period of time, do not develop addiction, it is very likely that our knowledge concerns neurobiological adaptations that protect from addiction and not those that determine it.

More pertinent for the addiction process is our knowledge on the biological basis of individual vulnerabilities. However, this knowledge exclusively concerns the first step of the addiction process, that is, that leading to acquire and maintain sustained drug use. Over the past 20 years, biological determinants of vulnerability to psychostimulants and alcohol use have been intensively studied by our group and a few others. This research has led to a consistent conclusion: vulnerability to drug use, which is spontaneously present in some individuals (HR rats) or induced by stress in others, involves an interaction between mesencephalic dopaminergic (DA) neurons, one of the major substrates of the reinforcing effects of drugs, and glucocorticoid hormones, one of the major biological responses to stress. The activity of the DA projection to the nucleus accumbens is higher in

drug-vulnerable subjects than in resistant ones. This increase in DA activity appears to depend on glucocorticoid hormones. Thus, by activating the glucocorticoid receptors in the nucleus accumbens, glucocorticoids enhance the DA response to drugs of abuse and increase the propensity to self-administer drugs.

Clearly, this is not the only neurobiological substrate proposed in the literature. Koob and Le Moal, Robinson and Berridge, Hyman *et al.*, Everitt *et al.*, Kalivas *et al.*, and Shaham and Hope proposed differently elaborated and theoretically valid hypotheses of the mechanisms leading to addiction. However, the interaction among stress, glucocorticoid, and dopaminergic neurons is the only substrate that can be considered as a pathophysiological mechanism of vulnerability to drugs within the framework of classical experimental medicine. In this conceptual framework, already clearly stated by Claude Bernard, a biological factor or a biological chain of events can be considered a pathophysiological mechanism when (1) changes in its activity can be correlated with the predisposition of vulnerable subjects, (2) known etiological or predisposing external conditions modify the activity of such a factor, and (3) opposite manipulations of such a factor can induce and reverse the pathology. These three conditions have been convincingly and extensively demonstrated only for the interaction between stress, glucocorticoid, and dopamine that consequently stands as the only known pathophysiological mechanism of vulnerability to initiate and maintain a sustained drug intake.

Our knowledge is more restricted concerning the pathophysiological mechanisms of the addiction-prone phenotype. Thus, we are still completely lacking the most important knowledge: What are the neurobiological changes that, during prolonged drug intake, appear specifically in a few individuals and lead them to addiction? As the investigative tools and the appropriate experimental models to address this question are now available, we are confident that the answer will surely be found in the coming years.

See also: Cellular Plasticity in Cocaine and Alcohol Addiction; Drug Sensitization and Drug Abuse; Incentive

Motivation and Incentive Salience; Molecular Neurobiology of Addiction; Neural Systems of Motivation.

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Vulnerability Factors in Addiction Disorders

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Glossary

Adaptive model or individual-centered model –

Individual differences exist prior to the first exposure and contribute to the propensity to use drugs or to enter into the addiction cycle. These individual differences are attributable to inherent or acquired vulnerabilities. This model is validated by clinical observations and research.

Comorbidities – Epidemiological studies and clinical observations state that most (if not all) individuals who will succumb had, prior to exposure to the addictive objects, a ‘proaddictive phenotype,’ observable psychopathological and behavioral pathologies, and emotional pain. Drug use is a response to these sufferings.

Epigenetics – Refers to the regulation of various genomic functions, including gene expression, that are elicited by heritable (in some cases), but potentially reversible, changes in DNA methylation and various modifications of histones. Epigenetic mechanisms correspond to biological traces left by environmental insults and lead to vulnerabilities and individual differences.

Exposure model or drug-centered model – Drugs (and addictive objects in general) are the primary and unique causes of addiction, in which only the use of addictive substances (or objects) induces addiction. This model is widely used in laboratory research.

Genetic factors – In psychiatry and non-Mendelian diseases, there is no specific gene for addiction but rather only susceptibility or vulnerability genes that participate indirectly to the disorder.

Vulnerability – A construct that covers all the fields of medicine. After a period devoted to description of symptoms and diagnoses, the next step was to identify the pathophysiological bases of the diseases, and then (now) to find the causes, and find a cure. Predictive medicine will be the last step, that is, to discover markers and prodromic states of vulnerabilities. Some vulnerabilities are specific for a given class of disease, others are, in the state of our knowledges, nonspecific.

Introduction

The main goal of current research on addiction is to understand the neuroadaptive mechanisms within specific neurocircuits that mediate the transitions between occasional, controlled drug use, abuse or harmful use, and finally the loss of behavioral control over drugseeking and drug taking that defines addiction as a chronic relapsing disease. However, epidemiological data and everyday clinical practice highlight considerable individual differences at each step of the process. For a given drug, a small percentage of users become addicted. Approximately 15.6% (29 million) of the US adult population will engage in nonmedical or illicit drug use at some time in their lives, with approximately 3.1% (5.8 million) of the US adult population going on to drug abuse and 2.9% (5.4 million) progressing to addiction on illicit drugs. For alcohol, 51% (120 million) of people over the age of 12 were current users, 23% (54 million) engaged in binge drinking, and 7% (16 million) were defined as heavy drinkers. Of these current users, 7.7% (18 million) met the criteria for substance abuse or dependence on alcohol. For tobacco, 30% (71.5 million) of people aged 12 and older reported past-month use of a tobacco product. Furthermore, 19% (45 million) of persons in the US smoked every day in the past month. Of the 15- to 54-year-olds, 75% had used tobacco at some time in their lives, with 24.1% meeting the criteria for addiction. In other words, some individuals are more at risk than others and even some are resilient. They display an inherent predisposition, propensity, or proneness to enter into or succumb to addiction and relapse at one or more stages of the addiction spiral.

The underlying reasons of this vulnerability have not raised extensive research, especially at the experimental and animal levels. Conversely, drugs also have effects that depend on the intrinsic and differential hazard of the drugs. The number of individuals meeting the criteria for addiction on a given drug as a function of ever having used the drug varies between drugs. Recent data derived from the National Household Survey on Drug Abuse showed that the percentage of subjects addicted to a given drug, of those who ever used, decreases in the following order: heroin > cocaine > marijuana > alcohol.

The percentage of users with addiction or abuse for heroin is 57.4%, cocaine 25.6%, and marijuana 15.6%.

Vulnerability refers to an inherent trait or acquired state of psychobiological nature for which an object (here a drug) that is neutral by nature and by essence not addictive becomes reinforcing and addictive. This occurs because of its conjunction with a specific individual, or one who is vulnerable. Most often, vulnerability is revealed empirically *a posteriori*. Clinical and experimental research has been aimed the past few years at revealing markers of vulnerability and predicting its consequences.

The origin of vulnerability are numerous and interactive and include genetic and environmental factors and aversive life events. All have left psychological and physiopathological traces in the organism. Vulnerability participates in psychopathological syndromes and comorbid factors diagnosed in most of the psychiatric disorders. Each individual has his or her own history that uniquely contributes to the entrance into the addiction process. However, such a view based on clinical observations and studies contrasts with most of the experimental investigations and raises methodological considerations about animal and biological models and the neuroscience of addiction in general.

Methodological Considerations: Individual-Centered versus Drug-Centered Approaches

The field of addiction research shares the same basic biopsychosocial frameworks with most of the psychopathological

and behavioral disorders. Contributing factors relate to individual history and are multifactorial: living conditions, education, stressful life events, genetic/environment interplay, comorbidities, etc. One or a combination of factors contributes to psychobiological traits or states that render the subject intrinsically more vulnerable. Such an individual-centered approach (**Figure 1**) places the individual at the focal point of research and interest.

Addiction is a behavioral disorder occurring in a vulnerable phenotype. This adaptive perspective is based on predisposition and explains why subjects are at risk. The intrinsic predisposed state determines the neuroplasticity induced by the drugs. To understand and detect the sources of vulnerability would lead to a predictive medicine and toward prevention and detection of such vulnerable subjects and psychosocial interventions. Translation from the real world and from clinical psychiatry to the laboratory must discover and develop models that will present for elucidation, (1) gradual individual differences from the resilient to the most vulnerable phenotypes, (2) a transition to misuse only for some animals prone to enter in to the disease process, and (3) the main symptoms of the disease at the end of the process. An individual-centered approach is basically a historic approach. Patients and animals are considered different and more or less vulnerable because of their past, developmental characteristics, or genetic background. The sequences of the process are represented in **Figure 2**.

This paradigm contrasts with classic pharmacological approaches (e.g., drug-centered or exposure model) based on acquired drug-induced neuroplasticity vulnerability.

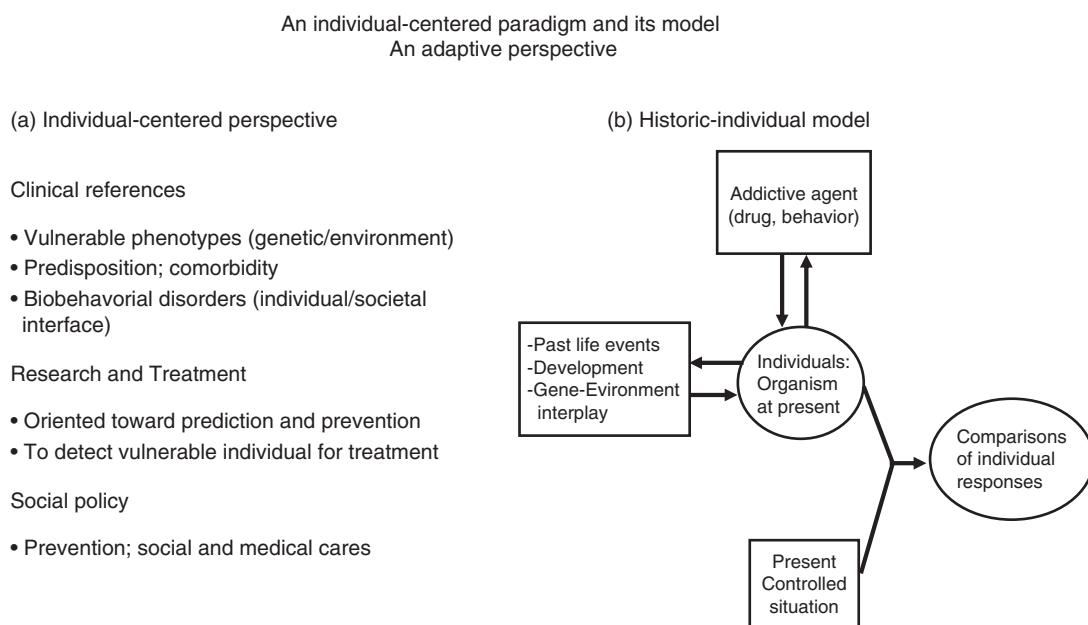


Figure 1 An individual-centered research perspective (a) to a historic-individual animal model (b), considering individuals as different from the point of view of their past life events, developmental characteristics, and genetic background. Individual comparisons require nonparametric statistics. Reproduced from Le Moal M (2009) Drug abuse: Vulnerability and transition to addiction. *Pharmacopsychiatry* 42: S42–S55, with permission from © Georg Thieme Verlag Stuttgart, New York.

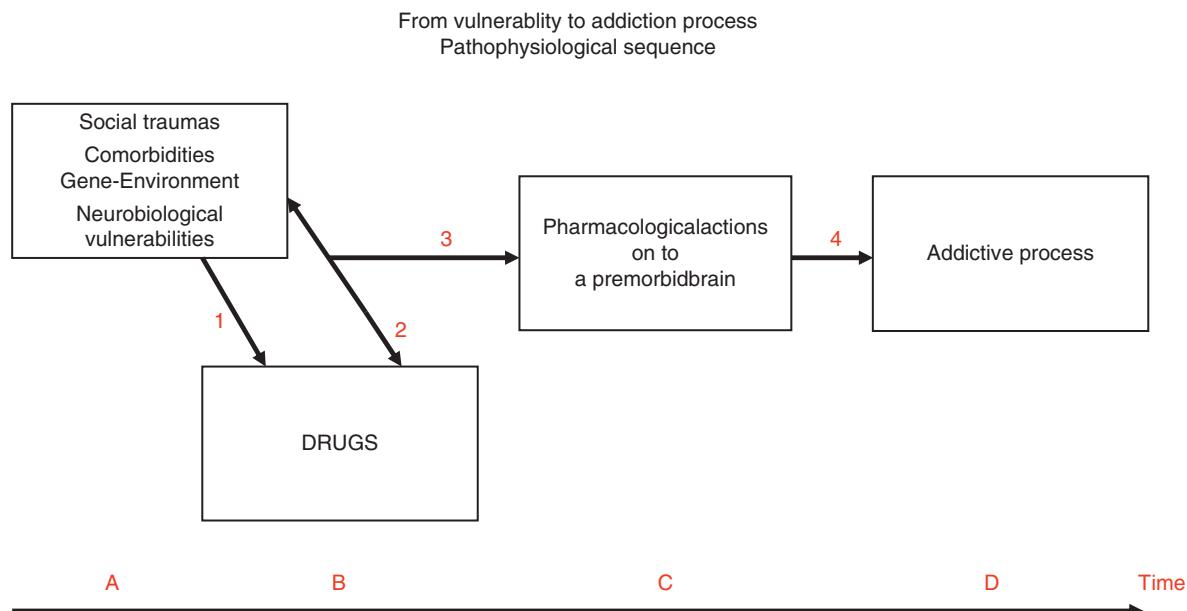


Figure 2 A given drug or behavior (e.g., eating, gambling, and sex) is addictive because of its exposure to a vulnerable individual (1) who will be prone to repeat the drug use (2). Drug use then will induce neuropharmacological and neurotoxicological effects and profound neuronal changes (3) and then addiction (4). A, B, C, and D: Process sequence. Reproduced from Le Moal M (2009) Drug abuse: Vulnerability and transition to addiction. *Pharmacopsychiatry* 42: S42–S55, with permission from © Georg Thieme Verlag Stuttgart, New York.

Here, drug abuse is, in many ways, an iatrogenic disorder, and both research and therapeutics are oriented toward understanding pharmacological drug properties, toxicological actions on brain substrates, and counteracting these effects by other pharmacological means. This paradigm is largely dominant in laboratory research. Animals are considered not in relation with their past (i.e., ahistoric models) but rather with the amount of drug taken (**Figure 3**). Individual differences are hidden under statistical standard errors or considered as protocol artifacts.

These two research interests and practices have their own logic and necessities and are complementary. Discovering the neuroplasticities induced by drugs is important. These neurobiological changes explain the transition from impulsive to compulsive behaviors and loss of control. A trivial observation is that frequent drug usage or the repetition of specific behaviors, combined with the intrinsic dangerousness of each drug (e.g., gambling, eating, and sex), is addictive by itself, leading to compulsive disorders and loss of control.

The characterization of such vulnerabilities in laboratory settings has been rather recent. Research has demonstrated that it was possible to show in rats: (1) marked individual differences in the development of psychostimulant self-administration, (2) differential propensity to drug taking predicted by individual reactivity to novelty, a robust and permanent trait, and (3) a significant positive correlation between the magnitude of reactivity and the amount of the drug self-administered during an acquisition session. Subsequent research

demonstrated that rats allowed short access (1 h per daily session) to a psychostimulant maintained low, stable, and controlled intake for many weeks, whereas long access (6 h) drug intake gradually escalated over days, with increased early drug loading, suggesting an increase in hedonic set point. Here the gradual transition from use to abuse correlated with the amount of drug taken. However, possible individual differences within groups were not investigated in their studies. More recently, it was demonstrated that three of the essential diagnostic criteria (i.e., loss of control, resistance to punishment, and motivation for drug) appeared over time in rats trained for months to self-administer cocaine. Interestingly, only 17% of the animals presented the three criteria and 41% no criterion (i.e., resilience), demonstrating significant individual differences in the propensity to become addicted.

Genetic Factors Involved in Vulnerability

Genetic factors are generally proposed to contribute to individual differences in vulnerability to initiating use of addictive substances and in vulnerability to shift from use to addiction. Genetic research in addiction, as well as in psychiatry in general, has been controversial.

Genetic contributions to addiction have long been postulated, and these can result from complex genetic differences that range from alleles that control drug metabolism to hypothesized genetic control over drug

A drug-centered paradigm and its model
An exposure perspective

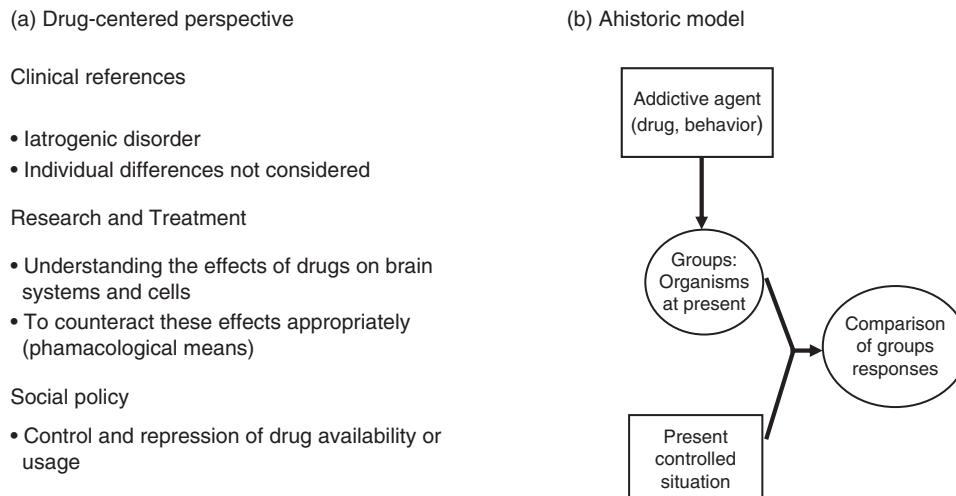


Figure 3 A drug-centered research paradigm (a) to an ahistoric animal model (b) with group comparisons and parametric statistics. The subjects are not considered from their individual characteristics – they are equal or similar. Reproduced from Le Moal M (2009) Drug abuse: Vulnerability and transition to addiction. *Pharmacopsychiatry* 42: S42–S55, with permission from © Georg Thieme Verlag Stuttgart, New York.

sensitivity and environmental influences. Complex genetic influences are those that are genetic but are not due to single-gene effects that produce Mendelian inheritance patterns. The classical approaches to complex trait genetics have been the examination of co-occurrence or comorbidity for the trait in monozygotic versus dizygotic twins, reared together or apart, and in analogous family studies with other types of biological relatives. Twin and adoption studies can provide researchers with estimates of the extent of genetic influences, termed heritability (i.e., the proportion of observed variation in a particular trait that can be attributed to inherited genetic factors in contrast to environmental factors). Using such estimates, genetic studies have demonstrated that genetic factors can account for approximately 40% of the total variability of the phenotype. In brief, and to summarize a great amount of studies, heritability estimates for drug dependence are for cocaine 44% (male) and 65% (female); opiates 43% (male); marijuana 33% (male) and 79% (female); tobacco 53% (male) and 62% (female); and alcohol 49% (male) and 64% (female). The specificity and selectivity of these data, however, are questionable. First, no ‘gene for’ exists when the various psychiatric traits and behavioral pathologies are considered. Genes have contributory roles in influencing individual variations in the liability of those traits, and the genetic effects are weak and indirect, and not causal for a given addiction. Gene variation has been shown to partially underlie complex personality and physiological traits, such as impulsivity, risk taking, and stress responsivity, as well as a substantial proportion of vulnerability to addictive

diseases. Second, evidence suggests substantial genetic and environmental nonspecificity across addictive behaviors. Genetic vulnerabilities for drugs or other types of addictions are shared with many other behavioral traits. Moreover, no evidence has specifically demonstrated genetic factors that increase risk to abuse substance A but not substances B, C, and D. A combination of environmental and genetic influences is shared between diseases. Poly-drug use is widespread, raising the additional possibility for a general addictive tendency or a general nonspecific vulnerability linked to comorbid factors. Third, addiction depends initially on individual’s decision to use an addictive substance (under the assumption that the choice of a person is not heritable) and wide variations in addictive liability are observed across time and space. Fourth, addictions are theoretically preventable by law, religious beliefs, and individual choices, imagining that a genetic shift accounts for the various drug epidemics observed over the past 40 years. Fifth, most individuals who succumb to addiction share comorbid disorders, such as impulsivity, externalized behaviors, anxiety, and dysphoria. An individual’s socio-economic status also is a decisive factor in initiating drug use, and psychosocial influences are integral to the syndrome.

Over the past few years, genetic background and, particularly, gene/environment interactions have received renewed interest by taking into account epigenetic mechanisms. These mechanisms account for many stable changes attributable to environmental events (and drugs) and subsequent psychiatric symptoms. Epigenetics exert lasting control without altering the genetic code, the

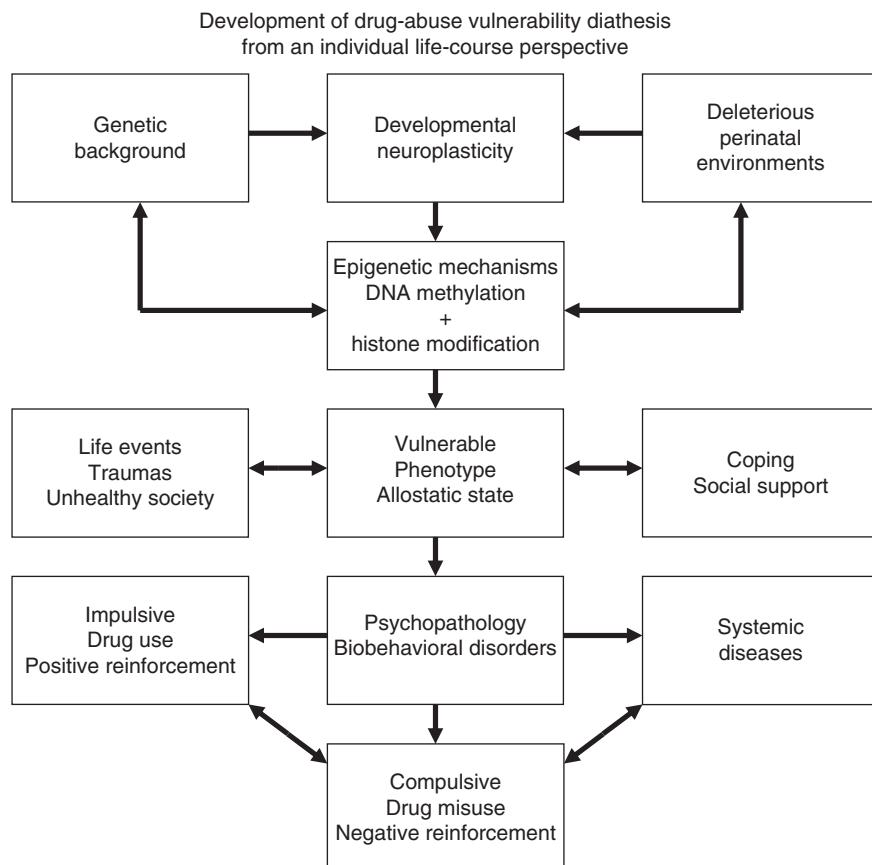


Figure 4 Development of drug-abuse vulnerability diathesis from an individual life-course perspective. Reproduced from Le Moal M (2007) Historical approach and evolution of the stress concept: A personal account. *Psychoneuroendocrinology* 32: S3–S9 (with modifications).

DNA sequence. Epigenetic changes affect chromatin modifications and methylations that occur at the carbon-5 position of cytosine in CpG nucleotides and impact chromatin packaging of DNA by posttranslational histone modifications and nucleosomes remodeling. Importantly, these epigenetic changes have not only been demonstrated in disease susceptibility, but also may be heritable. Heritable environmentally induced epigenetic modification may underlie reversible transgenerational alterations in phenotype. Epigenetic mechanisms correspond to biological traces left by environmental insult, which eventually lead to vulnerabilities (Figure 4).

Disrupted Development and Stressful Life Events

In humans, children of mothers who experienced stress during gestation display alterations in motor and cognitive development, increased anxiety, anomalies in brain and neuroendocrine function, and increased risk of developing a range of physiological and behavioral abnormalities. Extensive data from animal studies parallel

these findings. Moreover, offspring of rat mothers exposed to maternal restraint stress during the last week of gestation have a highly significant response to drugs and are prone to initiate drug use. These behavioral pathologies are linked to enduring stress axis dysregulations, among many other neurochemical changes. Prenatal stress-induced changes are reversed by postnatal handling or adoption. During infancy, disrupted maternal care, early adverse experience, and childhood sexual or physical abuse are important factors associated with a vulnerability to substance initiation and abuse. The prevailing view is that stressors influence the development of neural and neuroendocrine systems to regulate the expression of responses to stress and reinforcement processes. These clinical observations are consistent with results obtained in nonhuman primate models of disrupted maternal care. Infant rhesus macaques peer-reared, rather than mother-reared, exhibit neurobehavioral dysfunctions as adults, such as increased fear and anxiety and limbic–hypothalamic–pituitary–adrenal axis hyperreactivity following stressful situations, and they consume more alcohol than mother-reared offspring. Notably, however, acute social separation stress increases

alcohol consumption in mother-reared animals to the level of their peer-reared counterparts. Excessive alcohol intake correlates positively with plasma cortisol levels in peer-reared rhesus macaques and negatively with 5-HIAA concentrations in cerebrospinal fluid. A functional variant of the rhesus serotonin transporter-linked polymorphism has been shown to interact with rearing condition and gender to influence the adrenocorticotropic hormone response to stress, suggesting differential sensitivity to stress dependent on a gene/environment interaction. In rodents, disrupted maternal care with repeated episodes of maternal separation during the first 2 postnatal weeks provokes the same endocrine, functional, and behavioral disorders that are causal in adulthood for the individual differences in susceptibility to drug self-administration. Glucocorticoids interact directly with dopamine transmission. However, these deleterious effects resulting from maternal separation and isolation rearing of infants lead to disruptions in a variety of reward-related behaviors and neurotransmitter systems in adulthood. These effects also include reduced 3,4-dihydroxyphenylacetic acid (DOPAC)/dopamine turnover in the frontal cortex (but a larger turnover in ventral striatum) and increased self-administration of a variety of abused drugs. Enriched environments, by contrast, have been shown to decrease the propensity to initiate drug intake. Disrupted development and stress depend also on early aversive social experiences that are defined largely by social and economic disadvantages to contribute to adult psychopathologies and drug-abuse vulnerability.

Age and Gender Influences

Effects of Age and Early Drug Exposure

Adolescence (12–25 years of age) is characterized by pronounced changes in behavior with increased risk taking (e.g., hazardous driving, unprotected sex, and substance abuse), novelty and sensation seeking, social interactions, high activity and play behaviors accompanied with low levels of anxiety regarding the potential for harm, reduction in reward sensitivity, sensitivity to stress, strong emotional states, and mood instability. Self-regulatory executive functions are still maturing during adolescence, and impulsivity is supposed to normalize at the end of this period with the appearance of reasoning and planning abilities. These behavioral characteristics correlate with changes in cortical development. The prefrontal cortex and the limbic system undergo prominent reorganization, myelination, neuronal plasticity, and structural rearrangements. Sexual and stress neurohormonal systems and major neurotransmitter systems also undergo similar changes. Behavior and brain maturation interact, and the resulting traits are deeply influenced by

appropriate environments, particularly self-regulation abilities, executive function stabilization, and impulsivity control. Sociologic changes in Western societies, including individualism, early autonomy of children and adolescents, disrupted family environments, and the disappearance of social and civic connections and traditions during the second part of the twentieth century, have led to early and uncontrolled drug experiences. For example, among the US high school students, 12% of eighth graders (13–14 years of age), 22% of 10th graders, and 28% of 12th graders reported heavy episodic drinking within the past 2 weeks. According to the National Institute of Drug Abuse, 82% of adolescents have tried alcohol by the time they reach their senior year in high school. For college students, 44% binge drink every 2 weeks, and 19% are frequent binge drinkers, having more than three binge drinking episodes per week. Thus, adolescents are often drinking large quantities of alcohol. Drug taking during critical periods of cortical development may lead to increased vulnerability to drug abuse and dependence and to life-long changes in executive functions. Drug actions interfere with cortical remodeling. Persons first intoxicated at the age of 16 or younger were more likely to become heavy drinkers and 2–3 times more likely to develop substance dependence on alcohol. Similarly, persons who smoked their first cigarette during the age of 14–16 were 1.6 times more likely to become dependent than those who initiated at a later age. Others have argued that regular smoking during adolescence raises the risk for adult smoking by a factor of 16 compared to nonsmoking during adolescence. Most smoking initiation occurs during the transition from junior high school to high school (14–15 years of age). The age at which smoking begins influences the total years of smoking, the number of cigarettes smoked in adulthood, and the likelihood of quitting. When prevalence of lifetime illicit or nonmedical drug abuse and substance dependence was estimated for each year of onset of drug use from ages 13 and younger to 21 and older, early onset of drug use was a significant predictor of the subsequent development of drug abuse over lifetime. Drugs included sedatives, tranquilizers, opioids other than heroin, amphetamines, cocaine and crack cocaine, cannabis, heroin, methadone, hallucinogens, and inhalants. In general, the lifetime prevalence of dependence among those who started using drugs under the age of 14 years was 34%; this percentage dropped to 14% for those who started using at age 21 or older.

In adolescents, stages and pathways of drug involvement have been proposed in which initiation begins with legal drugs (e.g., alcohol and tobacco) and the involvement with illicit drugs occurs later in the developmental sequence. Marijuana is often cited as the bridge between licit and illicit drug. However, although this sequence is common, it does not represent an inevitable progression.

Only a very small percentage of youth progress from one stage to the next and on to later stage of addiction. Again, previous aversive experiences are responsible of their vulnerability.

Influence of Gender

Clinical and epidemiological data are now accumulating to suggest increased vulnerability to substance dependence in women. Gender differences have been reported across all phases of the dependence process, including initiation, maintenance, withdrawal, and relapse. Males and females report dissimilar motives for drug use. Females experience different subjective effects, including greater severity of withdrawal, and progress more rapidly than do men through more addictive routes. Moreover, women are more sensitive than men to stress disorders and trauma, and a link has been demonstrated between stress hormone, subsequent comorbidities, and vulnerability to substance use and abuse. Menstrual cycle phase modulates reward-related neural function in women, demonstrated by magnetic resonance imaging (MRI) techniques, with augmented reactivity of the reward system during mid-follicular phase when estrogen is unopposed by progesterone. A correlation between brain activity and gonadal steroid levels reveals that the activity in the amygdala–hippocampal complex is positively correlated with estradiol level. These data provide neurobiological foundations for understanding their impact on vulnerability to drug abuse and psychiatric comorbidity, including hormonally mediated mood disorders.

Analogous differences are observed in animals and data concur with findings, indicating that estrogen is a main factor influencing the enhanced vulnerability to initiation and relapse of drug abuse. Moreover, basal dopamine levels in the frontal cortex are dependent on the phase of the estrous cycle and attributable to the effect of ovarian steroid hormones. These data confirm MRI investigations performed in humans.

Comorbidity: Epidemiological Data

Proaddictive phenotypes depend upon multicausal factors and are heterogeneous by nature. A classic assertion is that the initiation is more associated with social disadvantage and developmental and environmental factors, whereas the transition to misuse and addiction are more associated with neurobiological factors. However, no separation exists between mental-behavioral events and their neurobiological translation (and vice versa; i.e., the magnitude of one impact on the magnitude of another). For most practitioners in addiction medicine, a normal individual will not become addicted, whereas psychopathological traits can be detected most of the times

prior to drug misuse. Patients recognized that they were not normal before drug use and that drugs were not the problem but rather a solution. Here, again, opposite positions are found in the literature. Researchers and public policymakers describe certain drugs or objects (game of chance) as addictive, tacitly implying that the cause of addiction resides in the properties of drugs or other objects. In fact, clinicians recognize that individuals who have been or who will be diagnosed with an addiction problem exhibit, prior to the onset of addiction symptoms, one or more other observable manifestations of biobehavioral pathologies, such as symptoms of another psychiatric disorder or dysfunctional behavior patterns. Individuals are concerned with lifetime comorbidity. The existence of *DSM-IV* disorders in the general population is not uncommon. Lifetime prevalence estimates are as follow: 28.8% for mood disorders; 20.8% for impulse-control disorders; 24.8% for substance use disorders; 24.6% and additional various other biobehavioral disorders: 46.4%. The median age of onset is much earlier for anxiety and impulse disorders (11 years) than for substance use (20 years) or mood disorders (30 years). Lifetime prevalence estimates were higher in a recent cohort than in earlier ones. When prevalence is estimated over a 12-month period, the percentage are the following: anxiety (18.1%), mood disorder (9.5%), impulse control disorder (8.9%), substance use disorder (3.8%), and any disorder (26.2%). Considering the severity of the cases, 22.3% were considered as serious, 37.3% as moderate, and 40.4% mild. Moreover, 55% had only a simple diagnosis, 22% two diagnoses, and 23% three or more diagnosis. Not surprising is that the strongest associations are found with mood, anxiety, conduct and personality disorders. Data from the International Consortium in Psychiatry Epidemiology that assessed approximately 30 000 subjects revealed that approximately 35% of the sample with drug dependence met lifetime criteria for a mood disorder, about 45% met criteria for an anxiety disorder, and 50% met criteria for either conduct or antisocial personality disorder. More recent data on 12-month prevalence of comorbidity in 43 000 respondents showed similar results: 21–29% for comorbidity of mood disorders, 22–25% for anxiety disorders, 32–70% for personality disorders.

An adaptive-clinical view of addiction focuses on the factors leading to vulnerability to addiction. The core focus of this approach is on developmental difficulties, emotional disturbances, structural factors, personality organization, and the building of the self. Two critical elements (disordered emotions and disordered self-care) and two contributory elements (disordered self-esteem and disordered relationships) have been identified, and have evolved into a modern self-medication hypothesis, in which individuals with substance use disorders take drugs as a means of coping with painful and threatening

emotions. In this conceptualization, individuals experience states of subjective distress and suffering or alexithymia and have feelings that are overwhelming and unbearable. Drug use is viewed as an attempt to medicate such a dysregulated affective state. The suffering of the patient is deep-rooted in disordered emotions characterized at their extremes by either an unbearable painful affect or a painful sense of emptiness. Interestingly, such self-medication may be drug specific. Patients may have a preferential use of drugs that fits with the nature of the painful affective states that they are self-medicating. Opiates might be effective in reducing psychopathological states of violent anger and feelings of rage. Others suffering from anhedonia, anergia, or lack of feelings, will prefer the activating properties of psychostimulants. Some who are flooded in their feelings, or cut off from feelings, will welcome repeated moderate doses of alcohol or depressants as medicine to express feelings that they are not otherwise able to communicate. Thus, in some cases, the subjects attempt to relieve painful feelings; others attempt to control or express feelings. The common element to this hypothesis is that each class of drugs serves as an antidote to dysphoric states and acts as a replacement for a defect in the psychological structure of such individuals. The paradox is that the choice of drugs with which to self-medicate will later by itself perpetuate the problem by facilitating entry into the addictive spiral, thereby continuing a life revolving around drugs.

General Conclusions

A significant proportion of the population takes drugs of abuse once or several times in their lifetime. Many individuals maintain repeated recreational use, but only a few develop the chronic relapsing disease named addiction. Who will succumb and why is it relevant to the problem of vulnerability? Laboratory research has only recently explored individual differences of these vulnerable phenotypes and genotypes predisposed to take drugs. The various origins of vulnerabilities have been reviewed from genetic to disrupted development and education, and stressful life-events perspectives. Most individuals who will succumb to addiction have a pro-addictive phenotype characterized by various psychopathological manifestations and emotional pain, possibly with underlying epigenetic mechanisms. Vulnerability remains a protean construct. More research is needed to understand if vulnerability is specific to drug abuse or nonspecific and relevant to psychopathology in general.

See also: Comorbidity – Depression; Drug Addiction; Human Fear and Anxiety; Perinatal Influences on Behavior and Neuroendocrine Functions; Psychiatric and

Substance Use Disorder Comorbidity; Stress and Drug Craving; Stress and Emotionality; Stress and Reward; Transition to Addiction.

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Compulsive Buying

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Glossary

Compulsive buying disorder (CBD) – Frequent buying episodes or impulses to buy that are experienced as irresistible or senseless. The spending behavior and impulses lead to personal distress, social, marital, or occupational dysfunction, and to financial or legal problems. The excessive buying behavior does not occur exclusively during episodes of mania or hypomania.

Compulsive Buying Scale (CBS) – A well-validated seven-item screening instrument for CBD. CBS-total scores can be calculated from the responses to the seven items through a regression formula. Lower scores on the scale indicate higher levels of compulsive buying.

Compulsive hoarding – Acquisition of and failure to discard a large number of possessions that appear to be useless. Living places are cluttered. Hoarding causes significant distress or impairment in functioning.

Impulse control disorders – A group of impulsive behaviors that have been included in the *Diagnostic and Statistical Manual*, Fourth Edition, Text Revision (DSM-IV-TR). These disorders are characterized by an inability to resist the impulse to perform an action that is harmful to one's self or others. The diagnostic category consists of the following disorders: trichotillomania, intermittent explosive disorder, pathological gambling, kleptomania, and pyromania.

Impulse-control disorders, not otherwise specified (NOS) – The NOS category comprises impulse-control disorders that do not meet the criteria for any specific impulse-control disorder or for another mental disorder having features involving high impulsivity and low impulse control.

Compulsive buying disorder (CBD) is a culturally bound excessive behavior wherein affected individuals engage in excessive buying. Historically, about one century ago, Kraepelin and Bleuler described the phenomenon termed 'oniomania' as a clinical entity in their psychiatric textbooks. Although the phenomenon

has received increasing attention in both consumer and psychiatric research, it has largely been ignored in clinical practice.

Phenomenology

Compulsive buying is characterized by shopping and spending behavior that seems out of one's control, wherein unnecessary items or large numbers of items are purchased. Individuals with CBD are preoccupied with buying impulses. They shop for longer periods of time and spend more money than intended. Repeated efforts to stop the excessive buying are unsuccessful, despite the negative consequences. The shopping episodes are typically preceded by negative feelings of tension, depression, anxiety, or boredom. Shopping typically leads to a short-term improvement in affect (i.e., a sense of relief, pleasure, well-being, and power), while the long-term negative consequences of the buying behavior are largely ignored. When the buying episode ends, most individuals experience guilt and shame. Typically, the purchased items are not used, but instead are hidden, hoarded, given away, or forgotten. These maladaptive patterns are at first minimized, then rationalized or dissimulated, and eventually produce further substantial negative psychological, social, occupational, and financial consequences. Sometimes, the pathological purchasing behavior also leads to legal problems. The extent of the shopping episode (i.e., amount of time spent shopping, amount of money spent during buying episodes), the negative consequences of the consumer behavior, and the stress individuals suffer from their behavior differentiate compulsive buying as a unique psychiatric entity when compared to occasional, unnecessary purchases. The excessive shopping behavior does not occur exclusively during periods of mania or hypomania. The following case example illustrates the behavioral excess.

Case Example

Anne, a 22-year-old student, suffers from repetitive, pre-occupying thoughts of buying. Her debt has increased substantially because of her purchases of clothes,

decorations, and cosmetics. Anne always enjoyed shopping and while buying her new purchases she imagined how attractive she would look wearing her new clothes and how other people would admire her. Her closets are overflowing with her purchases, which for the most part she does not use. In fact, many of the items are unwrapped and still have the sales tags on them. She cannot enjoy the purchases. Instead she feels depressed, ashamed, and angry with herself because of her uncontrolled shopping habits. It frightens her that she rarely uses what she buys and can hardly remember acquiring all of the items she had. Anne bought nearly the entire clothing line of one specific brand name. Her boyfriend and her parents, who have supported her financially in the past, are ashamed and refuse to offer financial help to her. Arguments are frequent between Anne and her family because Anne hides her purchases and lies to her family about her finances. In addition, she cannot pay the rent for her apartment because of her debts. Over the last few weeks, she has tried to stay at home and avoid shopping. However, she cannot resist the urge to visit the Internet homepage of different clothing companies several times daily.

Diagnosis and Assessment

Preliminary operational criteria for CBD were based on similar DSM-III-R criteria for impulse-control, obsessive-compulsive, and substance-use disorders.

Several questionnaires and diagnostic interviews have been developed to identify CBD (**Table 1**). The most widely used instrument in empirical studies is the Compulsive Buying Scale (CBS). For clarification purposes, a diagnostic interview should be administered in addition to a CBD questionnaire. To measure severity and clinical change in persons with CBD, the Yale-Brown Obsessive-Compulsive Scale-Shopping Version (Y-BOCS-SV) is recommended.

Table 1 Screening instruments for compulsive buying

	<i>Screening instruments</i>	<i>Authors</i>
<i>Questionnaires</i>	Compulsive Buying Scale Edwards Compulsive Buying Scale Canadian Compulsive Buying Measurement Scale Buying Impulsiveness Scale Compulsive Acquisition Scale	Faber & O'Guinn (1992) Edwards (1993) Valence <i>et al.</i> (1988) Rook and Fisher (1995) Frost <i>et al.</i> (2002)
<i>Interviews</i>	Minnesota Impulsive Disorder Interview (MIDI) Yale-Brown Obsessive-Compulsive Scale-Shopping Version (Y-BOCS-SV) SCID-I Impulse Control Disorders SCID-I Obsessive Compulsive Spectrum Disorders (SCID-OCSD)	Christenson <i>et al.</i> (1994) Monahan <i>et al.</i> (1996) First <i>et al.</i> (1996) du Toit <i>et al.</i> (2001)

For details on reference, see Further Reading section.

Epidemiology

Current research suggests that CBD is common in maturing consumer societies. Using the CBS in a national representative sample survey, researchers estimated the United States lifetime prevalence of CBD at 5.8%. German representative studies reported a point prevalence of CBD in Germany of about 7%. It should be noted that the use of only questionnaires is a limitation in these studies, and could result in an overestimation of CBD because of a response bias. Even so these findings demonstrate the high propensity to engage in compulsive buying in population-based samples.

There is also evidence that CBD has increased in prevalence in the past few decades. In Germany, two representative population-based surveys investigating CBD were conducted 10 years apart (1991 and 2001). In the first study, 1% of the East German population and 5.1% of the West German population were identified as having CBD. Ten years later the percentages had increased, with 6.5% of the population in East Germany and 8% of the population in West Germany reporting compulsive buying.

Clinical surveys suggest that women are more frequently affected by CBD than men, with the percentages of women in treatment-seeking samples being about 90%. However, population-based studies have not confirmed this gender difference. In fact, these population-based studies have found a near equal percentage of women and men in their surveys. Given these findings, it appears that CBD may not only affect women and that other gender-related factors (e.g., in propensities for treatment seeking) may influence the composition of clinical samples.

Psychiatric Comorbidity

Previous research indicated that CBD may be associated with elevated rates of *Diagnostic and Statistical Manual*,

Fourth Edition, Text Revision (DSM-IV-TR) Axis I disorders. Comparison of treatment-seeking individuals with CBD in Germany and the United States showed that almost all participants in both samples met criteria for at least one lifetime Axis I disorder. A few controlled trials were conducted comparing psychiatric comorbidity among individuals with and without CBD. Some researchers have reported elevated frequencies of lifetime mood disorders among those with CBD. Others have found higher frequencies of lifetime anxiety, substance-use, binge-eating, and impulse-control disorders. Another study described only substance dependence as occurring more frequently among patients with CBD. In a German study, treatment-seeking women with CBD showed significantly higher frequencies of affective, anxiety, and eating disorders compared to a nonclinical control group.

Two studies have assessed personality disorders among persons with CBD. One study found that 59% of 46 compulsive buyers met DSM-III-R criteria for at least one personality disorder. The most frequently identified Axis II disorders were obsessive-compulsive, avoidant, and borderline personality disorders. A second study reported that, compared to community controls, individuals with CBD presented with a significantly higher frequency of any personality disorder (73%), most commonly avoidant, depressive, obsessive-compulsive, and borderline personality disorder. Further, 23% of the CBD sample described at least one further impulse-control disorder, most frequently intermittent explosive disorder.

A strong link has been observed between CBD and compulsive hoarding. Previous findings have indicated that many patients with CBD suffer from high levels of compulsive hoarding. In one investigation, it was observed that about two-thirds of a treatment-seeking CBD sample reported substantial hoarding. In addition, CBD patients exhibiting hoarding reported more severe compulsive buying and obsessive-compulsive symptoms and presented more frequently with psychiatric comorbidity, especially current affective, anxiety, and eating disorders.

Because of the high frequencies of psychiatric comorbidity, there exists some skepticism about the recognition of CBD as an independent psychiatric entity. Alternatively, CBD could be conceptualized as an epiphenomenon of other psychiatric disorders. Nonconsideration of CBD as an independent psychiatric condition may result in further neglect and minimization of this disorder in clinical practice. Clinical experiences suggest that CBT typically does not resolve with the successful treatment of comorbid disorders. Because of the psychiatric comorbidity, patients with CBD represent a very heterogeneous patient sample. In summary, these findings could be interpreted to support the idea that a subtyping approach might be useful in classifying CBD. The development of specific therapeutic

interventions for those with CBD who also report comorbid psychiatric disorders may also be indicated.

Etiology

The etiology of CBD appears multifactorial, and models have been suggested based on psychological, social, neurobiological, and cultural influences. Patients with CBD report urges to buy, preoccupation with buying and shopping impulses, and in general increased impulsivity in the sense of acting without thinking. In addition, low self-regulatory resource availability and low self-control are associated with CBD. The negative consequences of CBD are often not considered by those affected. Furthermore, a close association between negative mood states and buying has been found. Using as a model short-term positive and negative reinforcement, compulsive buying is used as a way of escaping from problems and to relieve negative mood states. Individuals with CBD suffer from low self-esteem, and they often describe themselves mostly as socially anxious, depressed, and having decision-making problems.

Consumerism and money attitudes appear to play a key role in the development and maintenance of CBD. For example, younger age and higher materialistic values are associated with compulsive buying. The relationship between narcissism and compulsive consumption appears to be mediated by both low impulse control and high materialism values, and credit card use moderates the effect of money attitudes on CBD. These considerations involving factors influencing the development and maintenance of CBD are schematized in **Figure 1**.

In addition to psychological, social, and cultural influences, the biological model posits serotonergic, dopaminergic, and opioidergic contributions and a role for the brain reward systems. Currently, there is no direct evidence to support these neurobiological hypotheses, and it should be noted that the high comorbidity could influence the specificity of future neurobiological findings.

CBD is probably a culture-specific excessive behavior that occurs in industrialized countries. Technological developments, including television and Internet shopping, allow individuals to buy a vast array of products rapidly. In this cultural context, some authors warn against the labeling and medicalization of the CBD. Indeed, such an approach minimizes the clinical observations that individuals with CBD may suffer significantly from their shopping and buying behaviors, and do not seem to be able to easily stop the behavior. To understand CBD, and to create and validate etiological models and disorder-specific treatment strategies, both the psychological and consumer-research approaches should be considered.

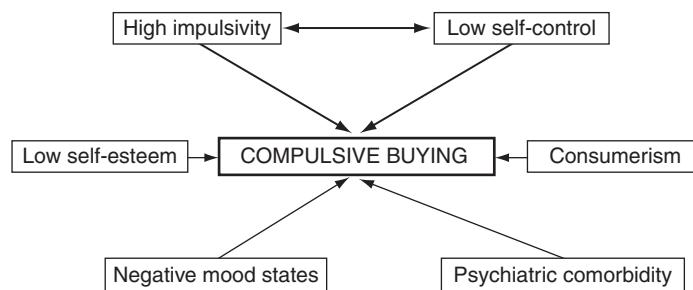


Figure 1 Proposed factors influencing CBD.

Considerations on Classification

The appropriate method of categorizing CBD continues to be debated. According to the preliminary diagnostic criteria, the inclusion of CBD in DSM-IV and ICD-10 as an “impulse control disorder not otherwise specified” is preferred. Some researchers have attempted to examine the link between CBD and obsessive-compulsive spectrum disorders (OCSDs) or addictive disorders. At present, there is a suggestion to create a category called ‘behavioral and substance addictions’ that would include both excessive behavioral disorders and substance-use disorders. The commonalities and a high comorbidity with substance-use disorders, the craving to buy, the sense of lack of self-control, the needs to spend increasing amounts of time shopping or spend increasing amounts of money in order to achieve the desired feelings (i.e., tolerance), and shopping-/buying-related withdrawal symptoms all support the categorization of CBD as an addiction. The similarities in phenomenological features, their courses, and their psychological and social consequences also cannot be ignored. In our experience, however, there exist crucial differences between substance addictions and excessive behaviors. A real dose-increase or tolerance (‘more and more/more often/more expensive’) is seen only in some of the CBD patients treated in our CBT studies. With regard to withdrawal symptoms, the patients describe negative feelings like depressive symptoms, pressure, and restlessness, if they begin to normalize their buying behavior. Detailed behavior analyses show, however, that these negative feelings are usually a matter of the emergence of negative mood states which had been suppressed through shopping behavior. The extent to which these reflect differences between CBD and substance-use disorders requires direct examination.

Treatment

Medication

A few case reports and successful open-label medication trials were reported using antidepressant, anxiolytic, mood stabilizer, anticonvulsant, and opioid antagonist

drugs. Some smaller controlled trials examined the efficacies and tolerabilities of fluvoxamine, citalopram, and escitalopram. These trials have not found superiority for these selective serotonin-reuptake inhibitors over placebo. However, the results are limited by small sample sizes, high dropout rates, and placebo-response rates ranging up to 60%. In summary, data do not support the sole use of any one medication in the treatment of patients with CBD at this time.

Psychotherapy

Some authors have described successful individual psychodynamic psychotherapy treatment and cue exposure plus response prevention for patients with CBD. Moreover, several specific group therapy and self-help approaches have been described.

The effectiveness of disorder-specific, manualized, group CBT was supported in two controlled psychotherapy studies. In both studies, the treatment was specifically aimed at interrupting and controlling the problematic buying behavior, establishing appropriate purchasing patterns, identifying and restructuring maladaptive thoughts and feelings associated with shopping and buying, developing healthy coping skills and communication patterns, and implementing relapse-prevention techniques. In addition, more general sessions about self-esteem, stress management, and problem solving were included. Group participants were expected to complete homework assignments and read and review manual materials regularly as assigned. The psychotherapy treatment focused both on current factors that maintain the excessive buying behavior and on strategies for controlling buying problems. Treatment lasted 10–12 weeks with one 90-min group session per week. Groups were conducted with 5–8 participants. The treatment manuals have been described in detail elsewhere. Further research is needed to determine whether specific individual, group, or family interventions have the most substantial impact on helping individuals with CBD.

Conclusions

CBD is a relatively prevalent disorder, particularly in developed societies. Individuals with CBD often experience significant personal distress and interference in areas of life functioning, including within financial and interpersonal domains. More research is needed into the biological basis of the disorder and more effective preventive and therapeutic strategies.

See also: Depression; Drug Addiction; Impulsive–Compulsive Sexual Behavior; Intermittent Explosive Disorder; Kleptomania; Pathological Gambling; Problematic Internet Use; Pyromania; Trichotillomania (Compulsive Hair Pulling) and Compulsive Skin Picking.

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Impulsive–Compulsive Sexual Behavior

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Glossary

Hyperactive sexual desire disorder – An interest or involvement in normative sexual activity that is so excessive as to cause distress or impairment in functioning.

Hyperphagia – Excessive appetite and excessive consumption of food.

Hypersexuality – Excessive interest or involvement in sexual activity. It is synonymous with hyperphilia.

Hypogonadism – Inadequate functioning of the testes or ovaries resulting in impaired sexual development or withdrawal effects (e.g., premature menopause) in adults and/or defective egg or sperm development leading to impaired fertility.

Nonparaphilic sexual addiction – Sexual thoughts, urges, and behaviors of normative content (but) that are unusually intense and persistent and are associated with distress or impaired functioning. Nonparaphilic sexual addiction (often simply referred to as sexual addiction) has been conceived of as a form of behavioral addiction.

Paraphilia – Powerful and persistent sexual urges, fantasies, or behaviors that involve: nonhuman objects; the suffering or humiliation of oneself or one's partner; or children or other nonconsenting individuals.

Sexual fetishism – Sexually arousing fantasies, sexual urges, or sexual behaviors focused on nonliving objects or on body parts not conventionally viewed as being sexual in nature, considered a paraphilia.

The term impulsive–compulsive sexual behavior, the focus of this article, is more often used to refer to sexual thoughts, urges, and behaviors that are normative in content but occur with such frequency or intensity that they are distressing or cause impairment in functioning. Other terms used to describe this problem include nonparaphilia-related sexual disorders or nonparaphilic sexual addictions, to differentiate them from the paraphilic. Terms such as compulsive sexual behaviors, hyperactive sexual desire disorder, hyperphilia, hypersexuality, sexual addiction, and out-of-control sexual behavior have also been used to describe this disorder. We use the term impulsive–compulsive sexual behavior throughout this article. Although impulsive–compulsive sexual behavior, under one name or another, has been discussed in the scientific literature since the early 1800s, there has been little rigorous research on the topic, so our understanding is limited. This category is not designated as specific disorder in the DSM-IV-TR; however, if an official diagnosis is needed, impulsive–compulsive sexual behaviors can be classified as impulse control disorders, not otherwise specified; or as sexual disorders, not otherwise specified.

Since it is not a formally recognized disorder, there are no official diagnostic criteria for impulsive–compulsive sexual behavior. However, there is some general agreement among experts. Impulsive–compulsive sexual behavior is operationally defined similarly to the paraphilic and to pathological gambling. The impulsive–compulsive sexual behavior diagnosis requires recurrent and intense sexually arousing fantasies, sexual urges, and sexual behaviors with a focus on normative sexual behaviors. It has been proposed that the behavior must have been present for a minimum duration of 6 months. The proposed diagnosis also requires standard DSM criteria of severity (frequency and/or intensity severe enough to cause clinically significant distress or impairment in social, occupational, or other important areas of functioning) and specificity (not due to a medical condition, medication or substance use, or better accounted for by another Axis I or II disorder).

Although the sexual behaviors themselves are normative, it is their excessive and poorly controlled nature that leads to serious problems such as exposure to human immunodeficiency virus and other sexually transmitted diseases, job loss, legal consequences, problems in maintaining romantic relationships or marriage, and unwanted pregnancies. Eli Coleman described seven subtypes of

Impulsive–compulsive sexual behavior refers to sexual thoughts, urges, and behaviors that are extremely difficult to resist. These thoughts and behaviors are generally divided into two types, paraphilic (which involve socially deviant sexuality) and more conventional but excessive sexuality. The paraphilic are better researched and better understood; they involve sexual interests that focus on nonhuman objects, children, or other nonconsenting persons, or the suffering or humiliation of oneself or one's partner. Paraphilic are a recognized category of disorders in the current *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition, Text Revision (DSM-IV-TR) and include disorders such as pedophilia, fetishism, exhibitionism, voyeurism, sexual sadism, and masochism among others.

impulsive–compulsive sexual behavior: compulsive cruising and multiple partners, compulsive fixation on an unattainable partner, compulsive autoeroticism (masturbation), compulsive use of erotica, compulsive use of the Internet for sexual purposes, compulsive multiple love relationships, and compulsive sexuality in a relationship. Studies have shown that the most common impulsive–compulsive sexual behaviors are promiscuity, masturbation, pornography dependence, and phone sex, but the use of the Internet for sexual purposes certainly may be increasing.

Based on Alfred Kinsey's concept of total sexual outlet (TSO), the total number of orgasms achieved per week by any means is often used to indicate severity of impulsive–compulsive sexual behavior, with a TSO of 7 per week persistently for at least 6 months usually considered the minimum for the diagnosis. Although a TSO of 7 may seem low as a criterion for hypersexuality, a TSO of 1–3 is typical of males in the general population and most estimates place the percent of males having a TSO of 7 or more at 3–7.5%, though estimates have been as high as 15%. Recall that diagnostic clinical and research criteria also require that the sexual behavior, thoughts, and/or urges need to cause distress or functional impairment.

TSO or the total time spent in sexual activities overall are considered by many to be better measures to use to quantify impulsive–compulsive sexual behavior, as compared to the frequency or time spent in specific kinds of sexual behaviors; however, the amount of time spent seeking sexual activities can also be considerable and problematic. Studies of males with impulsive–compulsive sexual behavior or paraphilic disorders found that the large majority of both groups have a TSO ≥ 7 per week and that the groups do not differ substantially from one another. Males with impulsive–compulsive sexual behavior spend 1–2 h a day occupied by their sexual urges, fantasies, or activities.

Most commonly, impulsive–compulsive sexual behavior is conceptualized either as an impulse control disorder, perhaps on the obsessive–compulsive spectrum, or as a behavioral addiction. Phenomenologically, at least initially, there is an impulsive component (pleasure, arousal, or gratification) at behavioral onset, and a compulsive component that leads to the persistence of the behavior; thus, the phenomenology can fit with either conceptualization. In the next version of the *Diagnostic and Statistical Manual of Mental Disorders* (the Fifth Edition; DSM-V), impulsive–compulsive sexual behavior may be recognized as a specific disorder. One new category under consideration for DSM-V into which impulsive–compulsive sexual behavior may be included is the behavioral and substance addictions. This category could include the disorders currently in the ‘substance-related disorders’ category as well as several putative impulse-control disorders (e.g., gambling addiction, sexual addiction, shopping addiction, and Internet addiction).

Epidemiology and Demographics

There have been no systematic epidemiological studies of impulsive–compulsive sexual behavior. Based on clinical samples, the estimate is that it affects 5–6% of the general population.

Impulsive–compulsive sexual behavior is believed to be approximately 3 times more common in males than females; the paraphilic disorders are rarely diagnosed in females except for sexual masochism, which is about 20 times more common in males than females. Virtually all research on these disorders has been conducted among males and little is known about impulsive–compulsive sexual behavior in females. It may be that the disorder presents differently in males than in females. Some consider females who fantasize about sex and seem to be addicted to romantic relationships to be suffering from impulsive–compulsive sexual behavior, a difference being that males are focused on the physical aspects of sexuality, while females focus more on the relationship.

It seems likely that impulsive–compulsive sexual behaviors, such as paraphilic disorders, begin by late adolescence and peak between ages 20 and 30. Although the severity may wax and wane, it may often be chronic. The typical outpatient in treatment for impulsive–compulsive sexual behaviors is a white, college-educated, employed man, with a middle-class income.

Individuals with impulsive–compulsive sexual behavior have a higher-than-expected rate of childhood sexual abuse; they differ from those with paraphilic disorders in that the latter have a significantly higher rate of childhood physical abuse. Those with paraphilic disorders have also often completed fewer years of education and are more likely to be unemployed or on disability.

Differential Diagnosis

Impulsive–compulsive sexual behavior may be a symptom of medical conditions (such as traumatic brain injury, stroke, epilepsy, and other conditions noted later in this article); in addition, substance intoxication, manic episodes, and schizophrenia may present with impaired judgment and diminished impulse control that may result in excessive sexual behavior. Onset in conjunction with an injury or change in health status, or at later than typical age, should be carefully evaluated for potential contributions from medical conditions.

Sexual obsessions can be a presentation of obsessive compulsive disorder (OCD) but can be distinguished from the obsessions that are part of impulsive–compulsive sexual behavior in several ways. Sexual obsessions in OCD are characterized by being ego dystonic and morally repugnant; the sexual thoughts induce anxiety

and often include uncertainty as to whether the feared sexual behavior may have actually been committed without the patient knowing it. In OCD, the sexual behaviors are not carried out and the compulsive behaviors related to these obsessions are not generally sexual. Rather, OCD patients frequently avoid specific situations or people in order to prevent the feared sexual acts from occurring and they may also perform rituals that are repetitive behaviors of a nonsexual nature meant to prevent or undo the distressing sexual thoughts or fears (such as by confessing to acts that didn't actually occur).

In addition, OCD rituals are not pleasurable activities engaged in for their own sake; rather, they are neutral or often irritating and unpleasant behaviors that are engaged in to reduce anxiety. In contrast, impulsive–compulsive sexual behaviors generally have an element of pleasure, at least initially, although they may lose their pleasurable quality over time; in this regard, they are more similar to addictions and to impulse control disorders such as pathological gambling.

Comorbidities

Individuals with impulsive–compulsive sexual behaviors tend to have multiple additional Axis I disorders. A majority of these males have a lifetime diagnosis of depression, and substantial minorities have lifetime diagnoses of substance abuse, anxiety disorders (especially social phobia), and impulse control disorders (such as pathological gambling, compulsive buying, kleptomania, trichotillomania, pyromania, compulsive exercise, and intermittent explosive disorder). Interestingly, the major comorbidity difference between males with impulsive–compulsive sexual behavior and those with paraphilic disorders is childhood attention deficit hyperactivity disorder (ADHD) (with corresponding ratios of approximately 1:6 vs. as many as 1:2, respectively).

By definition, those with impulsive–compulsive sexual behavior do not also have paraphilic disorders; however, a majority of individuals with paraphilic disorders also have impulsive–compulsive sexual behaviors; a majority report compulsive masturbation and notable minorities report promiscuity and pornography dependence.

Note that these individuals are not necessarily more ill than those with other psychiatric disorders; they simply differ from the general population in the pattern just described. Thus, patients with impulsive–compulsive sexual behavior should be screened for depression, anxiety disorders, other impulse control disorders, and substance abuse.

Neuroanatomical Features

Most knowledge of what brain structures may be involved in aberrant sexual behavior has come from animal

research and from studies of traumatic brain injury and disease processes in humans. As with most complex human behavior, it has become clear from this and other research that neural circuits are as or more important than specific structures or locations. Two circuits most clearly implicated in human sexual behavior are the frontal–striatal and the temporo-limbic circuits. Some conditions can affect either circuit depending on the disease locus; these include traumatic brain injury, stroke, seizures, and dementias. Other conditions may primarily affect only one of these circuits.

Fronto-striatal abnormalities have been linked to increased sexual desire but this seems to be due to general disinhibition rather than sexuality specifically. For example, there are many reports of hypersexuality following traumatic brain injury to frontal regions. Although disinhibition is one common consequence of frontal lesions, not all frontal lesions will result in hypersexuality, and its occurrence cannot be predicted at this time based on the injury site or severity. Likewise, in multiple sclerosis, cases of hypersexuality have occurred with frontal lesions, but those patients may have also had lesions elsewhere. Similarly, hypersexuality may occur in dementias that affect this circuit or in epilepsy with frontal seizures. Striatal disease can also result in hypersexuality (e.g., Huntington's disease, although hyposexuality is more frequent in this disorder).

The frontal lobe is an interesting candidate for a role in impulsive–compulsive sexual behavior because, in addition to being involved in impulse control in general, the orbital frontal cortex is specifically implicated in social cognition, decision making, working memory, and emotional processing, particularly in deficits in recognition of emotional expressions and relative insensitivity to potential consequences. The prefrontal cortex is also critical to the acquisition of moral and social knowledge; injuries in this brain region during childhood have been associated with limitations in this knowledge and deficits in moral reasoning. Injuries that occur after this knowledge is acquired will generally leave the knowledge base intact, but the patient may be unable to behave in accordance with this knowledge.

The single condition most associated with hypersexuality, Kluver–Bucy syndrome, seems to be related to temporo-limbic circuit dysfunction. It results most often from lesions in the amygdala, a temporal lobe structure, but can also result if there is a disruption of the temporo-limbic circuits. Kluver–Bucy syndrome has many symptoms but two of particular interest here, hypersexuality and hyperphagia, may reflect a loss of the ability to discriminate appropriate objects rather than an increase in drive, *per se*. Kluver–Bucy syndrome can result from many different disease processes, including Alzheimer's disease, adrenoleukodystrophy, anoxia or ischemia, carbon monoxide intoxication, herpes simplex encephalitis, limbic encephalitis, multicentric glioblastoma multiforme,

porphyria, progressive subcortical gliosis, Rhett's syndrome, systemic lupus erythematosus, trauma, and temporal lobectomies. Temporal lobe epilepsy and temporal lobe resection treatment of epilepsy have been related to changes in sexuality. The amygdala has been found to be a focal structure in emotion processing, that is where stimuli are given their emotional and motivational significance. Thus, if the amygdala is damaged, objects may lose their prior emotional significance.

It has been theorized that impulsive–compulsive sexual behavior may function similarly to substance addictions, that is, the intense, excessive sexual behavior may bring about changes to neural circuitry that may then perpetuate the behavior, just as the addictive substances may cause changes in neural circuitry.

Neurochemical Features

Strong biological influences are apparent in human sexual functioning, notably on the very basic phases: sexual desire, arousal, and orgasm. Endocrine, neurotransmitter, and neuropeptide influences on these phases have been specifically identified. However, there are also clear environmental/learned influences on sexual desire and behavior, and the relationship between these and biological factors is complex.

Among the endocrine factors that have been implicated, the androgens, in particular, play a role in sexual interest and activity in both males and females. Although there is a relationship between testosterone levels and the frequency of sexual thoughts in adolescent males, in adult males there seems to be no such relationship within a wide range of normal testosterone levels. In addition, hypogonadal and castrated males rapidly lose sexual interest, and this is reversed with testosterone administration. Similarly, females experience a decline in sexual interest as testosterone levels decrease, such as with natural or induced menopause. However, in females, estrogen has little effect on sexual interest while estrogens and progesterone seem to have an inhibiting effect on male sexual interest.

The monoamines (serotonin, dopamine, and norepinephrine) are considered important for human sexual functioning and there is reason to believe that hypersexuality may result from dysregulation of these monoamines. These neurotransmitters modulate sexual motivation. In that sexual side effects are associated with certain medications (antidepressants, psychostimulants, and neuroleptics), the neurotransmitter systems that they target influence sexual motivation and behavior. It has yet to be proven, however, that the monoamines are specifically involved in hypersexuality. Several relevant neurotransmitters are discussed below.

Serotonin

The role of serotonin in sexual functioning is complex and not yet fully understood. Activation of some receptors, such as 5-HT₂, may impair functioning while activation of other receptors, such as 5-HT_{1A}, may facilitate functioning. As noted earlier, medications that enhance central serotonin neurotransmission have dose-dependent side effects, including decreased desire and difficulties with arousal, although the most common sexual side effects are delayed ejaculation and delayed or absent orgasm. The importance of serotonin in sexual motivation and behavior is evident from animal research and records of human injury and disease. In addition, serotonin dysfunction has also been found in a number of other psychiatric disorders characterized by impaired impulse control (e.g., pathological gambling and substance abuse); this may reflect impaired frontal inhibition, which may result in a diminished ability to control desires. Impaired impulse control may result from overstimulated drive and/or impaired inhibition or reward processing.

Dopamine

Dopamine is important in several aspects of male sexuality ranging from basic sexual functioning (e.g., seminal emission and erectile function) to sexual arousal and motivation. The exact role of dopamine in female sexual behavior is less clear. In general, alterations in dopamine functioning may be associated with intense urges that override considerations of the consequences in impulse control disorders. As with serotonin, evidence for the role of dopamine in sexual interest and behavior comes from animal research as well as from observation of naturally occurring human injury and disease. For example, hypersexuality may result when Parkinson's disease is treated with dopaminergic medication. Dopamine is a key part of mammalian reward systems. Certain behaviors such as gambling and sexual arousal may result in a release of dopamine which may then lead to feelings of intense pleasure. Consequently, the dopamine system has been proposed as an important component of behavioral and chemical addictions.

Pharmacological Treatment

No pharmacological agents have been Food and Drug Administration (FDA)-approved for the treatment of impulsive–compulsive sexual behavior, and research on these treatments is limited with no convincing controlled trials in the literature. Given the absence of research and based on the knowledge regarding treatment of other impulse control disorders, it is important to consider

prominent symptoms and comorbidities in choosing a pharmacological approach.

Considerable research has been performed investigating pharmacotherapy and medical treatments of the paraphilic behaviors; however, these treatments are not necessarily appropriate for impulsive–compulsive sexual behavior. Notably, surgical and pharmacological techniques to lower testosterone levels do reduce paraphilic preoccupations, urges, and activities, but may be too drastic and risky to be used in impulsive–compulsive sexual behavior treatment.

Although there are no definitive controlled trials, one placebo-controlled trial, open-label studies, and case reports suggest that serotonin reuptake inhibitors (SRIs) are effective in reducing the symptoms of impulsive–compulsive sexual behavior and/or paraphilic behaviors. The SRIs reported to be effective in impulsive–compulsive sexual behavior include citalopram, clomipramine, fluoxetine, sertraline, and venlafaxine; however, there is no evidence that the SRIs differ from one another in efficacy. In clinical practice, SRIs are one of the first-line treatments for both impulsive–compulsive sexual behavior and paraphilic behaviors. They were initially tried in the hope that their sexual side effects, especially decreased libido but also impaired sexual functioning, would be helpful in impulsive–compulsive sexual behavior. The results have been better than expected in that reduction in impulsive–compulsive sexual behavior symptoms may be a result of their anti-obsessional effects causing a decrease in thoughts and urges, rather than the sexual side effects, which seem to be independent. The best treatment would suppress disturbing and out-of-control sexual drives and behaviors, while leaving other aspects of sexuality intact.

The sexual side effects of selective SRIs (SSRIs) can sometimes be reduced by titrating the dose. In some cases, nefazodone may be worth trying since it has a lower rate of sexual side effects than other SSRIs and there are some reports that it is effective in ameliorating impulsive–compulsive sexual behavior; however, the possibility of liver abnormalities must be considered. In line with the notion of using comorbidities and symptoms to choose a treatment for impulsive–compulsive sexual behavior, SSRIs can be an excellent treatment because depression and anxiety disorders are common comorbidities and SSRIs are effective treatments for these disorders.

Although an open-label trial of divalproex sodium showed no change in paraphilic symptoms, there is some evidence for the use of mood stabilizers (such as carbamazepine, lamotrigine, topiramate, and valproic acid) in impulsive–compulsive sexual behavior. They have been shown to be effective in some other impulse control disorders, such as pathological gambling. These mood stabilizing agents should be considered in patients who

are identified as bipolar or have prominent impulsivity. These drugs can also be used in combination with SRIs.

In line with the conceptualization of impulsive–compulsive sexual behavior as a behavioral addiction, if the SSRIs or mood stabilizers are not effective, opioid antagonists such as naltrexone and nalmefene may be helpful.

Case reports have noted other agents as successful in treating impulsive–compulsive sexual behavior or paraphilic behaviors; these include buspirone, tricyclic antidepressants, and atypical antipsychotics. Particular subgroups may benefit from adjunctive or other treatment. Patients with ADHD and impulsive–compulsive sexual behavior have been successfully treated with SSRIs augmented with the psychostimulant methylphenidate.

As noted, other agents widely used in the treatment of criminal sexual offenders, such as pedophiles, are often considered too drastic for most cases of impulsive–compulsive sexual behavior. These include anti-androgen treatment like medroxyprogesterone acetate and gonadotropin-releasing hormone. These agents may completely eliminate sexual interest.

Since benzodiazepines can be disinhibiting and increase impulsive behavior, they are generally not recommended for impulsive–compulsive sexual behavior, although they can be helpful in emergency situations.

In summary, when selecting a pharmacological treatment, it is probably best to refine the decision based on comorbidity and prominent symptoms. For individuals with depression or anxiety or prominent obsessive symptoms, an SSRI might be a good choice for a first trial. For those with a more addictive symptom profile, opiate antagonists might be tried. Those on the bipolar spectrum or with prominent impulsivity might be started on mood stabilizers. Patients with prominent ADHD symptoms might benefit from stimulants.

Psychological Treatment

Very little research has been conducted on psychological treatments for impulsive–compulsive sexual behavior. Most psychological treatments that have been studied in paraphilic behaviors might be appropriately applied to impulsive–compulsive sexual behavior. In addition, psychological treatment approaches used with behavioral and substance addictions show promise for impulsive–compulsive sexual behavior. Based on these two traditions, the most promising treatments might be 12-step programs, cognitive–behavioral therapy and motivational interviewing.

Treatments based on 12-step programs are widely used; these include Alcoholics Anonymous-type groups such as Sexaholics Anonymous, Sex Addicts Anonymous, Sex and Love Addicts Anonymous, Sexual Recovery Anonymous, and Sexual Compulsives Anonymous. As with Alcoholics Anonymous, there are also groups for

partners and families of those with impulsive–compulsive sexual behavior (co-dependents of sex addicts and S-Anon international family groups). In addition, formal inpatient and outpatient treatment programs also incorporate this approach.

Cognitive behavioral therapy (CBT) is often used. CBT can help individuals identify the triggers for their sexual thoughts, urges, and behavior. It can be useful in training them to manage stress, improve their coping skills, and managing symptoms of depression and anxiety; all these applications of CBT can decrease patients' susceptibility to their impulsive–compulsive sexual behavior. In general, CBT may also be very useful in preventing relapse. There is some evidence that combinations of CBT and 12-step approaches are effective in reducing impulsive–compulsive sexual behavior.

Other similar disorders, such as pathological gambling and substance addictions, respond to 12-step, CBT and motivational interviewing approaches so these approaches seem promising for impulsive–compulsive sexual behavior.

Conclusions

Impulsive–compulsive sexual behavior may be a serious, chronic, and difficult-to-treat disorder with public health and societal consequences. It is similar in many ways to substance addictions and addiction-like disorders such as pathological gambling. Although there has been little research to date on its pathogenesis, some promising treatment options are currently available, including pharmacological and psychotherapeutic approaches. The condition can be difficult to treat and may be even more challenging than similar disorders mentioned since sex is a basic human drive.

See also: Compulsive Buying; Pathological Gambling; Problematic Internet Use.

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Relevant Websites

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<http://www.sa.org> – Sexaholics anonymous.
<http://sash.net> – The Society for the Advancement of Sexual Health.

Intermittent Explosive Disorder

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Glossary

Diagnostic and Statistical Manual (DSM) – Published by the American Psychiatric Association. In the United States, it is the primary system used to classify and diagnose mental disorders. It is currently in its fourth edition (DSM-IV).

Epiphénomène – A secondary phenomenon that occurs alongside or in parallel to a primary phenomenon, but may not be causally linked to the primary phenomenon.

First line – Preferred, standard, or initially recommended approach; often used in reference to treatment selection.

Impulsive aggression – Also known as reactive or hostile aggression, impulsive aggression occurs in response to angering thoughts. The goal of impulsive aggression is to cause harm to another. This is in contrast to instrumental or premeditated aggression in which anger is not the primary motivator for the aggressive behavior and the aggression is seen merely as a means to an end (e.g., hitting someone to steal their wallet).

Morbid risk – The probability that an individual born into a population or group will develop a disorder.

Selective serotonin reuptake inhibitors (SSRIs) – A class of drugs that increase the amount of serotonin available in the synapse (space between nerve cells) by inhibiting the recapture or reuptake of serotonin by the nerve cells that released the serotonin into the synapse.

individuals who display intermittently violent behavior and who are “generally excitable, aggressive, and over-responsive to environmental pressures” with “gross outbursts of rage or of verbal or physical aggressiveness different from their usual behavior.” In DSM-III, ‘explosive personality’ was codified and operationalized as IED for the first time and assigned ‘clinical disorder’ status under Axis I. The diagnostic criteria, however, were not well operationalized (e.g., criterion A ‘assaultive’ and ‘destructive’ acts had no specific guidelines on which behaviors would satisfy the criteria from a severity, frequency, or time-frame standpoint) and were otherwise problematic. Subjects who were generally aggressive or impulsive in between the ill-defined aggressive episodes were excluded from receiving the diagnosis (criterion C). Since individuals with recurrent, problematic, impulsive aggression are also generally impulsive and aggressive between more severe outbursts, this exclusion ruled out the vast majority (e.g., 80%) of individuals who now would be diagnosed with IED.

With the introduction of the DSM-IV in 1994, the earlier exclusionary C criterion that omitted individuals with chronic aggression problems (effectively excluding most subjects now considered to meet criteria for IED) was removed. Regardless, DSM-IV still lacked objective criteria for the intensity, frequency, and nature of aggressive acts to meet criteria for IED ([Table 1](#)).

Noting the limitations of the DSM-IV criteria for IED, some investigators developed an alternative criteria set that integrated their research findings with DSM conceptualizations of IED ([Table 2](#)) ‘integrated research’ criteria for IED (IED-IR) differ from DSM-IV IED criteria on four key points. (1) IED-IR criteria operationalizes the severity and frequency of aggressive behavior required for the diagnosis. The inclusion of verbal aggression within the IED-IR construct reflects data showing that frequent verbal aggression occurs in over 85% of subjects with physical aggression, that subjects with frequent verbal aggression in the absence of more severe assaultive acts show the same core deficits and impairment as assaultive subjects and that anti-aggressive responses to serotonin reuptake inhibitors are seen in both groups. (2) IED-IR criteria explicitly require the aggressive behavior to be impulsive in nature. This was also informed by research showing psychosocial, biological, and treatment-response characteristics differentiated impulsive and premeditated aggression. (3) IED-IR

Intermittent explosive disorder (IED) is a DSM-IV diagnosis that describes the pathology of people with aggression, typically impulsive aggression. Although aggressive behavior is relatively common, many clinicians and researchers rarely consider the diagnosis of IED when faced with a patient with recurrent, problematic, aggressive behavior. This is notable because IED has been a part of the DSM since its third edition in 1980. Before that, this construct was referred to as ‘passive-aggressive personality (aggressive type)’ and was characterized as “persistent reaction to frustration with irritability, temper tantrums and destructive behavior.” This disorder evolved into ‘explosive personality’ in DSM-II at which time such patients were characterized as being aggressive

Table 1 DSM-IV criteria for intermittent explosive disorder

- A. Several discrete episodes of failure to resist aggressive impulses that result in serious assaultive acts or destruction of property.
- B. The degree of aggressiveness expressed during the episodes is grossly out of proportion to any precipitating psychosocial stressors.
- C. The aggressive episodes are not better accounted for by another mental disorder (e.g., antisocial personality disorder, borderline personality disorder, a psychotic disorder, a manic episode, conduct disorder, or attention-deficit/hyperactivity disorder) and are not due to the direct physiological effects of a substance (e.g., a drug of abuse and a medication) or a general medical condition (e.g., head trauma and dementia of the Alzheimer's type).

Table 2 Research criteria for IED: IED-IR

- A. Recurrent incidents of aggression manifest as either:
 - A1. Verbal or physical aggression towards other people, animals, or property occurring twice weekly on average for one month.
or
 - A2. Three episodes involving physical assault against other people or destruction of property over a one year period.
- B. The degree of aggressiveness expressed is grossly out of proportion to the provocation or any precipitating psychosocial stressors.
- C. The aggressive behavior is generally not premeditated (e.g., is impulsive) and is not committed in order to achieve some tangible objective (e.g., money, power, intimidation, etc.).
- D. The aggressive behavior causes either marked distress in the individual or impairment in occupational or interpersonal functioning.
- E. The aggressive behavior is not better accounted for by another mental disorder (e.g., major depressive/manic/psychotic disorder; ADHD); general medical condition (e.g., head trauma, Alzheimer's Dx); or to the direct physiological effects of a substance.

criteria explicitly require the presence of subjective distress (e.g., in the individual) and/or social or occupational dysfunction in order to specifically link distress/dysfunction to aggressive behavior. (4) IED-IR criteria allow subjects with borderline and/or antisocial personality disorder (BPD/AsPD) to have a comorbid IED diagnosis (i.e., if they otherwise meet the IED-IR criteria, see below). This decision was based on the finding that IED subjects with or without BPD/AsPD are similarly aggressive, and much more aggressive, than non-IED subjects with or without BPD/AsPD. Accordingly, high levels of aggression have been found to be associated with the presence of IED but not the presence of BPD/AsPD. These research criteria for IED have been used in studies of IED in several sites in the United States. Moreover, several of the suggested changes made by 'Research criteria' have been adopted in the text revisions of the current DSM-IV text revision.

criteria for IED, and as many as 6 million or 10 million have met criteria in any 1 month or year, respectively.

Clinical Presentation

Aggressive outbursts in IED have a rapid onset, often without a recognizable prodromal period. Episodes are short-lived – typically less than 30 min – and involve verbal assault, destructive and nondestructive property assault, or physical assault. Aggressive outbursts most commonly occur in response to a minor provocation by a close intimate or associate, and IED subjects may have less severe episodes of verbal and nondestructive property assault in between more severe assaultive/destructive episodes. Episodes are associated with substantial distress, impairment in social functioning, occupational difficulty, and legal or financial problems. In a recent community sample study of more than 9200 individuals, subjects meeting current IED criteria (i.e., defined as three high-severity episodes in the current year) were found to engage in direct interpersonal aggression (67.8%), threatened interpersonal aggression (20.9%), and aggression against objects (11.4%). These subjects reported engaging in, on average, 28 high-severity aggressive acts during their worst year, with two to three lifetime aggressive outbursts requiring medical attention. Mean dollar value of property damage due to IED aggressive outbursts lifetime was approximately \$1600.

Clinical Picture and Course of Illness

Prevalence

Initially IED was described as rare. However, this designation was based on limited empirical research. Four recently published epidemiological studies report that approximately 4–6% of individuals meet lifetime criteria for IED, and 1-month and 1-year point prevalence estimates of IED have been reported to be in the 2–3% range. If so, approximately 16 million Americans have met lifetime

Clinical Example

Roger is a 28-year-old married man who works as a community outreach provider. He was self-referred for anger problems after he found his 4-year-old daughter crying in her room in the wake of a heated argument between Roger and his wife in which Roger punched a wall. Roger acknowledged always being hot tempered, providing examples of getting into shoving matches and two fistfights when playing sports, as well as frequent aggressive behavior (honking horn, screaming at drivers, flipping them off) when driving. His aggressive behavior worsens when he is intoxicated, and he has a history of alcohol abuse. He significantly reduced his alcohol use after the birth of his daughter; despite this he continues to have problems with aggressive behavior. Roger has had to replace his cell phone twice in the past year due to him throwing it in anger. Roger also continues to verbally snap and yell at his wife and occasionally his daughter, which has placed stress on an otherwise happy marriage. He understands that his behavior is excessive, and afterwards often feels guilty and remorseful, but he adds that his anger feels overwhelming at times and that he does not know how to control it.

Quality of Life

High levels of hostility and aggression negatively impact quality of life across several dimensions, including interpersonal relationships, sleep quality, job satisfaction, and health problems. Similarly, the limited data suggest that individuals with IED have more health problems, are more impaired in overall functioning, and are less happy than healthy volunteers or psychiatric controls. Furthermore, their quality of life improves after successful treatment. There are little data on the impact of IED on the quality of life for family members. However, multiple studies have linked witnessing and experiencing aggression in childhood with adverse adult consequences, including intergenerational transmission of aggression.

Age of Onset, Gender and SES

IED appears as early as childhood and peaks in mid-adolescence with a mean age of onset in three separate studies ranging from 13.5 to 18.3 years. In one study, the age of onset was found to occur at a significantly earlier time in males as compared to females. While IED may be more common in males, recent data suggest that IED may occur with a more equal prevalence among men and women. In a large epidemiological survey, sociodemographic variables (e.g., sex, age, race, education, marital, occupational status, and family income) did not differ as a function of IED status.

Comorbidity

Available data suggest that IED is a chronic disorder whose onset precedes other comorbid Axis I disorders. If so, it is unlikely that IED develops into another disorder. More likely, IED promotes the development of other disorders by leading to divorce, financial difficulties, and stressful life experiences that promote onset of other disorders later on in adulthood. In clinical samples, IED has been reported to be highly comorbid with multiple Axis I disorders, including mood disorders, anxiety disorders, and alcohol and other substance-use disorders. In community samples, however, the relationship between current IED and such disorders was only significant for generalized anxiety disorder, alcohol abuse, and any substance-use disorder. The vast majority of subjects reported that IED began at an earlier age than these comorbid conditions.

Familial Correlates

A family history study comparing first-degree relatives of 30 IED, and 20 control, probands found significantly elevated morbid risk for IED in relatives of IED, compared to control, probands (0.26 vs. 0.08, $p < 0.01$). Elevation in the morbid risk for IED was not caused by the presence or absence of comorbid conditions among the IED probands (e.g., history of suicide attempt, major depression, alcoholism, and drug use disorder) and was not due to increases in morbid risk of other non-IED disorders in the relatives (e.g., major depression, alcoholism, drug use disorders, anxiety disorder, any disorder). Accordingly, familial aggregation of IED is not due to an epiphenomenon of the liability of either the proband or the relative to having non-IED comorbid conditions and suggests a clear familial signal. This supports research showing that aggressive behavior is under a substantial degree of genetic influence.

Biology

While laboratory studies clearly show a biobehavioral relationship between aggression and selected brain chemicals (e.g., serotonin), studies in IED, specifically, have been conducted only over the past few years. To date, published data have reported IED subjects as having altered serotonin function compared with non-IED subjects or healthy control subjects. Other studies supporting the IED-serotonin link demonstrate a reduction in: (1) prolactin responses to d-fenfluramine challenge in IED subjects compared with non-IED or healthy control subjects and (2) numbers of platelet 5-HT transporters (via H^3 -paroxetine binding) in IED subjects compared with non-IED or healthy control subjects. These findings are supported by imaging studies. Two fluoro-deoxy-glucose

(FDG) positron emission tomography (PET) studies found low FDG utilization after d,l-fenfluramine challenge in frontal areas of the brain and low FDG utilization after *meta*-chlorophenylpiperazine (*m*-CPP) challenge in the anterior cingulate in IED subjects compared with healthy control participants. A third, ligand-binding study of the serotonin (5-HT) transporter also reports reduced low 5-HT transporter availability in the anterior cingulate in IED versus control subjects. Finally, a functional magnetic resonance imaging (fMRI) study demonstrated increased activation of amygdala, and reduced activation of orbital medial prefrontal cortex, to anger faces in IED subjects as compared to healthy control subjects.

Treatment of IED

Psychopharmacological treatment

Classes of agents shown to have anti-aggressive effects in double-blind, placebo-controlled trials of individuals with primary aggression (i.e., not secondary to psychosis, severe mood disorder, or organic brain syndromes) include mood stabilizers (e.g., lithium), 5-HT reuptake inhibitors (e.g., fluoxetine), and anticonvulsants (e.g., diphenhydantoin and carbamazepine). While noradrenergic (NE) beta-blockers (e.g., propanolol/nadolol) have also been shown to reduce aggression, these agents have exclusively been tested in patient populations with secondary aggression (e.g., mental retardation, organic brain syndromes, etc.). Classes of agents which may have pro-aggressive effects include tricyclic antidepressants (e.g., amitriptyline), benzodiazepines, and stimulant and hallucinatory drugs of abuse (e.g., amphetamines, cocaine, and phencyclidine). Double-blind, placebo-controlled, clinical trials suggest that anti-aggressive efficacy is specific to impulsive, rather than nonimpulsive, aggression.

A double-blind, placebo-controlled trial of fluoxetine on impulsive aggressive behavior in 100 subjects with IED (by research criteria) demonstrated anti-aggressive efficacy for fluoxetine over placebo. This study observed reduction in overt aggressive behavior as reported by subjects, subjective/objective anger/aggression, and a response rate of 70% (CGI scores of much improved or very much improved). Notably, fluoxetine was not associated with any increase in aggression compared with those randomized to placebo. In contrast, placebo was associated with a greater frequency of increased aggression, and increased magnitude of aggression, after randomization compared with fluoxetine treated subjects. While positive in result, only 29% of IED subjects displayed no aggression at end of trial. This indicates that while fluoxetine can reduce impulsive aggressive behavior, remission from IED symptoms may take more than the drug itself. Another placebo-controlled study of IED involving divalproex reported a favorable effect of this

agent on overt aggression but only in IED subjects with comorbid cluster B personality disorder.

Psychotherapeutic Treatments

The authors recently completed a psychotherapy outcome study comparing the efficacy of a 12-week multicomponent CBT treatment presented in either group or individual format to a wait-list control group in the treatment of IED ($N = 45$). The treatment was modeled after the Cognitive Restructuring, Relaxation and Coping Skills Training (CRCST) treatment developed to treat anger, but was modified to serve as a more appropriate treatment for aggressive individuals (e.g., extend treatment from 8 to 12 session, include a time-out technique, and increase the emphasis on the prevention of and relapse to aggression). Aggression, anger, and associated symptoms were assessed at baseline, mid-treatment, posttreatment, and 3-month follow-up. Relative to the wait-list condition, both group and individual CRCST improved anger control and reduced aggression, anger, hostile thinking, and depressive symptoms. Post-treatment effect sizes were large and were maintained at 3-month follow-up, providing initial support for the efficacy of CBT in the treatment of IED.

Other Issues Regarding Treatment of IED

There have been no published studies regarding length of treatment for IED. Our experience is that impulsive aggression is a trait that can be suppressed, but not eliminated, by medication. We have found that within about 1 month of discontinuing fluoxetine that patients with impulsive aggressive behavior often demonstrate a return of impulsive aggressive behavior to pretreatment levels. The one study that examined the effect of lithium on impulsive aggression in prison inmates found that impulsive aggressive behavior returned to pretreatment level within 1 month of being switched to placebo. This finding contrasts with our findings with CRCST. CRCST effects continue at least 3 months after active treatment has ended, probably because treatment components (relaxation training, cognitive restructuring, and coping skills training) have been incorporated into the individual's life and are still active. In contrast, once medication is no longer in the body, its effects on behavior typically end.

There have been no published studies regarding the effect of combining modalities of treatment. Examining our own data with fluoxetine and CRCST in IED subjects, we note that both modalities yield a similar magnitude of improvement in outcome measures (e.g., ~30% remission and ~15% partial remission from IED). Since these two modalities work through different mechanisms, we would hypothesize that together they would be more effective than either alone. Our clinical

experience is consistent with this idea but these data are anecdotal. Whether medication or CRCST should be first-line may depend, in part, on the patient since some prefer medication (some individuals perceive this as easier) and others prefer psychotherapeutic treatment (some individuals wish to avoid being placed on medication). Severity of aggression may also influence choice of modality and one can envision medication (and/or both medication and CRCST) as being first-line in cases of severe aggressive behavior. While an attractive strategy, there are no currently little empiric data to support either approach at this time.

See also: Neural and Pharmacological Substrates of Aggression; Neural Bases of Defensive Aggression; Offensive and Defensive Aggression; Social Competition and Conflict Resolution.

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Kleptomania

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Glossary

Antisocial personality disorder – A psychiatric condition characterized by persistent disregard and violation of the rights of others and what society considers right and wrong.

Escitalopram – A drug in the selective serotonin reuptake inhibitor (SSRI) class which is an isomer of citalopram and is used to treat depression and anxiety.

Opioid antagonist – A class of medications which bind to the opioid receptors in the brain, effectively blocking the effects of opiates (e.g., heroin and morphine). Examples of this class of medication include naloxone hydrochloride and naltrexone hydrochloride.

Serotonergic dysfunction – Problems in the neurotransmission of serotonin.

Ventromedial prefrontal cortex – A brain region that contributes to decision-making and risk-taking.

White matter – Brain tissue consisting of myelinated nerve fibers (e.g., axons).

include significant legal consequences, reduced quality of life, and impaired functioning. Suicide attempts are also common and have been reported in 25–30% of persons in treatment for kleptomania.

Estimates of more than \$13 billion of retail sales lost due to shoplifting have been documented by the National Association for Shoplifting Prevention in 2009. Recent data provided through the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC) illustrate rates of lifetime shoplifting of 11.3% in the United States. Historically, the terms ‘shoplifting’ and ‘kleptomania’ have been used interchangeably but significant differences separate someone with kleptomania from one who shoplifts. Shoplifting is defined as stealing items from a store without a specific motivating factor. Kleptomania, on the other hand, is defined as a psychiatric diagnosis characterized by a diminished ability to resist recurrent impulses to steal objects that are not needed for their monetary or personal use. Although kleptomania has been documented for almost two centuries, it remains a poorly understood disorder with limited data regarding neurobiology or treatment. This article details what is currently known about the clinical characteristics, neurobiology, and treatment of kleptomania.

Introduction

Kleptomania is a psychiatric disorder characterized by persistent and recurrent patterns of stealing. The *Diagnostic and Statistical Manual* (Fourth Edition) defines kleptomania by the following criteria: (1) recurrent failure to resist impulses to steal objects that are not needed for personal use or for their monetary value; (2) increasing sense of tension immediately before committing the theft; (3) pleasure, gratification, or relief at the time of committing the theft; (4) the stealing is not committed to express anger or vengeance and is not in response to a delusion or a hallucination; and (5) the stealing is not better accounted for by conduct disorder, a manic episode, or antisocial personality disorder. Psychosocial problems are common among individuals with kleptomania and

History

Reports of uncontrolled, impulsive shoplifting behavior date back centuries and gained notoriety in the nineteenth century with the advent of the department store which made a variety of goods available in a concentrated area. Upper-middle-class women were caught stealing from these stores, creating a media-frenzy in the newspapers of both North America and Europe describing unbridled shoplifting behavior. It was at this time that the medical community began attempts to explain this behavior.

First coined in 1816 by the Swiss physician Andre Matthey, the term 'klopmannie' was derived from the Greek words 'kleptein' (to steal) and 'mania' (insanity) to describe a person who could not or did not control their stealing behavior. In 1838, Jean-Etienne Esquirol wrote the first detailed description of this seemingly nonvolitional and irresistible behavior. This article on kleptomania was important as it distinguished a person with this disorder from those who steal due to a lack of moral character.

Because of the extensive newspaper reports in the nineteenth century depicting women stealing, the medical community initially attributed this 'exclusively female' behavior to the female reproductive system, specifically uterine diseases and premenstrual tension. Following the dismissal of this explanation in the early twentieth century, partially due to the observation that more men were presenting with similar behavior, nearly all research pertaining to kleptomania ceased. The first *Diagnostic Manual of Mental Disorders* (DSM-I) in 1952 did not include kleptomania as a formal diagnostic illness but rather as a supplementary term. Kleptomania was not included in DSM-II. In 1980, DSM-III categorized kleptomania as an impulsecontrol disorder not elsewhere classified, the same clinical diagnostic category it currently holds in the DSM-IV-TR.

Epidemiology

Although kleptomania has been documented in case studies and newspaper articles for centuries, relatively little is known about its prevalence in the general population. In the only study assessing rates in a general community sample using DSM-IV criteria, researchers examined 791 college students and found that although 28.6% reported having stolen an item in his/her lifetime, only 0.4% met criteria for kleptomania.

Other studies have examined the rates of kleptomania in treatment samples. An adult psychiatric inpatient study ($n=204$) revealed lifetime rates of 9.3% ($n=19$) while 7.8% ($n=16$) had symptoms consistent with a current diagnosis of kleptomania. In a recent study of 102 adolescent psychiatric inpatients, researchers noted that 9 (8.8%) met diagnostic criteria for current kleptomania.

The prevalence of kleptomania in specific psychiatric populations has also been assessed. A study of 79 patients with alcohol dependence found that 3.8% ($n=3$) endorsed symptoms consistent with kleptomania while a study of 107 depressed patients found that 3.7% ($n=4$) suffered from kleptomania. In two studies of individuals with pathological gambling, rates of co-occurring kleptomania were 2.1% and 5%.

A Comparison of Kleptomania and Shoplifting

Individuals with kleptomania differ from 'ordinary' shoplifters in that they do not steal for personal gain, but rather for symptomatic relief. It is estimated that more than \$13 billion worth of goods are stolen from retailers each year, which translates into more than \$35 million per day. Vast majority of shoplifters are described as amateurs with sporadic activity and no known history of criminal activity who steal for their own consumption rather than for resale. Studies involving apprehended, legally referred shoplifters indicate that shoplifting may be more common in women (ranging from 52% to 100%) than in men. However as with kleptomania, these rates may be falsely elevated because women may be more likely to be referred for psychiatric evaluation or seek psychiatric treatment than are men. Male shoplifters are more likely to be apprehended during adolescence and early adulthood, whereas women shoplifters are more likely to be apprehended during puberty/early adulthood and around the age of menopause. Some of these same studies also revealed that shoplifting was not related to lower socio-economic level and that most stole for personal gain. Rates of kleptomania among people who are arrested for shoplifting have ranged from 0% to 8%. A study that compared individuals with kleptomania to shoplifters interviewed directly after apprehension found that 58% of the shoplifters were male compared to only 32.4% of kleptomania patients. The mean age among shoplifters was 27 years and among the kleptomaniacs, 41 years. Although none of the shoplifters met DSM criteria for kleptomania, approximately one-fifth had not stolen for personal use and had eventually discarded the object. The study also found that both groups reported the same degree of impulsivity and 'a feeling of not being oneself.' On the other hand, individuals with kleptomania reported a relatively greater number of previous thefts compared to shoplifters, a finding consistent with a compulsive aspect of kleptomania.

Childhood and Adolescent Stealing

Both typical shoplifting and kleptomania may start at a relatively early age. A young child generally has little, if any, concept of stealing – for him or her, desiring or wanting means possession of the object. By the age of 6 or 7, children begin to realize they are doing something wrong when they take something that does not belong to them. Children may steal because they are unhappy, lonesome, jealous, fearful, or craving attention. For older children and adolescents, stealing can be used to gain acceptance from a group, but is also a strong predictor

of future delinquency and a marker for families lacking in warmth and personal stimulation. A strong attachment to parents decreases involvement in shoplifting.

Overall, studies have shown that roughly 40% of apprehended shoplifters are adolescents. A study involving almost 1700 adolescents found that 37% reported shoplifting at least once in the prior 12 months. The percentage of subjects acknowledging shoplifting peaked around the 10th grade and then declined, consistent with official crime statistics. One hypothesis for adolescent theft involves multiple, non-mutually-exclusive factors involving a function of immaturity during a stressful transition to adulthood, an inability to purchase certain items, and an increased opportunity (the steepest gain of independence occurs around age 16 when most adolescents are allowed to drive and work). On the other hand, adolescents report that they shoplift because of the associated novelty and risk, for social reasons, and because they desire for the stolen items. Additionally, no relationship has been found between family occupational status and adolescent shoplifting. How many of these adolescent shoplifters currently suffer from, or will develop a problem with, kleptomania is not clear. Longitudinal studies of this nature, to help clinicians better assess who should receive treatment, are needed.

Clinical Characteristics of Kleptomania

Case Vignette

Jennifer, a 54-year-old married female, described a history of uncontrollable shoplifting beginning at about age 16 years of age. She started by stealing a scarf ‘in a dare’ from a friend but found the ‘high’ was intense and overwhelming. Over the course of about 1 year, she reports that she progressed from occasional theft to being unable to control herself when she entered a store. Although she has had periods of a year or two without any shoplifting, Jennifer reports that she currently shoplifts once or twice a week. She describes a ‘rush’ each time she steals. In fact, the ‘rush is short-lived’ and when she leaves the store she usually throws away the stolen item or leaves it outside the store. Although she usually steals clothing, she denies really wanting the items and she could easily afford to buy them. Jennifer also describes daily thoughts and urges to shoplift that interfere with her ability to concentrate at work. In addition, she has never told her husband about her behavior. The behavior and the lying cause significant depression and feelings of worthless. Jennifer tried to commit suicide on one occasion, never telling anyone the reason behind her suicide attempt. She also reports drinking in the evening to ‘deaden’ the pain and guilt over her shoplifting.

Kleptomania usually begins in adolescence or early adulthood, with males tending to start at an earlier age,

though kleptomania has been documented in patients as young as 4 years old and as old as 77 years old. Although prospective studies are largely lacking, kleptomania appears to follow a similar trajectory as substance dependence, with high rates in adolescent and young adult groups, lower rates in older adults, and periods of abstinence and relapse.

One study found that individuals with kleptomania stole, on average, twice or thrice a week. The places from which items are stolen and the value of those stolen items generally change over time for those with kleptomania. Although stealing from stores is the most common place of theft, stealing from friends, relatives, and work is not uncommon. The objects stolen by the individual are usually affordable and are typically discarded, hoarded, thrown away, discreetly returned to the store, or given away. Most individuals with kleptomania report that the value of a stolen item increases over time. Examples of commonly stolen objects include sweets, newspapers, food, books, and clothes. The excitement and rush associated with the act of committing the theft and getting away with it are typically immediately followed by feelings of shame and remorse.

Gender Differences

Clinical samples of patients have reported that two-thirds of patients with kleptomania are female. Women with kleptomania are more likely to be married, tend to have a later onset of shoplifting, are more likely to steal household items, and hoard the items stolen. They are also more likely to have a comorbid eating disorder. Men with kleptomania are more likely to steal electronic goods and more likely to have another co-occurring impulse control disorder (most commonly intermittent explosive disorder or compulsive sexual behavior).

Quality of Life Issues and Legal Consequences

Individuals with kleptomania endorse a significant amount of impairment in their daily lives. Independent of comorbidity, they report significantly poorer life satisfaction compared to a general, nonclinical adult sample. Perhaps because many individuals with kleptomania report significant amounts of shame and embarrassment, patients may not tell their significant other about the behavior or endorse kleptomania symptoms until after being arrested or after responding to treating for a co-occurring psychiatric condition.

Along with impairment in the social and occupational realms, legal repercussions are common for individuals with kleptomania. Among clinical samples of patients who meet criteria for kleptomania, a majority have been arrested. In fact, many individuals with kleptomania

have been apprehended multiple times. Because kleptomania often goes undiagnosed for years, it is important upon initial presentation to consider the involvement and education of psychiatric, psychological, and legal professionals.

Psychiatric Comorbidity

Comorbid Axis I psychiatric conditions are common in kleptomania. Substance use (15–50%), eating (9–25%), impulse control (20–47%), and mood (45–100%) disorders are frequently observed. Suicidal ideation among individuals with kleptomania is common.

Personality disorders also appear to be common in kleptomania. High frequencies of paranoid (17.9%), schizoid (10.7%), and borderline (10.3%) personalities have been reported in individuals with kleptomania.

Family History

Few family history and genetics studies of kleptomania have been performed. In the only study to use a control group, the first-degree relatives of subjects with kleptomania had a significantly higher frequency of alcoholism (15.1%) as compared to healthy controls (5.1%). Other studies have reported frequent depression, bipolar, and obsessive-compulsive disorder in the first-degree relatives of individuals with kleptomania.

Neurobiology

Although the etiology of kleptomania is unknown, serotonergic dysfunction in the ventromedial prefrontal cortex has been suggested as one contributing factor underlying the poor decision making seen in individuals with kleptomania. One study examining the platelet serotonin transporter in patients with kleptomania found that the number of platelet 5-HT transporters, evaluated by means of binding of ³H-paroxetine, was lower in kleptomania subjects compared to healthy controls, thereby suggesting some nonspecific serotonergic dysfunction.

Neurocognitive assessment of women with kleptomania revealed, as a group, no significant deficits in tests of frontal lobe functioning when compared to normative values. Those individuals with greater kleptomania symptom severity, however, had significantly below-average scores on at least one measure of executive functioning. Significantly higher rates of cognitive impulsivity were found in kleptomania subjects compared to a control group of psychiatric patients without kleptomania.

Damage to the orbitofrontal–subcortical circuits of the brain has been reported to result in kleptomania. Neuroimaging techniques have demonstrated decreased white matter microstructural integrity in the ventral-medial frontal brain regions of individuals with kleptomania compared to controls. These findings are consistent with reports of increased impulsivity in individuals with kleptomania. These studies also support the hypothesis that specific brain-based differences contribute to some individuals with kleptomania demonstrating a diminished ability to control their impulses to steal.

Treatment

There are limited data regarding effective treatments for kleptomania. Most available data are confined to case reports and case series with small samples of subjects. Although there are no medications approved by the Food and Drug Administration in the United States to treat kleptomania, pharmacotherapy and psychotherapy have shown some early promise in treating this disorder.

Pharmacotherapy

Case series and case reports have suggested a benefit of mono- or combination pharmacotherapy in the treatment of kleptomania. In the case of monotherapy, the following medications have shown preliminary benefit: topiramate, naltrexone, escitalopram, paroxetine, fluoxetine, valproic acid, and fluvoxamine.

In terms of combination therapy the following have been preliminarily reported as successful in treating kleptomania: paroxetine plus valproic acid plus naltrexone; topiramate plus paroxetine; naltrexone plus venlafaxine; lithium plus fluoxetine; trazodone plus tranylcypromine; sertraline plus methylphenidate; and imipramine plus fluoxetine.

To date, there are only two published trials of pharmacotherapy for the treatment of kleptomania. In the first of these trials, 24 subjects received open-label escitalopram. After 7 weeks of treatment, escitalopram was shown to reduce shoplifting urges in 19 (79%) of the participants. Responders were then randomized to a double-blind discontinuation phase where patients either received active medication or placebo. At the end of this portion of the study, no significant differences were seen between active medication and placebo as 50% of those on placebo and 43% on active medication maintained their improvement from the open-label portion of the study.

The other open-label study involved the use of the opioid antagonist naltrexone in the treatment of kleptomania. After 12 weeks, at a mean dose of 145 mg/day, 20% of subjects reported full remission of symptoms and 80% overall had significant improvement in their shoplifting urges and behavior.

A naturalistic study of naltrexone produced similar results. Seventeen subjects were followed over a 3-year period while being treated with naltrexone (mean dose of 135.3 mg/day). The study showed that 41% of subjects reported complete abstinence from stealing and 76% of subjects reported significant reductions in their urges to steal.

Pharmacotherapy and Combination Treatments

Although there have been no studies evaluating the efficacy of psychotherapy for kleptomania, case reports have illustrated the benefit of combining medication with cognitive-behavioral therapy (CBT). Aversion therapy, covert sensitization, and systematic desensitization are CBT techniques shown in case reports to benefit patients with kleptomania. In addition, techniques such as a self-imposed ban on shopping appear to be the most common intervention which allows the patient to treat the behavior without seeking help from a medical professional.

Promising examples of combined psychotherapy and pharmacology for the treatment of kleptomania include: fluoxetine 40 mg/day combined with supportive psychotherapy; fluoxetine 40 mg/day combined with problem-oriented psychotherapy; fluoxetine 20 mg/day plus cognitive therapy; combination of CBT, sertraline 50 mg/day, and a self-imposed shopping ban; and a combination of CBT and citalopram 40 mg/day.

Conclusions

Kleptomania, a disorder currently receiving scant attention from the psychiatric community, may present as a chronic illness for many individuals and cause significant psychological, social, and legal repercussions. Since presentation specifically for kleptomania is rare, it is important that clinicians recognize the disorder and screen patients appropriately. Various treatments have been helpful in case studies and small treatment studies but more research examining etiology and treatment is needed.

See also: Compulsive Buying; Impulsive–Compulsive Sexual Behavior; Intermittent Explosive Disorder; Obesity and Binge Eating Disorder; Pathological Gambling; Problematic Internet Use; Pyromania.

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Relevant Websites

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- <http://shopliftingprevention.org> – National Association for Shoplifting Prevention (NASP).
- <http://www.impulsecontroldisorders.org> – University of Minnesota Impulse-Control disorders Clinic.

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Obesity and Binge Eating Disorder

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Glossary

Body mass index (BMI) – A ratio derived by dividing weight in kilograms by height in meters-squared; useful in the determination of overweight and obesity.

Bulimia nervosa – An eating disorder characterized by bingeing and purging of food.

Cannabinoid – Relating to cannabis. For example, cannabinoid receptors bind cannabis when the drug is introduced in the body. Cannabinoid receptors have been implicated in regulating hunger and this feature may explain patterns of eating behavior (e.g., having the munchies) when smoking cannabis.

Cardiac valvulopathy – A disease involving the valves of the heart.

Diagnostic and statistical manual – A book widely used in psychiatry that categorizes and defines mental health disorders.

FDA – Food and Drug Administration, the US federal agency that regulates drugs and related products, including approving drugs for specific therapeutic indications.

Lipase – An enzyme that breaks down lipids or fats.

Obesity – The condition defined by a BMI ratio above 30; often marked by a co-occurrence of such health complications as hypertension, cardiovascular disease, and type II diabetes.

Overweight – The condition defined by a BMI ratio between 25 and 30; often associated with adverse health measures, albeit to a lesser extent than with obesity.

Type II diabetes – The form of diabetes that typically has its onset in adulthood and is associated with obesity. Type II contrasts with type I diabetes that typically has a childhood onset and is typically not associated with obesity as it has a different pathophysiology.

Introduction

Obesity represents a major public health concern. Although questions remain regarding optimal prevention and treatment approaches, research has illuminated many characteristics of obesity. Binge eating disorder (BED) is characterized by seemingly impulsive or excessive bouts of eating, and BED typically co-occurs with obesity. Both obesity and BED are addressed in the following sections.

Epidemiology of Obesity

Several calculated ratios are used to define overweight states and obesity. The body mass index (BMI) – which is derived by dividing weight in kilograms by height in meters-squared – is the most widely accepted and used measure for the determination and classification of obesity and overweight. Using this system, a BMI value in the 19–25 range generally indicates a healthy, normal weight, while a ratio of 25–30 defines overweight, and a calculation over 30 classifies obesity. Obesity has been subclassified into three types according to BMI: type I (30–34.9); type II (35–39.9), and type III or extreme (≥ 40). However, BMI might not accurately reflect obesity status in some cases; certain athletes, for example, may have high BMIs related to increased muscle mass. The waist-to-hip ratio serves as another useful tool in the diagnosis of overweight and obese states, with measures over 88 cm in women and greater than 102 cm in men indicating abdominal obesity.

According to the World Health Organization (WHO), the global rate of obesity continues to increase at an alarming rate. Over 60% of the US adult population is now classified as overweight. Similar findings are indicated in Europe, Canada, and growing numbers of South American countries. Such startling obesity rates are not

localized to Western, industrialized nations. Rather, a growing tendency toward obesity has also been seen in several African nations – despite strained regional demands on limited food supplies – particularly among black African women in areas that have recently experienced socioeconomic growth and increased urbanization. Many Asian countries, also, have demonstrated similar statistics. By WHO estimates, almost 10% of the global population meets the criteria for obesity.

Clinical Characteristics and Co-Occurring Disorders

The increasing prevalence of obesity has been linked with a rise in illness and death from obesity-related health complications. Over a quarter of a million annual obesity-related deaths occur in the United States alone. Being overweight or obese has been linked to multiple health complications including hypertension, cardiovascular disease, stroke, heart attack, congestive heart failure, hypercholesterolemia, type II diabetes, osteoarthritis, obstructive sleep apnea, and certain cancers.

In addition to lowering life expectancy, the relationship of obesity to disease may negatively impact quality of life. Overweight or obese individuals experience higher levels of stigmatization and social discrimination than those falling within healthy-weight ranges, perhaps accounting for the markedly increased prevalence of anxiety and affective disorders, especially depression, in overweight individuals as compared to their healthy-weight counterparts.

Binge Eating Disorder

BED is characterized by episodes of extreme or out-of-control eating. Majority of individuals with BED are either overweight or obese. BED has been proposed as a separate diagnostic category distinct from obesity and bulimia nervosa. Currently, BED is recognized as a provisional diagnosis in need of further study in the *Diagnostic and Statistical Manual of Mental Disorder*, Edition 4, Text Revision (DSM-IV-TR) and is defined in Appendix B by the following criteria:

- recurrent episodes of binge eating in which an individual consumes an abnormally large quantity in a short time; these episodes are typically associated with eating more rapidly than normal, eating until feeling uncomfortably full, eating in the absence of physical hunger, or eating alone due to embarrassment;
- subjective experience of loss of control over eating behaviors during binge-eating;

- distress regarding eating behavior, often marked by feelings of guilt, disgust, or depression after overeating, as well as concern over weight and shape.

Importantly, the occurrence of such behaviors is not seen in conjunction with regular compensatory measures in response to binge episodes, such as purging, laxative abuse, or excessive exercise, thereby distinguishing BED from bulimia nervosa.

Although BED is associated with obesity, findings have indicated differences between overweight/obese individuals with and without BED. Individuals with BED more frequently demonstrate co-occurring Axis I and Axis II psychiatric conditions than do overweight/obese individuals without BED, particularly with respect to major depression and borderline personality disorder. The association between binge eating and depression seems to operate bidirectionally: while the onset of binge eating may follow periods of depression in many individuals, affective disorders often have their onset prior to BED. Other diagnoses frequently seen in people with BED include other mood disorders, obsessive-compulsive personality disorder, and avoidant personality disorder. Body image disturbances and weight and shape concerns also seem more prominent in obese individuals with BED as compared to those without BED.

Although methodological constraints in the identification of BED (such as the inconsistent and varied use of assessment techniques across different studies) have hindered the precise determination of BED prevalence in the general population, it is estimated that approximately 3% of all adults in the United States, and roughly 8% of all obese adults, suffer from BED. The disorder is seen across ethnic and racial groups, with BED having a slightly higher, although not statistically significant, prevalence in women than in men. Such gender-related differences, however, should be considered preliminary as much of the existing BED research has thus far been limited to exclusively female samples.

As few longitudinal studies have been performed, the natural history of BED is poorly understood. Among clinical samples, many individuals with BED report a long history of the disorder, with a fluctuating course defined by intermittent remission and recurrence.

Etiology of Obesity and Binge Eating Disorder

Although many questions exist regarding the etiologies of obesity and BED, genetic, neurobiological, and behavioral features that may influence the course of these disorders have been identified. From a physiological perspective, obesity may be described as a homeostatic energy

imbalance in which energy intake significantly exceeds energy expenditure. This imbalance, in turn, is modulated by a complex interaction of factors regulating hunger, satiety, and metabolic rate. Specific genetic factors, when combined with certain environmental factors (such as high-caloric diets, low physical activity, or certain *in utero* factors) may significantly increase the likelihood of developing obesity and BED.

Genetics

Twin and family studies investigating the heritability of obesity and BED suggest significant genetic contributions. Obesity was found to occur twice as frequently among individuals with at least one relative who was overweight or obese. These findings varied with severity of obesity: individuals whose relatives fell within the top 1% of BMI distributions were up to 5 times as likely to be overweight or obese as individuals in the general population.

Similar studies suggest a heritability component of approximately 50% in BED. However, only a limited number of studies have thus far explored the genetic transmission of BED, and methodological concerns – such as the heterogeneity of criteria used for defining BED across investigations – leave precise heritability estimates in question.

Molecular investigations have identified associations between extreme obesity and specific genetic factors. A variant of the gene encoding the melanocortin-4 receptor (Mc4r) has been identified in approximately 3–5% of all morbidly obese individuals. However, not all individuals demonstrating this genetic variation are found to be obese, suggesting that environmental factors and/or multiple genes – such as those contributing toward metabolic regulation, energy conversion, and fat storage – may contribute to determining an individual's propensity to obesity. For example, individuals with overweight or obese spouses or cohabitating partners are frequently overweight or obese themselves; thus, existing data suggest a complex interaction between genetic and environmental factors.

Neuroanatomical and Neurochemical Features

Factors governing hunger, food intake, and body weight play important roles in the etiology of obesity. Multiple appetite-related hormones and signaling factors act on receptors in the hypothalamus to trigger hunger responses and indicate satiety upon sufficient food absorption. These signaling factors include leptin, ghrelin, serotonin, dopamine, norepinephrine (NE), corticotropin-releasing factor, neuropeptide Y, gonadotropin-releasing hormone, and thyroid-stimulating hormone. Dysfunction involving any of these factors or the excitatory or inhibitory pathways they influence may potentially impact upon food-intake regulation and contribute to the development of obesity.

Other potential neurochemical elements implicated in the etiology of obesity include dopamine and related systems that influence motivational and emotional states, responses to reward, and reinforcement of behaviors. Prenatal environmental factors may also influence metabolic functioning, with extreme caloric deprivation *in utero*, for example, showing an association with high BMI in childhood.

While no specific neurochemical or structural anomalies have as yet been definitively identified in BED, it has been hypothesized that dysregulation of serotonin pathways regulating mood, appetite, and impulse control may contribute to the disorder. Although few imaging studies have been conducted thus far in BED, early functional imaging data suggest reduced serotonin transporter binding in obese individuals with BED. When exposed to food cues, individuals with BED report a greater subjective desire to eat and demonstrate greater regional cerebral blood flow in the left as compared to the right hemisphere, particularly in frontal brain regions, perhaps suggesting increased reinforcement sensitivity to food-based reward cues in BED. Notably, these findings also seem to indicate that activation patterns in individuals suffering from BED also differ significantly from those seen in subjects diagnosed with bulimia nervosa. Although further imaging work in BED remains to be done, such differential responses to visual food cues between BED and bulimia nervosa subjects seem to highlight the distinctiveness of BED as a condition independent of BN.

Neurocognitive and Behavioral Features

While multiple influences impact disease etiology, environmental factors and lifestyle choices (governed by motivational behavioral decision-making) seem particularly important in the development of obesity. Among these behavioral elements, dietary habits, eating patterns, and physical activity seem particularly relevant.

Obesity co-occurs frequently with mental health disorders, and this relationship seems especially salient for women. In particular, depression and anxiety are reported at higher rates among individuals who are overweight or obese, and the extent to which this relationship reflects genetic influences, environmental contributions such as those related to stigmatization and social discrimination, or an interaction between genetic and environmental factors remains to be determined.

While an imbalance between energy intake and expenditure may summarize obesity's etiology, caloric intake does not in, and of, itself predict obesity. Rather, the nutritional nature of food eaten seems particularly important, with BMI correlating with dietary fat consumption.

Decreased physical activity is also associated with obesity. Lifestyle changes over the past few decades include substantially decreased levels of average daily physical activity. Frequent usage of motorized

transportation, for example, has drastically reduced the reliance on more active methods of travel such as walking or cycling. Daily television viewing hours have significantly increased in many industrialized nations. These factors may contribute to the rapidly increasing rates of childhood obesity.

Factors associated with BED include adverse childhood experiences, including physical or sexual abuse, family problems, bullying, and teasing or negative criticism about weight, shape, or eating. These findings suggest that binge eating may function as a type of maladaptive emotional coping mechanism.

Treatment of Obesity and BED

Today, the three most widely accepted strategies for the treatment of obesity and BED are behavioral therapies such as cognitive behavioral restructuring (often employed in conjunction with training for healthier diet and increased physical activity), pharmacotherapy, and, in extreme cases of obesity, surgery.

Cognitive Behavioral Treatment of Obesity

A goal-oriented treatment strategy focusing on the direct modification of dietary and exercise habits, cognitive behavioral therapy (CBT) is widely used in the treatment of obesity. With a clearly defined objective of behavioral change, this approach centers on building new skill sets and advocates steady, reasonable progress to change, rather than sudden, drastic (and typically short-lived) behavioral modification. Cognitive behavioral approaches for obesity often include a nutritional education component and strategies for increasing physical activity.

Cognitive restructuring is also typically incorporated within a CBT program to minimize self-defeating thoughts or unrealistic goals which, when not met, may deflate self-confidence and hinder treatment efficacy. The strengthening of skill sets such as communication and self-control in situations which have previously led to overeating is also addressed. Special attention is often given to teaching self-monitoring, or the thoughtful observation of one's behavior, especially with respect to food selection and intake, physical activity, and environmental and emotional cues associated with periods of overeating. By focusing on the development of such skills, CBT in the treatment of obesity aims to help individuals understand circumstances and motivations which may lead to overeating and learn to use a variety of different problem-solving strategies to recognize and avoid such lapses in the future, thereby helping patients gain improved control over eating behaviors.

Behavioral modifications of diet and exercise are presented to help individuals reach a reasonable weight loss

goal. Through the course of treatment, individuals learn the importance of well-balanced diets and are encouraged to gradually modify eating patterns to establish healthier, more nutritious eating habits. An integrated exercise component is perhaps one of the most critical aspects of a CBT program for obesity, as sustained increased physical activity seems to be a strong predictor of long-term weight control. Lifestyle changes which may directly lead to increased activity, such as encouraging using the stairs instead of an elevator or walking to a nearby destination instead of riding in the car, are generally advocated over the sudden introduction of more high-intensity exercise approaches. Such small behavioral changes leading to increased energy expenditure have proven more successful in encouraging a long-term commitment to habitual activity.

CBT has shown short-term efficiency in terms of weight loss, with many individuals losing an average of 10% of their initial weight in the short term, although further research remains necessary to examine the long-term success of such treatment strategies. Preliminary findings suggest a typical weight regain of approximately one-third of initial loss in the first year following treatment. Furthermore, almost 50% of patients completing a CBT program for obesity return to baseline weight within 5 years. While studies suggest that follow-up weight-maintenance programs may help individuals continue to utilize weight control skills by emphasizing positive coping with slips and relapses, such courses seem to delay, not prevent, weight regain in the future. To this end, the incorporation of a therapeutic pharmacological component may prove useful in the continued treatment of obesity.

CBT Treatment of BED

Arguably even more so than in obesity, CBT appears helpful for individuals with BED. Because BED shares features with bulimia nervosa, some treatment approaches for BED have been modeled after those for bulimia nervosa.

While focusing primarily on the reduction and control of binge eating episodes, CBT in BED aims to improve an individual's body image and simultaneously address co-occurring psychological disorders. Toward these ends, structured patterns of food intake may be emphasized with the specific goal of reducing instances of binge eating. Concurrently, cognitive restructuring techniques may be employed to help reshape an individual's often negative attitudes toward weight, shape, body image, and food in general. Like with CBT in obesity, other elements may include the teaching of self-monitoring skills, nutritional education, and useful techniques for relapse prevention.

Weight loss, while also important in CBT for BED, is often postponed until an effort has been made toward controlling binge eating behavior. Thus, in addition to nutritional education and an emphasis on regular, structured eating habits, CBT in BED may encourage exercise and increased physical activity. CBT has shown positive results in the treatment of BED, although, as with the treatment of obesity, there may exist limited long-term success in maintaining weight loss and controlling binge eating in obese individuals with BED.

In addition to CBT, other psychotherapy strategies, including interpersonal and dialectical behavior therapy (DBT), have shown promise in the treatment of BED. Modeled after therapeutic treatment programs for depression, interpersonal therapy (IPT) aims to address negative mood and interpersonal conflict arising from repeated episodes of binge eating. DBT, in turn, is structured on the basis of an affect regulation model of binge eating. Under this paradigm, binge episodes are viewed as maladaptive strategies for coping with unpleasant affect. Therefore, DBT aims to help individuals learn to regulate emotion by using healthier coping mechanisms. While both techniques have shown some efficacy in reducing the frequency of binge eating, further investigation is necessary to optimize treatment strategies for BED.

Pharmacological Treatment of Obesity

Pharmacotherapies for obesity might be broadly categorized into two groups: drugs that produce weight loss by interfering with intestinal digestion of fat and those that lead to weight reduction through appetite suppression or other central, brain-based mechanisms.

Of the lipase-inhibiting drugs, orlistat is currently the most widely prescribed. Treatment programs incorporating orlistat have shown modest weight reductions in obese patients and may help reduce the risk of type II diabetes. Due to its mechanism of action, orlistat is not recommended for individuals whose dietary intake is composed of less than 30% fat, as little weight loss is typically seen when these subjects are treated with the drug. Adverse events seen with the use of orlistat may include a decrease in absorption of fat-soluble vitamins presumably related to the drug's inhibition of intestinal triglyceride digestion.

The appetite-suppressing class of drugs, in contrast, reduces weight in obesity by decreasing food intake through several different paths of action. Benzphetamine, phendimetrazine, and diethylpropion stimulate the release of NE and have only been approved by Food and Drug Administration (FDA) for short-term use. Rimonabant, a cannabinoid antagonist which binds CB1a receptors, is indicated for use as an anti-obesity agent. Mazindol acts by blocking NE reuptake, and sibutramine – which has approval for long-term, maintenance

treatment of obesity – operates by blocking both NE and serotonin (5-HT) reuptake. Adverse effects related to appetite-suppressing drugs vary and may include dry mouth, constipation, and insomnia. Sibutramine may also produce a small but medically significant increase in pulse and blood pressure, therefore warranting careful observation and contraindicating the drug's use in some individuals with hypertension and heart disease.

In addition to the lipase inhibitors and appetite suppressants currently in use for the treatment of obesity, several other drugs are actively being explored for their potential efficacy. Not currently approved for weight loss, bupropion has been associated with weight loss in the treatment of depression, perhaps related to the drug's noradrenergic properties. Topiramate, a carbonic anhydrase inhibitor used in the treatment of epilepsy and bipolar disorder, has also been associated with weight loss. Antagonists to the melanin-concentrating hormone peptide receptor – which are found primarily in the lateral hypothalamus and regulate food intake – also present a potential future course in the treatment of obesity.

While current and developing pharmacotherapy approaches offer encouraging prospects, drug therapy courses are not without complications and undesirable consequences. For example, fenfluramine, dexfenfluramine, and phentermine have been associated with instances of cardiac valvulopathy and withdrawn from the market. The prescription of other drugs, especially amphetamines and methamphetamines which decrease body weight by stimulating thermogenesis and reducing appetite, should be carefully considered given their addictive potential.

Although pharmacotherapy has shown efficacy in the treatment of obesity, the most effective strategy for the treatment of obesity may involve the combination of medication and behavioral interventions, like CBT, that integrate elements of dietary modification and increased physical activity.

Pharmacological Treatment of BED

While psychotherapeutic approaches such as CBT, IPT, and DBT have shown efficacy in reducing binge frequency, they have demonstrated little success in long-term maintenance of weight loss in BED. To this end, the pursuit of pharmacological approaches to treating BED has been examined.

Although no definitive pharmacological treatment has yet been established for the treatment of BED, at least three different classes are being investigated: serotonergic and noradrenergic drugs used in the treatment of depression, appetite suppressants currently used in the treatment of obesity, and anticonvulsants which have shown efficacy in the treatment of bipolar disorder.

Selective serotonin reuptake inhibitor (SSRI) antidepressant medications, including fluoxetine, fluvoxamine, sertraline, and citalopram, have been found to reduce the frequency of bingeing episodes and contribute toward a decrease in body weight. Tricyclic antidepressants, such as desipramine and imipramine, which block the reuptake of NE, have also shown some efficacy in the treatment of BED.

In addition to antidepressant medications, centrally acting appetite suppressants that have serotonergic and noradrenergic mechanisms of action, such as sibutramine, have shown some preliminary success in the treatment of BED. Furthermore, anticonvulsants have been associated with modest reductions in both weight and frequency of binge episodes in preliminary investigations. Zonisamide, for example, has shown peripheral weight loss in its use as an antiepileptic and has been found to reduce episodes of binge eating, although further clinical investigations are necessary to explore the efficacy of this drug in the treatment of BED. While these initial findings are encouraging, further research is needed to demonstrate the short- and long-term effectiveness of pharmacotherapies in the treatment of BED and to identify who might respond best to which treatments.

Surgical Approaches to the Treatment of Extreme Obesity

In cases of extreme obesity (BMI greater than 40 or greater than 35 with some comorbid disorders such as type II diabetes, hypertension, heart disease, and significant arthritis in weight-bearing joints) where repeated dietary and exercise modification efforts have been ineffective, surgical techniques may offer a promising treatment alternative. There are three central classes of surgical techniques in practice today for the treatment of extreme obesity: restrictive surgery, malabsorptive procedures, and mixed procedures such as gastric bypass which combine elements of both restrictive and malabsorptive interventions. While all three types of surgeries aim to reduce weight, each has differences with respect to efficacy and adverse effect profiles.

In restrictive procedures – which limit food intake to induce weight loss – surgical techniques are used to reduce stomach size. On average, patients who undergo the procedure lose between 40% and 60% of excess weight within the first 2 years postsurgery. While such surgeries have shown success in treating extreme obesity, they are not encouraged in the treatment of overweight individuals who also suffer from BED, nor in patients whose BMI exceeds 50, in part related to surgical risks. Unfortunately, individuals with extreme obesity who undergo restrictive operations may experience subsequent weight gain and complications, including band

slippages or erosions and pouch dilations which may necessitate further corrective surgery.

Malabsorptive procedures, in contrast, reduce nutritional absorption by diminishing the amount of mucosal surface encountered in the process of digestion through a bypass of certain areas of the intestinal tract, thereby resulting in weight loss. Strictly, malabsorptive procedures are now implemented more rarely as they may be associated with malnutrition problems.

Mixed procedures describe an array of surgical techniques which combine elements of both restrictive and malabsorptive surgical strategies. Of these, gastric bypass is the most common operation in use today. In this procedure, a reduced stomach capacity decreases food intake, while an intestinal shortening and bypass incorporates the advantages of malabsorptive procedures. While leading to weight loss, gastric bypass procedures may improve symptoms of diabetes as well. Data suggest that gastric bypass procedures may be superior to restrictive procedures alone in weight loss and in reduction of obesity-related health problems.

Although surgical interventions in the treatment of obesity have been used for several decades, there remains little standardization, and criteria for choosing one intervention over another remain to be established. Toward this end, further research directly comparing the efficacies of various surgical approaches is needed. Such procedures, however, represent a promising development in the treatment of individuals with extreme obesity that fails to respond to other therapeutic strategies.

Obesity in Children and Adolescents

In recent years, the prevalence rates of obesity in children and adolescents have increased at an alarming rate. Today, BMI represents the most effective tool for assessing obesity in children and adolescents, where age- and gender-specific BMI ranges are used to categorize underweight, normal weight, overweight, and obese individuals. One approach uses percentile values along an estimated normal distribution curve of BMI values for each respective age and gender group, with values falling between the projected 85th and 95th percentiles of BMI indicating overweight, and BMI ratings above the 95th percentile describing obesity.

However, current estimates suggest that a much larger percentage of children have BMI ratings falling above the projected 85th percentile mark than would be expected under a pattern of normal distribution. By this classification standard, approximately one-third of all American children are overweight, as are roughly 14–22% of their European counterparts, with about 9–13% of all children falling within the classification ranges for obesity.

Childhood obesity has been associated with multiple negative health measures, including hypertension, glucose intolerance and insulin resistance (both conditions often seen as a precursor to type II diabetes), and respiratory abnormalities. Childhood obesity is linked with both obesity and obesity-related health complications in adulthood. Overweight and obese children may experience stigmatization which may hinder development of social skills in adolescence and negatively impact self-esteem. Emotional and behavioral problems are also often seen in overweight children.

Since being overweight as a child appears to predict adult obesity, early implementation of preventative strategies is important. Behaviorally oriented programs may teach children regarding the importance of proper nutrition and healthy diets while also encouraging physical activity over pastimes such as watching television and playing video games. Therapeutic approaches, such as those based on CBT approaches used in the treatment of adults with obesity, have shown some success when implemented with overweight and obese children. Such programs may be particularly helpful with the involvement of parents (and other family members), who are encouraged to help their children continue at home the techniques learned in treatment sessions.

Binge Eating in Children and Adolescents

While patterns of binge eating may be seen early in life, it remains difficult to assess the prevalence of BED in this population, principally because diagnostic criteria for identifying BED have not been adequately established or validated in children and adolescents. Findings suggest a 7–28% occurrence rate of binge eating episodes in children and adolescent (the broadness of this range is perhaps accounted for by the use of different definitions of binge eating across different investigations). BED as defined in the DSM-IV-TR may be seen in roughly 1–3% of children and adolescents. The relationship between childhood binge eating and adult BED requires further research.

Case Example

The following account of a BED patient's history illustrates one possible course of the illness. Amy, a 29-year-old single female, describes frequent episodes of extreme eating in which she often feels out of control over the quantity of food she consumes, eats more rapidly than normal, and habitually eats when not physically hungry or until she feels uncomfortably full. She does not report ever engaging in any sort of compensatory behaviors

(such as purging or excessive exercise) following binge episodes, although she does endorse feelings of guilt, embarrassment, and/or depression after excessive eating. Her medical history reveals that she has experienced at least one major depressive episode in the past 3 years and has previously sought treatment for anxiety. The patient has a BMI of 31 and is being treated for hypertension. Treatment approaches in this case could include cognitive behavioral restructuring coupled with SSRI-based pharmacotherapy to jointly target the reduction of binge episodes. While the primary goal of CBT would be to manage the frequency binge eating, a secondary objective of weight loss through training for healthier diet and increased physical activity would also be pursued once the occurrence of binge episodes has been controlled. The patient's body image and co-occurring psychological disorders would also be addressed.

Conclusion

Although recent research has contributed to an improved understanding of obesity and BED and their symptoms, related features, underlying causes, and potential treatments, many questions still remain. With continued research in this field, however, the development in the near future of new and effective approaches to the prevention and treatment of these conditions seems promising.

See also: Control of Food Intake; Gastrointestinal Peptides and the Control of Food Intake; Stress and Energy Homeostasis.

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Pathological Gambling

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Glossary

Appetitive – Driven by a hedonic or pleasurable feeling or motivation.

Diagnostic and Statistical Manual – A book widely used in psychiatry that categorizes and defines mental health disorders.

Ego-dystonic – In opposition with the ego, or unpleasurable.

Ego-syntonic – In conjunction with the ego, or pleasurable.

Gambling – Placing something of value at risk in the hope of gaining something of greater value.

Microdialysis – A method for measuring levels of chemicals within the brain.

Pathological gambling – A mental health disorder characterized by excessive and interfering patterns of gambling.

Polymorphism – In genetics, a term used to describe a variant form of a gene, gene segment, or other segments of DNA.

knowledge that there is a high probability of severe social and economic consequences. This may be particularly true when aspects of tolerance develop, akin to and suggestive of drug tolerance in drug dependence.

One conceptual framework has been proposed in which impulsive features of PG are considered along an impulsive-compulsive spectrum similar to obsessive-compulsive disorder (OCD). In this framework, affected individuals are purported to experience an intense unpleasantness resulting in attendant neurophysiological compensation, and these processes, in turn, may promote an intense drive to perform the specific behavior, namely gambling. A key characteristic hypothesized to distinguish these obsessive-compulsive spectrum disorders is the degree to which an ego-syntonic component of PG is related to an underestimation of risk. By contrast, ego-dystonic feelings in OCD may lead to an overestimation of harm, risk aversion, and anticipatory anxiety. Alternate models consider the relationship between PG and mood regulation. Given the frequent co-occurrence of PG and depression and suicidal ideation and impaired judgment, as is also seen in bipolar disorder, PG has been conceptualized as an affective spectrum disorder. A third conceptual model posits PG as a non-substance-related addiction, consistent with its frequent co-occurrence with substance use disorders (SUDs).

Although the term addiction was not initially linked to excessive patterns of substance use, it has been specifically used to define ‘impaired control over substance use behaviors.’ However, recent arguments bolstered by biological data have been forwarded to consider non-substance-use illnesses such as PG as addictive disorders. One element proposed as a common characteristic has been loss of control or impaired control over a particular problem behavior with the attendant adverse consequences.

Case Example

James is a 43-year-old, married African-American man who suffers from compulsive thoughts of gambling, an inability to resist the urge to gamble, alcohol dependence, and high blood pressure. James began gambling and experimenting with drugs at age 16. Despite being a talented student-athlete, he frequently smoked pot on the weekends and drank beer with his friends, who would gamble and get involved in petty criminal mischief. When James went to college, his academic performance began to decline as he frequented a popular

Phenomenology

Of the formal group of impulse control disorders (ICDs) not elsewhere classified, which also includes intermittent explosive disorder, kleptomania, pyromania, trichotillomania, and ICDs not otherwise specified, pathological gambling (PG) arguably represents the disorder studied in greatest detail. ICDs have been characterized as obsessive-compulsive spectrum disorders and behavioral addictions, and diagnostically they are grouped in the *Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (DSM-IV)* as a heterogeneous cluster of disorders linked by failure-to-resist impulses to engage in harmful, disturbing, or distressing behaviors. Thus, a core set of clinical features characterize these disorders: (1) compulsive and repetitive performance of the problematic behavior despite adverse consequences; (2) diminished control over the behavior; (3) craving or appetitive urge states that precede the performance of the behavior; and (4) pleasure or release of tension derived from the behavior. These features are exhibited by individuals with PG who continue to gamble until financial resources are exhausted, and who do so with the

athlete-oriented, off-campus nightclub. He spent the majority of his time socializing, getting high, and skimping on his studies. After college, he married. Thereafter, his doctor sternly advised him to lose weight and cut down on alcohol intake, because of his positive family history of coronary artery disease and borderline hypertensive readings. Eventually, James resolved to get back into shape and was able to reduce his alcohol consumption and avoid gambling.

However, over the past 2 years, he has steadily become more engaged in high-stakes gambling activities, is preoccupied with gambling, always planning his next gambling excursion, and seeking ways to obtain money for gambling. He has begun to drink more heavily as the frequency of his gambling has escalated. His tolerance to alcohol has increased and he drinks daily to control symptoms of withdrawal, such as late-afternoon tremors.

James' drinking and gambling forays have left the family's finances in ruins and have had an impact on his job performance. Often, he arrives late for work and is exhausted due to gambling. He and his boss have clashed over the poor quality of his work. At home, he and his wife bicker constantly over their finances and the increasingly large cash withdrawals he makes before the weekend. At the time of his evaluation, James reported gambling about 3 times during the workweek and the entire weekend. When not gambling, he reports intense urges to gamble. When he is gambling, he reports alcohol cravings and a feeling of relief that comes over him when he has his first drink of the day, usually immediately after work at his favorite watering hole. When he goes to the casinos, he is mesmerized by the atmosphere and gambles up to 8 h continuously.

Over the past several months, his wife has become more resentful because of his worsening gambling and alcohol addiction, his lack of attention to his family, and his refusal to get help. This has left him feeling hopeless,

guilty, anxious, and depressed. He fights these feelings by drinking more heavily, and resolving to hit it big the next time. He has borrowed money from his family, and as it became obvious that he had a gambling addiction, they refused additional loans. He insists that his behavior is not problematic but has become desperate, resorting to illegal activity, including placing bets with a local bookie. Recently, he started having an affair and justifies this behavior by stating that his wife is distant because "she is hung up on material possessions."

Diagnosis and Assessment

In 1980, PG was introduced into the *DSM*. Having undergone several revisions since its inception, a current listing of diagnostic criteria is included in **Table 1**. As the above case vignette illustrates, current diagnostic criteria for PG share similar features with substance dependence, including interference in major areas of life functioning, tolerance, withdrawal, and repeated unsuccessful attempts to cut back or quit. It is characterized by persistent and recurrent maladaptive patterns of gambling. Screening instruments include the South Oaks Gambling Screen (SOGS) and symptom severity instruments include the Yale Brown Obsessive–Compulsive Scale for Pathological Gambling (PG-YBOCS) and Gambling-Symptom Assessment Scale (G-SAS).

Epidemiology

In surveys conducted throughout the United States and Canada, prevalence rates of PG ranged between 0.4% and 2.0%; in Australia, New Zealand, and Europe, 0.2–2.1%; and in Asian countries, 1–2%. A meta-analysis of prevalence studies performed in North America over several decades estimated the lifetime prevalence rates of problem gambling and PG at 3.9% and 1.6%, respectively.

Table 1 Diagnostic criteria for Pathological Gambling

- A. Persistent and recurrent maladaptive gambling behavior as indicated by five (or more) of the following:
 - 1. is preoccupied with gambling (e.g., preoccupied with reliving past gambling experiences, handicapping or planning the next venture, or thinking of ways to get money with which to gamble)
 - 2. needs to gamble with increasing amounts of money in order to achieve the desired excitement
 - 3. has repeated unsuccessful efforts to control, cut back, or stop gambling
 - 4. is restless or irritable when attempting to cut down or stop gambling
 - 5. gambles as a way of escaping from problems or of relieving a dysphoric mood (e.g., feelings of helplessness, guilt, anxiety, depression)
 - 6. after losing money gambling, often returns another day to get even (chasing one's losses)
 - 7. lies to family members, therapist, or others to conceal the extent of involvement with gambling
 - 8. has committed illegal acts such as forgery, fraud, theft, or embezzlement to finance gambling
 - 9. has jeopardized or lost significant relationship, job, or educational or career opportunity because of gambling
 - 10. relies on others to provide money to relieve a desperate financial situation caused by gambling
- B. The gambling behavior is not better accounted for by a Manic Episode.

Hence, while a majority of people (86%) are thought to have engaged in some form of gambling activity, only a relatively small fraction develops gambling problems. In adolescents and young adults, higher lifetime prevalence rates of problem gambling and PG have been reported, 9.45% and 3.88%, respectively. Males may represent a high-risk group, with most studies reporting a male-to-female ratio of approximately 2:1. However, the recently increased accessibility to legalized gambling, particularly, forms that appear more problematic for women such as slot machines, presages a decline in this ratio. Some studies have suggested that minority groups, particularly African-Americans, represent a high-risk group for PG. For example, African-Americans comprised 31% of problem gamblers as compared with 15% of recreational and 21% of nongamblers in the St. Louis Epidemiologic Catchment Study. The limited data on Hispanic, Asian-American, and Native American groups suggest that they may be at heightened risk for developing problem gambling or PG.

Psychiatric Comorbidity

An important factor in establishing effective treatment regimes is having reliable data on comorbidity of PG and other psychiatric disorders. In a placebo-controlled, double-blinded, randomized, parallel-arm trial, lithium was superior to placebo in reducing symptoms of gambling and mania in individuals with PG and co-occurring bipolar spectrum symptomatology. Furthermore, in an independent trial in individuals with PG and co-occurring anxiety disorders using open-label escitalopram, followed by double-blind discontinuation, reductions in gambling and anxiety occurred during the open-label phase that persisted after double-blind discontinuation in the active condition but not the placebo one.

Individuals with SUDs have been reported to have 4- to 10-fold higher rates of PG (rates of 5–15% depending on the substance and the study). Studies have also found high rates of SUDs in PG cohorts, with rates as high as 45–55%, and nicotine dependence in the range of 70%. Some data suggest that PG and certain SUDs result from shared genetic factors representing a common etiologic basis. The presence of a co-occurring SUD may have a significant impact on clinical outcome. A study of individuals with alcohol dependence and a co-occurring nongambling disorder found that the presence of PG symptomatology was associated with a poorer treatment outcome. Therefore, it is important that concurrent treatment of gambling and comorbid disorders be tested further such that more precise treatment algorithms can be developed and validated.

Clinical Course, Pathophysiology, and Classification of Pathological Gambling

PG has been associated with impaired social functioning, bankruptcy, divorce, and incarceration. PG often begins early in the teen and young adult years, particularly among males. The progression of PG appears similar to that seen in SUDs, with high rates among youth, low rates among older adults, and periods of gambling interspersed with periods of abstinence. A ‘telescoping’ pattern, defined as a foreshortened time-period between initiation and problematic levels of behavioral engagement seen in women as compared to men, is observed in both PG and SUDs.

PG and other ICDs may go unrecognized in clinical settings. In one study that actively screened for ICDs following admission, over 30% of patients hospitalized for psychiatric care were found to have a current ICD, and this percentage contrasted with the less than 2% who were diagnosed with an ICD at the time of their hospitalization. Improved identification of ICDs with brief screening instruments may thus help to identify patients who could benefit from treatment of ICDs including PG.

Proposed Models of Addiction, Impulsivity, and Pathological Gambling

The core clinical features of addiction overlap with those for impulsivity. Impulsivity has been defined as “a predisposition toward rapid, unplanned reactions to internal or external stimuli with diminished regard to the negative consequences of these reactions to the individual or others.” Thus, PG has been described as a “behavioral addiction” or an “addiction without the drug” because of shared similar features with substance dependence. Blum and colleagues have conceptualized a mechanism underlying impulsivity and addiction, using the term ‘reward deficiency syndrome.’ They hypothesize that diminished dopamine (DA) function in mesolimbic reward pathways renders affected individuals sensitive, potentially placing them at elevated risk for addictive, impulsive, and compulsive behaviors. Alternatively, the “impaired response inhibition and salience attribution” model of addiction and the allostatic addiction model proposed by Koob and Le Moal suggest that, among other aspects, there exist alterations in the DA reward pathways in people who are vulnerable to addiction. Each model incorporates important environmental and genetic contributions to the development of addictions.

Neurobiology

The DA neurotransmitter system represents an important component of the brain reward and reinforcement processes, particularly as related to drug addiction. DA neurons project from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc). These neurons are important for reward reinforcement because interruptions in DA impulse trafficking along axonal routes or at the receptor level decrease the rewarding influences of VTA-related DA stimulation. This system may be perturbed in addictions wherein pleasurable sensation may be associated with DA release, and altered dopaminergic pathways may stimulate reward-seeking behavior. For example, acute ingestion of cocaine increases DA transmission in the basal ganglia, which may enhance the behavioral reinforcement and learned associations encoding the addictive behavior.

In addition to the DA system, other monoamine systems have been implicated in addictions. Serotonin (5-HT) has been proposed to be particularly relevant to the initiation and cessation of the problematic behavior, and norepinephrine (NE) appears particularly relevant to arousal and excitement. Abnormalities in DA, 5-HT, and NE neurotransmitter systems have been reported in PG. Hypotheses that drive the investigation of medications in the treatment of PG are typically based on the neurobiology of PG. Effective pharmacological treatments may also provide insights into the pathophysiology of PG.

Animal Models of Impulsivity and Neuronal Activation

Animal models have been used to assess impulse control. Preferred-choice paradigms of impulsivity may involve simultaneous measurements of real-time neurophysiological and neurochemical data. Such models suggest that ventral and dorsal prefrontal regions represent distinct neuroanatomical substrates of impulsive behavior related to specific aspects of monoamine neurotransmission. The discrete ventral and dorsal areas of frontal cortex implicated in impulse control have been further divided into functionally dissociable areas. DA, 5-HT, and their metabolite concentrations in rat medial prefrontal cortex (mPFC) and orbitofrontal cortex (OFC) were measured using *in vivo* microdialysis during a model of impulsive choice, with mPFC-related 5-HT efflux specifically implicated. By contrast, increased 3,4-di-hydroxyphenylacetic acid (DOPAC, a DA metabolite) levels were observed in OFC, suggesting a double-dissociation, implicating frontocortical 5-HT and DA neuromodulation during impulsive decision making. In a similar experiment designed to measure DA release in PFC, Listar-hooded rats were tested on a visual attention task based on a behavioral disinhibition

model of impulsivity. Compared to their pretask levels, DA and DOPAC efflux increased significantly (~100%) during performance runs. The increase in DA release observed in the PFC was associated with performance measures. Those animals demonstrating a greater tendency for impulsive behavior were noted to demonstrate a similar magnitude of DA response. Primate studies of impulsive decision making have also implicated medial PFC function. Together, these animal data complement those from human studies suggesting an important role for ventral and medial prefrontal cortical function in impulsive behaviors.

Brain Imaging

Relatively few brain-imaging studies have investigated the ways in which brain function in individuals with PG differs from that in those without PG. One early functional magnetic resonance imaging (fMRI) study found that during exposure to gambling videotapes, men with PG, as compared with control comparison men, show relatively diminished activation of cortical, basal ganglia, and thalamic brain regions. These findings contrast with cue provocation studies in OCD in which relatively increased activation of these regions was reported. During the period of most intense gambling stimulus presentation, subjects with PG were distinguished from those without by showing diminished activation of the ventromedial prefrontal cortex (vmPFC), a brain region implicated in emotional regulation and decision making. A separate study of cognitive control used the Stroop color-word interference task and found that subjects with PG were distinguished most from those without by showing less activation of vmPFC. Subsequent fMRI studies investigating simulated gambling and decision making have found that individuals with PG (with or without SUDs) show less activation of the vmPFC when compared with nonaffected control subjects. Thus, these studies indicate that PG subjects show less activation of the vmPFC during performance of multiple cognitive and behavioral processes. These findings extend data from stroke patients with vmPFC lesions, who demonstrated disadvantageous decision making. Another brain region implicated in PG is the ventral striatum, a region including the NAcc, wherein less activation was seen during a simulated gambling task. Taken together, these studies suggest an important role for ventral cortico-striatal function in the pathophysiology of PG.

Genetics

PG is heritable, with twin studies suggesting that over 50% of the variance for PG is related to genetic contributions. These findings are similar to those for drug addictions in which 30–60% of the contributing variance is estimated to be genetic in nature. There is also overlap

in genetic and environmental contributions between PG and other comorbid disorders such as alcohol dependence and antisocial personality disorder. In comparison, a similar study found that the overlap between PG and major depression was accounted for predominantly by shared genetic factors.

Candidate genes have been proposed for PG and impulsivity in general. Genetic variations in the gene encoding the DA D4 receptor (DRD4) have been associated with novelty seeking and PG, and variations in the DRD2 allele have been associated with PG and drug addiction, albeit inconsistently. Preliminary data investigating influences of allelic variants of the DRD2 on reward processing have suggested differences in medial OFC, amygdala, hippocampus, and NAcc function. The extent to which such findings are observed in PG subjects requires further investigation.

While these results are intriguing, findings from genetic association studies should be viewed with caution. This is particularly true since there have been significant methodological limitations, such as the lack of definitive diagnoses or stratification by racial/ethnic identity. Perhaps given the preliminary nature and methodological limitations of early studies, some initial findings (e.g., an association between PG and DRD2 variation) have not been replicated in subsequent, controlled studies. Larger population-based studies using accurate diagnostic assessments and genome-wide interrogation should provide a framework for a more precise understanding of the genetic influences contributing to PG.

Treatment

Over the past decade, multiple pharmacotherapies have been tested in the treatment of PG. Placebo-controlled trials indicate frequent placebo responses so the findings from open-label trials should be interpreted cautiously. Serotonin reuptake inhibitors such as paroxetine and fluvoxamine have shown mixed findings, with some placebo-controlled trials observing superiority of active drug over placebo and others not. Preliminary data suggest that serotonin reuptake inhibitors may be particularly helpful for individuals with PG and co-occurring internalizing disorders (e.g., anxiety disorders). Mood-stabilizing drugs have been found to be helpful for some individuals. In one trial, lithium was found to be superior to placebo in diminishing gambling and manic symptoms in individuals with PG and co-occurring bipolar spectrum disorders (e.g., bipolar II disorder). Arguably, the most consistent findings have emerged from studies of opioid antagonists. In three separate placebo-controlled trials, the opioid antagonists nalmefene and naltrexone have been reported to be superior to placebo in the treatment of PG. Individuals with strong gambling urges at treatment

onset or a family history of alcoholism appear to respond particularly well to opioid antagonists. The glutamatergic neutriceutical *n*-acetyl cysteine has been found to be superior to placebo in one controlled trial. Although encouraging, the majority of pharmacotherapy studies have limitations with respect to short-term durations, small sample sizes, and exclusion of subjects with co-occurring disorders. As such, more study is needed to identify treatments with long-term benefits and to understand who might respond best to specific treatments.

As with SUDs, behavioral therapies are currently a cornerstone of treatment for PG. Self-help approaches like Gambler's Anonymous have been associated with improved outcomes. Among formal therapies, imaginal desensitization, motivational approaches, cognitive behavioral therapy, and brief interventions have support in controlled studies. However, in some instances, long-term follow-up data have not been as encouraging as short-term data. More research is needed to investigate the possible predictors of treatment outcome and evaluate combinations of behavioral and pharmacological therapies.

Conclusions

PG and SUDs share features including tolerance, withdrawal, repeated attempts to quit or cut back, and impairments in life functioning. Phenomenological similarities exist, including age-related prevalence estimates and telescoping patterns in women. Multiple neurotransmitter systems (e.g., 5-HT, NE, DA, and opioids) appear to contribute to PG. Treatments targeting these neurotransmitter systems have demonstrated varying degrees of promise. Ventral cortico-striatal brain pathways appear particularly relevant to impulsive decision making and PG. The relationships between PG and SUDs, as well as those between PG and other psychiatric disorders, may be important considerations in the development of improved prevention and treatment strategies.

See also: Compulsive Buying; Impulsive–Compulsive Sexual Behavior; Problematic Internet Use.

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Problematic Internet Use

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Glossary

Impulse-control disorders – As defined in the fourth-edition text revision of the *Diagnostic and Statistical Manual* (DSM-IV-TR), these are disorders of impulse control that are not categorized in groupings of other major psychiatric disorders. The essential feature of impulse-control disorders is the failure to resist an impulse, drive, or temptation to perform an act that is harmful to the person or to others. The individual with the disorder typically feels an increasing sense of tension or arousal before committing the act and then experiences pleasure, gratification, or relief at the time of committing the act.

Impulsivity – Impulsivity has been defined as a predisposition toward rapid, unplanned reactions to internal or external stimuli with diminished regard to the negative consequences of these reactions to the impulsive individual or to others.

Intermediary phenotype – A measurable characteristic, one that may not be immediately obvious (e.g., impulsivity), that may be closer to biological phenomena than other phenotypes (e.g., diagnostic categories used in mental healthcare settings). Intermediary phenotypes may facilitate the identification of clinically relevant genes and the elucidation of their functions. An intermediary phenotype can involve a simpler behavior or an underlying endocrine or neural mechanism.

Internet-enabled sexual behavior (IESB) – Alternatively termed Internet sex and cybersex, it encompasses a wide range of sexual behaviors expressed on the Internet, including Internet pornography (solitary), on-line sexually charged relationships (bidirectional), and their variants such as webcam strip-shows and role-playing sex games.

Massively multiplayer on-line games (MMOGs) – These encompass a genre of computer games in which hundreds or thousands of players can cooperate and compete with one another in the same game in real time. They typically feature at least one persistent virtual world, and typically require players to invest large amounts of time.

Problematic Internet use – Also termed Internet addiction, pathological Internet use, and compulsive-impulsive Internet-use disorder, this term describes poorly controlled or excessive use of the Internet that is associated with significant psychosocial and functional impairments.

Time distortion – It describes an individual's experience that time seems to pass slower or faster than it actually does. It is revealed by the discrepancy between the perceived length of time and the measured amount of time spent on a certain activity. It is often associated with computer and Internet uses, and with other pleasurable activities as well.

Introduction

The Electrical Numerical Integrator and Calculator (ENIAC), constructed in the United States (US) in 1946 by John Mauchly and John Presper Eckert, is widely regarded as the first functionally useful electrical computer. It weighed almost 30 tons and covered 1800 square feet of floor space. The invention of the microprocessor in the 1970s made the development of consumer computers possible. By the 1990s, the computer became a common household appliance. It is estimated that at present over 70% of Americans own a personal computer. While computers may have been originally invented to be calculating machines, with the advent of the Internet the modern computer can now perform virtually unlimited varieties of functions and provide an unparalleled interactive experience. The significance of the computer and the Internet in human life has also assumed different dimensions. To many, the Internet enlivens the computer to much more than an inanimate device. The ways in which some individuals seem attached to or enslaved by computer activities, in particular Internet-based functions, led to the hypothesis of the addictive potential of such activities.

Internet addiction or problematic Internet use are two of the most commonly used terms for this behavioral syndrome. Both terms appear, in this article, to adhere to the terms used by different authors in describing their findings. Addiction is not a term used or defined in the current *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition, Text Revision (DSM-IV-TR). Although the term problematic Internet use is widely used to characterize a variety of Internet-related behaviors, there exists no unified definition for the disorder. In this article, a descriptive approach is employed and the term problematic Internet use is used to describe poorly controlled or

excessive use of the Internet that is associated with significant psychosocial and functional impairments. Computer activities are of a large variety and can be on- or off-line. Problematic patterns of computer use had been observed even before the Internet became widely available. However, since the advent of the Internet, it has been observed that the activities most typically associated with problematic use are Internet-based (e.g., Internet gambling, Internet-enabled sexual behavior (IESB), on-line gaming), and most existing research addresses problematic and compulsive use of the Internet.

Epidemiology

The true prevalence of problematic Internet use is not known. This is largely due to the lack of unified diagnostic criteria and assessment instruments, and the scarcity of epidemiological studies. One concern with regard to assessment instruments is that it is unclear how well they differentiate between normal and problematic uses, and their thresholds may greatly influence the prevalence estimates. Another problem is that most existing studies focus on children, adolescents, and college students based on the presumption that problematic Internet use is primarily a phenomenon of the younger population, although epidemiological evidence in support of this premise is largely lacking. Given the reports of problematic Internet use in people of different ages and professions, such as home-makers and working professionals, further study of the prevalence in the general population is needed.

The telephone survey conducted by Aboujaoude *et al.* in 2004 is probably the most methodological rigorous prevalence-estimate study to date. They conducted a nationwide, random-sample telephone survey of over 2500 US adults (≥ 18 years) utilizing a structured interview that elicited responses to eight suggested measures of problematic Internet use, which were extrapolations from established diagnostic criteria for impulse-control disorders, obsessive-compulsive disorder, and substance abuse. Four sets of possible diagnostic criteria were generated, and the least restrictive one required that Internet use interferes with relationships; the respondent be preoccupied with the Internet when off-line; and, has either tried unsuccessfully to cut down on Internet use, or often stayed on-line longer than intended. Merely 0.7% of respondents endorsed all of these four criteria. However, considerably larger proportions (3.7–13.7%) endorsed one or more of the suggested measures. The study did not include people younger than 18 years of age and had a relatively small proportion of younger adults. As such, the study design might have biased the prevalence estimates downward. Nevertheless, the results suggest that potential markers of problematic Internet use are present in a sizeable proportion of the population.

Multiple observations can be made from the existing epidemiological studies of problematic Internet use. Studies have been performed in multiple geographical regions and significant prevalence rates have been reported in countries across multiple continents and countries (e.g., the United States, Korea, China, Norway, and Italy) indicating that the phenomenon is recognized worldwide. The disorder may be more prevalent among children, adolescents, and college students, with prevalence estimates from off-line studies of younger populations ranging approximately from 2% to 8%, higher than the <1% in adults reported by Aboujaoude *et al.* Problematic Internet use also appears to have a male preponderance, with most, but not all, off-line studies reporting higher prevalence rates and heavier computer use in male subjects. One possible explanation is that men are more likely to engage in computer activities that are associated with a strong emotional-motivational state that contributes to addictive behaviors, such as computer games, Internet pornography, and gambling. Given that multiple gender differences have been reported in other behavioral addictions such as pathological gambling, differences in computer-use patterns between the sexes are likely to be complex. In a sample of about 700 young people in Hong Kong, being a young female student, endorsement of being emotionally open on-line, and using Internet chat function heavily were all associated with problematic Internet use. Men and women may be attracted to, and experience problems with, different computer activities. The above-mentioned observations require replication in future studies of large, representative samples with the use of standardized criteria and assessment instruments and structured interviews.

Clinical Characteristics

Subjective Experiences and Behavioral Patterns

Individuals with problematic Internet use typically spend large amounts of time on Internet-related activities, in some cases objectively measured to be over 35 h a week. Time spent on the Internet is frequently longer than originally intended. Individuals may excessively participate solely in specific Internet activities, and may have made unsuccessful efforts to control or stop Internet use. Lying to family members or therapists to conceal the extent of Internet use is commonly reported – a behavior also seen in pathological gambling.

The above-mentioned study by Aboujaoude *et al.* found that the subjective experience of feeling preoccupied with the Internet when off-line was most highly associated with other suggested clinical markers for problematic Internet use. Consistent with the description of impulse-control disorders, individuals may report difficulty resisting an impulse to use the Internet, an increased

tension prior to the desired activity, a release of the tension after the activity starts, and feeling stimulated and euphoric while engaging in the activity. Analogous to the phenomena of tolerance to and withdrawal from a chemical substance, individuals often report needing to use the Internet for increasing amounts of time in order to feel satisfied, and feeling restless, depressed, or irritable when Internet use is being cut down. Time distortion is also a common subjective experience, as documented by discrepancies between self-reported and measured amounts of time spent on the Internet. Subjects often report finding relief from dysphoric, unpleasant states through Internet activities, and thus may choose them as a way of coping or escaping.

Many of the above-mentioned clinical features are captured in proposed sets of Internet-addiction diagnostic criteria, and a commonly cited set was proposed by Young and modeled on the DSM-IV criteria for pathological gambling. The specificity and sensitivity of various symptoms and combinations of symptoms in differentiating normal versus problematic Internet use in the general population are yet to be established.

Physical Health Consequences

Problematic Internet use could potentially cause health problems secondary to the individual's general disregard of his or her health and reduction in physical activities. Poor personal hygiene, irregular eating and digestive system symptoms, declines in cardiovascular fitness, and sleep deprivation may occur in association with problematic Internet use. Other physical complaints related to excessive computer use may include musculoskeletal pain of the back and the wrist, eyestrain, and headache.

Psychosocial Impairments

Individuals with problematic Internet use may neglect family and friends outside the Internet world and thus may jeopardize significant relationships and work and educational opportunities. Individuals with problematic Internet use may be disciplined at work for nonproductive use of the Internet, and this may result in job loss. Other serious psychosocial problems, such as incurring debts and bankruptcy, academic failures, marital discord, and divorce, have also been associated with problematic Internet use.

Co-Occurring Disorders

As is the case in substance-dependence and impulse-control disorders such as pathological gambling, psychiatric disorders frequently co-occur with problematic Internet use. Common co-occurring psychiatric disorders

appear to include mood disorders, attention deficit and hyperactivity disorder (ADHD), other impulse-control disorders, social phobia, and substance-use disorders. Comorbid psychotic disorders appear less common. Comorbidity might indicate causal relationships among two or more disorders – that they share common etiological factors, or some other relationship. The study of comorbidities in problematic Internet use may shed light on the underlying mechanisms and further inform prevention and treatment efforts.

Mood Disorders

The association between depressive disorders and problematic Internet use appears to be robust and has been observed in adolescents, college students, and adults. Approximately 10–25% of individuals with problematic Internet use have been reported to be depressed, but larger epidemiological studies are needed to determine the precise rate. Excessive Internet use could lead to stressful social circumstances, such as academic failures and marital discord, which could contribute to depression. Moreover, increased time devoted to the Internet means decreased time available for social engagement, which may lead to isolation and depression, as suggested by a longitudinal study by Kraut *et al.*, which gave randomly selected families Internet access and found higher levels of depression and loneliness in respondents who used the Internet as little as a few hours a week after a 2-year follow-up. On the other hand, depressed people may be drawn to computer games and the Internet for consolation. Precisely how the use of the Internet as a coping strategy may progress to become compulsive or excessive is not well understood. Irrespective of the nature of the relationship, it is important to effectively evaluate and treat depressive disorders in individuals with problematic Internet use.

With regard to the potential role of hypomania and mania, the case series by Shapira *et al.*, in 2000, found that 70% of subjects with Internet addiction had a lifetime diagnosis of bipolar affective disorder I or II, but all had a current or recent depressive or mixed episode, and none had current or recent mania. Poorly controlled Internet use could be a manifestation of hypomania or mania, but this does not seem to be the case in the majority of subjects encountered clinically.

Attention Deficit and Hyperactivity Disorder (ADHD)

ADHD has been associated with problematic Internet use in children and in adults. In a sample of over 500 Korean elementary-school students, over 20% of those identified as problematic Internet users had co-occurring ADHD. The majority of those children used the computer to play

on-line games. Those with ADHD and problematic Internet use had more severe ADHD symptoms in that study, as reflected by reports by parents, teachers, and children. Further, in a college-student study with subjects older than 18 years of age, adult ADHD – as ascertained via diagnostic interviews – was the most significantly associated factor with Internet addiction in a logistic regression analysis. Since ADHD predated Internet use, Internet addiction may be a manifestation of ADHD or the two conditions may share common intermediary phenotypes such as a shortened delay gradient. This feature, which has been termed delay discounting and is related to impulsivity, manifests as the tendency to select an immediate reward over a larger but delayed reward. Immediate gratification is often a central experience of using the Internet, where objects of desire are just one-click away. ADHD patients may also have impaired impulse control and hence face difficulties restraining the urge to engage in activities like on-line games.

Obsessive-Compulsive Disorder (OCD) and Impulse-Control Disorders

Problematic Internet use has phenomenological similarities to OCD and impulse-control disorders, in particular pathological gambling. Impulse-control disorders and OCD have been proposed to lie along ends of an impulsive/compulsive spectrum. While obsessive-compulsive symptoms and the trait of harm-avoidance have been associated with excessive Internet use in some cases, patients typically report Internet use as more impulsive and ego-syntonic rather than ego-dystonic (as in OCD), and the remorse from losses secondary to problematic Internet use appears different from the irrational fears often present in OCD. In small case series in which clinical diagnoses were made, the frequencies of co-occurrence of OCD with problematic Internet use varied substantially (0–15%), but were low when compared to those of any non-computer-related impulse-control disorder (up to 50%). Multiple impulse-control disorders have been identified in association with problematic Internet use, and these include intermittent explosive disorder, kleptomania, pathological gambling, pyromania, compulsive buying, compulsive sexual behavior, and compulsive exercising.

Social Phobia

Shyness has been associated with heavy Internet use in college students. It has been contended that the anonymity of the Internet may draw socially fearful individuals to participate in low-risk social interactions via the web. In the case series of 20 patients reported by Shapira and colleagues (2000), concurrent social phobia was found in 40% of subjects, and social phobia was the most common

anxiety disorder in the series. However, social phobia did not predict problematic Internet use in a large epidemiological study in Taiwan, nor in a sample of college students after depression and ADHD were controlled. It appears that social phobia, although it may contribute to heavier Internet use, may not fully explain or account for problematic Internet use.

Substance-Use Disorders

Given the phenomenological similarities between substance dependence and problematic Internet use, the co-occurrence of the two conditions might be anticipated. Moreover, pathological gambling, to which problematic Internet use has been likened, is commonly comorbid with substance-use disorders. However, epidemiological data are almost nonexistent in this regard, with one adolescent survey finding no association between Internet addiction and alcohol and nicotine dependence. On the other hand, small case series have found that up to 50% of subjects with Internet addiction have a lifetime diagnosis of substance abuse. Further epidemiological research is needed.

Internet Gambling, IESB, and On-Line Gaming

Internet gambling, IESB, and on-line gaming are behaviors most frequently associated with problematic patterns of use, although other computer-based behaviors such as on-line chatting and e-mailing may also become problematic. These forms of Internet use appear to share the propensity to alter mood states, a feature which has been proposed to contribute to addictive behaviors. Gambling, sexual behavior, and computer-gaming are not Internet-specific activities. However, certain distinctive properties of the Internet, including interactivity, accessibility, affordability, and anonymity, may potentially exacerbate or promote excessive involvement in specific individuals, although more research is needed to examine these possibilities. From the limited epidemiological data available, problematic forms of these behaviors appear to be more common in males than in females.

Internet Gambling

The majority of adults in the US have gambled within the past-year, and epidemiological studies suggest that problem and pathological gambling occur in a small proportion of the population, probably up to 4.0%. The hypothesis that Internet gambling exacerbates the problem of pathological gambling because of the above-mentioned properties has not been confirmed by

empirical studies. Two large-scale Internet gambling studies which recorded Internet betting activities from more than 4000 subscribers to an Internet gambling website over a 2-year period found that gambling behaviors were moderate at the population level, with 1–5% of subjects exhibiting behaviors that deviated markedly from the norm. However, Internet gambling may be a larger problem in subsets of the population such as college students. In one study, almost 25% of college subjects reported having wagered on the Internet at least once in their lives. The study also found that markedly more regular Internet gamblers than off-line gamblers were pathological gamblers (61.6% vs. 5.0%), and Internet gambling was associated with poorer mental health ratings. The question remains whether Internet gambling leads to problematic gambling behaviors, problem gamblers are drawn to Internet gambling, or some other relationship exists.

Internet-Enabled Sexual Behavior

Sex addiction, or nonparaphilic compulsive sexual behavior, is one of the proposed conditions currently subsumed under the DSM-IV-TR category of impulse-control disorder not otherwise specified. IESBs include pornography viewing, on-line sexual relationships, and their variants. It has been estimated that the Internet pornography industry is worth well over \$1 billion, and most of the top word searches are related to Internet sex. Hence, IESBs appear to be very prevalent at the societal level. Internet sex replaces real-life intimacy in many cases, and has been related to reprimands at work, serious relationship problems, and divorce. However, given the secretive nature of the condition for the majority of individuals, the prevalence and extent of compulsive or problematic IESBs are difficult to determine. In a small outcome study of treatment for men involved in problematic IESBs, subjects with different co-occurring disorders responded differently to integrated group therapy – those with anxiety disorders responded best; those with depression showed a moderate response; and those with ADHD appeared to be most resistant to treatment, illustrating the heterogeneity of subjects with problematic IESBs.

On-Line Gaming

Computer gaming can be conducted on-line or off-line, but heavy or excessive playing is more frequently associated with on-line gaming modalities. Another observation repeatedly reported in multiple studies is that players and problematic players are predominantly males. Both quantitative and qualitative studies have shown that to socialize and interact with others is an important motivational factor in on-line gaming. This is

consistent with the observation that excessive playing is most often associated with massively multiplayer on-line games (MMOGs). The design of MMOGs inherently promotes heavy gaming, since the competition runs continuously in real time, making devotion to gaming mandatory. A study of Korean adolescents found that role-playing game users had significantly higher Internet-addiction ratings than players of other genres, such as sports games. Although computer games are traditionally regarded as a leisure activity of children and adolescents, an on-line study of players of the popular on-line game Everquest found that almost 60% of players were between the ages of 18 and 31, indicating that on-line gaming may be gaining a wider audience. Whether the prevalence of compulsive gaming is increasing in the older age group is not known. In clinical practice, it is still most commonly encountered in children and adolescents, and underlying ADHD is implicated in some cases.

Neurobiological Features

Knowledge about the neurobiology of problematic Internet use is currently very limited. The theoretical models of substance-addiction and impulse-control disorders, to which problematic Internet use has been likened, are not mutually exclusive, and studies have shown multiple neurobiological and genetic similarities between the two groups of disorders. Pathophysiological mechanisms implicated in substance-addiction and impulse-control disorders may also contribute to problematic Internet use, but direct evidence is lacking.

Dopaminergic projections from the ventral tegmental area to the nucleus accumbens are considered to be important in the expression of goal-directed behaviors, and this pathway has long been implicated in drug addiction. A current hypothesis posits that low dopamine D2-receptor availability may mediate vulnerability to addiction. Multiple clinical observations suggest that the mechanism might also underlie problematic Internet use. The phenomenon of *punding* – characterized by an intense fascination with repetitive handling of technical equipments seen in relation to the use of psychostimulants (e.g., amphetamine and cocaine) and dopaminergic anti-Parkinsonian medications – has also involved compulsive use of the computer in some patients. Computer-use behaviors in punding and problematic Internet use in subjects not under the influence of dopaminergic medications share phenomenological features. A case report documented the successful treatment of compulsive IESB with naltrexone, the hypothesis being that a non-chemical stimulus such as Internet pornography causes the release of endogenous opiates which enhances dopamine release, and naltrexone works via blocking opioid receptors. As mentioned, further research with direct

examination of the roles of dopaminergic and opioidergic systems in problematic Internet use is needed to examine these hypotheses.

A preliminary electroencephalographic study reported that as compared to nonaddicted computer-game players, addicted ones showed higher emotional arousal and stronger cortical reactivity in response to computer-game-related visual cues. In addicted computer-game players, game-related cues induced late positive complexes of significantly larger amplitudes. The findings are consistent with previous reports that individuals with Internet addiction may have a different Internet-use experience than do nonaddicted individuals, being more emotionally engaged with the Internet. Similar cue-induced responses had been described in alcohol- and drug-dependent subjects, as well as pathological gamblers confronted with related cues. Thus, problematic Internet use may share common pathophysiological processes with these other disorders.

Genetics

No genetic study has been published examining problematic Internet use. Genetic studies of pathological gambling showed that genetic factors contribute significantly, and pathological gambling shares genetic risk with alcohol dependence and antisocial behavior. All these conditions may be linked via common underlying pathways such as impulsivity. Hence, genetic study of problematic Internet use may help clarify its relationships with other impulse-control disorders and substance addictions.

Treatments

Given the recent recognition of problematic Internet use as a psychiatric problem, not much research has evaluated the efficacy of treatments for the disorder. No double-blind, controlled trials of pharmacotherapies or psychotherapies have been published. However, many patients seek help and the resulting treatment infrastructure is expanding. Multiple treatment centers and groups have emerged over time, both on- and off-line. For example, specialized outpatient treatment service is available at the Computer Addiction Study Center at McLean Hospital of Harvard Medical School. In some instances, patients have been admitted for inpatient rehabilitation. In China, a halfway house was recently opened for adolescents with problematic Internet use.

Since the Internet is an integral part of life for many, total abstinence may not be practical. Hence, many contend that moderated and controlled use is the most appropriate treatment goal. With regard to children and

adolescents, the input of parents is essential. It has been found that although most parents appear open to buying computers because of perceived positive features, few may discuss with their children their appropriate use in detail or carefully supervise their use, unless or until the use becomes problematic.

Psychopharmacological Treatments

Selective serotonin-reuptake inhibitors (SSRIs) are effective in the treatment of OCD. Studies of the use of SSRIs in impulse-control disorders have shown mixed results. Problematic Internet use appears to have compulsive and impulsive qualities, and treatment with escitalopram, an SSRI, has been studied. An open-label study in 19 subjects by Dell'Osso, in 2006, showed that 10 weeks of escitalopram treatment (20 mg per day) was associated with significant decreases in weekly hours spent on-line (from a mean of 36.8 to 16.5 h) and in measures of impulsivity and compulsion, as well as improvement in global functioning. After 10 weeks, subjects were blindly randomized to continue treatment with escitalopram or placebo. Following another 9 weeks of treatment, therapeutic gains achieved at week 10 were maintained in both treatment groups and no further gain was shown for either group. The medication was well tolerated. The most frequently reported side effects were drowsiness and nausea, which were modest and self-limited. In a case report, escitalopram successfully treated a depressed adult patient with compulsive on-line gaming. Another case study reported the successful use of an atypical antipsychotic (quetiapine) as augmentation for citalopram in the treatment of a subject with compulsive Internet use. This augmentation strategy has also been successfully used in treating OCD. Naltrexone has also been used with the reasoning that it may antagonize endogenous opiates that are induced by pleasurable stimuli, perhaps through modulation of dopamine pathways underlying motivated behaviors. A case report documented the successful use of naltrexone in the treatment of compulsive IESB. The patient's depressed mood initially responded to sertraline, but his urge to view Internet pornography was still uncontrolled until naltrexone was started. He reported complete control of his impulse at the dose of naltrexone 150 mg daily. Further clinical trials are needed to establish the efficacy and effectiveness of medication monotherapy and augmentation strategies.

Psychological Treatments

Cognitive behavioral therapy

Cognitive therapy is based on the underlying theoretical rationale that an individual's affective states and behaviors are largely determined by the way in which he or she structures the world on the basis of cognitions and

assumptions. Recognition, examination, and correction of faulty cognitions and assumptions may then lead to changes in regulating affective states and controlling behaviors. Principles of cognitive therapy have been applied to the treatment of problematic Internet use. Studies suggest that compulsive Internet users may be more likely to expect positive outcomes and relief from negative affective states from Internet use, which may not be true given the psychosocial impairments they suffer from excessive use of the Internet. They may presume that the Internet is the most effective way to alleviate depressive feelings and meet truthful friends ("unlike my dishonest husband..."). Such expectations represent potential targets for cognitive restructuring, as are other dysfunctional thoughts such as "Just a few more minutes won't hurt" which may maintain the pattern of Internet use.

Cognitive therapy is typically combined with a behavioral component in the initial phase. Such therapies may initially start with a daily log to evaluate Internet-use behaviors and a focus on establishing and achieving reasonable goals such as decreasing the amount of time of use, abstaining from problematic applications, and developing alternative activities and coping skills. Such therapies may be combined with the use of software to restrict Internet access.

The outcome of CBT in the form of on-line counseling for problematic Internet use has been evaluated in a study conducted by Young. Overall, subjects reported incremental or continuous improvements in symptoms and functioning throughout the course of therapy of 12 sessions, and maintenance of benefits at 6-month follow-up. The moderate sample size (114 treatment-seeking subjects) and its highly educated composition may limit the generalizability of the results.

Motivational interviewing and stages of change

A transtheoretical model of behavioral change was proposed and developed by Prochaska and DiClemente. Based on this model, motivational interviewing has been successful in the treatment of substance addictions. The model has been adopted in the psychotherapy of problematic Internet use. The principle states that behavioral change goes through successive stages of pre-contemplation, contemplation, preparation, action, maintenance, and termination, and individuals at different stages have different needs and levels of readiness. A therapist should be sensitive to the motivational stage of the patient and respond to his or her needs accordingly. If the patient does not recognize any problem with his or her Internet use, it would not be therapeutic for the therapist to propose an action plan. The goal at this precontemplation stage is to help the patient overcome denial by discussing pros and cons of Internet use and overuse. If the patient is already trying to make behavioral changes, it may be

unnecessary or inappropriate for the therapist to continue discussing negative ramifications of excessive Internet use. Support and educational materials are provided according to the patient's readiness to change. A small study of group therapy applying the principles of cognitive behavioral therapy (CBT), motivational interviewing, and stages of change showed improvements in quality of life and depression measures, but no significant changes in the subjects' computer-use behaviors.

Insight-oriented psychotherapy

While people have different motivations to use and overuse the Internet, qualitative studies and case reports illustrated common psychological themes in patients' stories. Internet activities may serve to fulfill some inherent psychological needs – the need for a sense of achievement, for interpersonal connectedness and a sense of belonging to a community, for entertainment, and to discharge frustration. The virtual self on-line can be an extension of the real self, or a compensatory identity to satisfy unfulfilled wishes. This interpretation may explain why role-playing games represent one of the most popular genres. Blogosphere may also provide a space to fulfill the wish to self-reveal. Insight-oriented psychotherapy aims to help patients understand the relationships among their anxieties, inner needs, motivations, and Internet-use behaviors so they can make their own choice to change behaviors and develop alternate ways of coping. Empirical data on this and other psychotherapeutic approaches for problematic Internet use are limited.

Conclusions

Problematic Internet use is a clinically recognizable behavioral syndrome that is associated with significant adverse psychosocial consequences. As is the case with substance-dependence and impulse-control disorders, comorbidity appears to be the rule rather than the exception. The study of the prevalence, phenomenology, pathophysiology, and treatment of problematic Internet use is hindered in part by the lack of standardized criteria and assessment instruments. This behavioral syndrome appears heterogeneous – people have different motivations to use the Internet to excess, and they have problems with different applications. Novelty-seeking individuals may be drawn to the Internet's incessant novelty, while people with harm-avoidance tendencies may find a sense of safety in the virtual world. Nonetheless, both types of individuals could develop problematic patterns of use. The study of intermediary phenotype constructs, such as impulsivity, across diagnostic groups should facilitate further elucidation of neurobiological and genetic bases of problematic Internet use and other theoretically related conditions. Clinically, efforts in subtyping problematic

Internet use may be performed in conjunction with understanding a patient's unique experience with the Internet, and helping him or her reflect on his relationship with the virtual world. Clinicians should be aware of the clinical features of problematic Internet use and its potential consequences, and be equipped to assess computer-use history in the clinical interview.

See also: Animal Models of Sexual Function; Cognition: Attention and Impulsivity; Brain–Machine Interfaces; Comorbidity – Depression; Compulsive Buying; Depression; Impulsive–Compulsive Sexual Behavior; Molecular Neurobiology of Addiction; Novelty; Pathological Gambling; Value of Animal Models for Predicting CNS Therapeutic Action.

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Pyromania

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Glossary

Affective disorder spectrum – A grouping of psychiatric disorders characterized by alterations in mood that share clinical and treatment-related characteristics.

Arachnoid cyst – A sac, filled with cerebrospinal fluid, that forms around the cranial base in the surface region of the brain, or on the arachnoid membrane.

Cerebellar vermis – A midline part of the cerebellum.

Ego-dystonic – The aspects of a person's thoughts, mood, impulses, and behavior which are repugnant and inconsistent with how the person normally views his or herself (opposite of ego-syntonic).

Nucleus accumbens – A brain region where dopamine-secreting neurons terminate, which has been implicated in various addictions, urge-driven behaviors, reward-based learning and motivation; considered part of the brain reward circuitry.

Obsessive-compulsive spectrum – A group of disorders hypothesized to share common clinical and biological characteristics of obsessive-compulsive disorder. These disorders include, but are not limited to, Tourette's syndrome, body dysmorphic disorder, hypochondriasis, depersonalization disorder, pathological skin picking, trichotillomania, and pathological gambling.

Psychopathy – A psychiatric condition characterized by chronic antisocial and immoral behavior.

Topiramate – A sulfamate-substituted monosaccharide derivative used as an anticonvulsant to treat partial seizures.

Ventral tegmental area – A region in the midbrain where the mesocortical and mesolimbic dopaminergic systems originate; dopamine neurons in the ventral tegmental area project to the nucleus accumbens.

deliberate and purposeful fire setting that has occurred on more than one occasion; (2) feelings of tension or arousal preceding a fire-setting act; (3) pleasure, gratification, or relief when setting fires or when watching/participating in the aftermath of the fire; (5) the act of fire setting is not done out of vengeance or for monetary gain; and (6) fire-setting cannot be directly attributed to another mental condition such as conduct or bipolar disorder or impairment due to substance use. Psychosocial problems are common among individuals with pyromania and include impaired functioning and thoughts of suicide. Although pyromania has been documented for almost two centuries, it remains a poorly understood disorder with limited data regarding neurobiology or treatment. This article details what is currently known about the clinical characteristics and treatment of pyromania.

History

Pyromania has been described in medical literature for at least two centuries. The term pyromania derives from the Greek, fire (*pyr*) and madness (*mania*). One of the first descriptions in medical texts was in 1838 by Jean-Etienne Esquirol who referred to the behavior as 'incendiary monomania.' Esquirol included pyromania with kleptomania and erotic monomania of examples of irresistible behaviors. Esquirol differentiated pyromania from simple fire setting by claiming that pyromania was due to an instinctive impulse independent of will. Since that time, although people have debated the validity of the disorder, surprisingly little has been written about pyromania.

Pyromania appeared in the first edition of DSM in 1952 as a supplemental term. Pyromania did not appear in DSM-II. In 1980, DSM-III categorized pyromania as an impulse control disorder not elsewhere classified, the same clinical diagnostic category it currently holds in the DSM-IV-TR.

Introduction

Pyromania, also referred to as pathological fire setting, is a disorder currently included in the *Diagnostic and Statistical Manual*, 4th edition (DSM-IV), as an impulse control disorder not elsewhere specified. Pyromania is defined by the following criteria according to DSM-IV: (1)

Epidemiology

Although documented cases have been reported for centuries, very little is known about the prevalence of pyromania. In the only study to examine the rate of pyromania in a community sample, 791 college students were asked about histories of fire setting. The study found

eight (1.01%) students who met DSM-IV criteria for lifetime pyromania. An additional 10 (1.26%) students met subsyndromal pyromania criteria, which was defined by the behavior causing significant distress and dysfunction but not meeting full criteria for DSM-IV pyromania. In general, the finding that over 2% of the sample had either clinical or subsyndromal pyromania suggests that pyromania may not be an uncommon disorder.

Several studies of clinical, noncriminal samples also suggest that pyromania may not be uncommon. One study of 107 patients with depression found that three (2.8%) met current DSM-IV criteria for pyromania. A recent study of 204 psychiatric inpatients revealed that 3.4% ($n=7$) endorsed current symptoms, and 5.9% ($n=12$) had lifetime symptoms meeting DSM-IV criteria for pyromania. Small studies of individuals with compulsive buying ($n=20$) and kleptomania ($n=20$) have also found frequencies of 10% ($n=2$) and 15% ($n=3$), respectively, for lifetime pyromania. Although adolescent fire setting may be a symptom of various psychiatric disorders, a recent study of 102 adolescent psychiatric inpatients found that after excluding those patients who set fires due to conduct disorder, substance use disorders, bipolar disorder, psychotic disorders, or developmental disorders, seven (6.9%) met the criteria for current pyromania. All seven adolescents with pyromania were girls.

Clinical Characteristics of Pyromania

Little data have been published from clinical samples describing the characteristics of individuals with pyromania. Age of onset for pyromania is generally late adolescence or early adulthood, although onset as late as the fourth decade of life is not uncommon. Pyromania appears to occur equally in men and women. Most individuals with pyromania are single, employed, and have at least a high school education. Some may work in jobs that allow them easy access to fires (e.g., firefighter).

Individuals with pyromania frequently report a rush when watching or setting fires. Stress and boredom are common triggers for starting fires. Many people with pyromania set what they consider to be controlled fires in dumpsters, their bathrooms, backyards, or vacant lots. Considerable time is spent on planning the fires, buying utensils to set the fires, and planning what items will burn well and most intensely. In addition, many individuals with pyromania spend considerable time watching fires and traveling to fires when they hear fire engines. The time spent on planning, setting, and watching fires significantly interferes with other responsibilities. Frequency of setting fires increases over the course of the illness, with less time between fires, and the intensity of the fires also increases over time. Many individuals report switching to other types of impulsive behavior

after stopping fire setting (e.g., compulsive buying, alcohol or drug addiction, pathological gambling).

Although individuals with pyromania report either pleasure or relief when setting fires, the vast majority also report significant distress following their behavior. Suicidal ideation is common subsequent to setting fires. Social or occupational impairment due to activities associated with setting fires is common. Although the behavior of pyromania often results in arson, most individuals have not been arrested. Histories of physical or sexual abuse while growing up are fairly common in individuals with pyromania.

Comorbidity

Psychiatric comorbidity appears to be quite common in persons with pyromania. Lifetime rates of mood (62%; most commonly major depressive disorder), impulse control (47%; most commonly kleptomania), anxiety (33%; most commonly posttraumatic stress disorder), and substance use (33%) disorders have been documented in those with pyromania. The majority of individuals with pyromania with mood or substance use disorders indicate that pyromania preceded the other disorders. In addition, pyromania has been associated with attention-deficit/hyperactivity disorder, learning disabilities, dementia, and mental retardation.

Relationship of Pyromania to Other Psychiatric Disorders

Approximately 10 years ago, researchers suggested that one way to understand an impulse control disorder, such as pyromania, was as part of an obsessive-compulsive spectrum. In addition, other models for understanding pyromania have been suggested, and research suggests that behavioral diagnoses such as pyromania may be far more heterogeneous than initially thought.

Pyromania is characterized by repetitive behavioral engagement and impaired inhibition. The difficult-to-resist and difficult-to-control fire-setting characteristics of pyromania suggest a similarity to the frequently excessive, unnecessary, and unwanted rituals of obsessive-compulsive disorder (OCD). There are, however some apparent differences between pyromania and OCD. For example, unlike individuals with OCD, majority of the individuals with pyromania report an urge prior to engaging in the problematic behavior and report their behavior as pleasurable. Individuals with OCD generally describe their behaviors as ego-dystonic and harm avoidant. Only one study has examined OCD in subjects with pyromania, and it found that a 4.8% co-occurrence of OCD in pyromania. When pyromania has been examined

in subjects with OCD, relatively low co-occurrence has also been found (0.3%). Controlled family studies with pyromania are currently lacking, and therefore any putative familial link between pyromania and OCD cannot be precisely determined.

The model of pyromania as a behavioral addiction also has support from recent research. Pyromania shares certain distinct features with substance use disorders: (1) an urge to engage in a behavior with negative consequences; (2) mounting tension unless the behavior is completed; (3), rapid but temporary reduction of the urge after completion of the behavior; (4) return of the urge over hours, days, or weeks; and (5) a pleasurable feeling or rush. In addition, the majority of individuals with pyromania report that the time between episodes of fire setting decreases over time and the intensity of the fires increases over the course of the illness. This element is reminiscent of tolerance in substance use disorders. Finally, pyromania frequently co-occurs with substance use disorders with a lifetime frequency of approximately 33%.

Mood disorders also co-occur frequently with pyromania and have led to the possible inclusion of pyromania within an affective spectrum. Lifetime mood disorders occur in approximately 62% of individuals with pyromania. However, a study of 107 patients with depression found that three (2.8%) met current criteria for pyromania. Individuals with pyromania often report that their symptoms worsen, or are triggered by negative mood states. Fire setting may have an antidepressant effect. In addition, because of the elevated rates of co-occurring bipolar disorder (14%) and because pyromania behavior is risky, pyromania may also be a symptom of subclinical hypomania or mania in some individuals.

Ultimately, an assessment of pyromania's relationship with other psychiatric disorders needs to consider the respective etiologies. Unfortunately, knowledge of this aspect of pyromania is not yet advanced enough to precisely address this topic.

Differences between Pyromania, Fire Setting, and Arson

There is often confusion about the terms used to describe the various types of fire setting. Pyromania is the proper term for fire-setting behavior only when the DSM-IV criteria have been met. In contrast to pyromania, arson is not a diagnosed psychiatric disorder or medical illness and has a distinct definition that differs from that of pyromania. Although state statutes may differ on the explicit language, arson is generally defined as a crime of maliciously, voluntarily, and willfully setting fire to a building or other property of another person or burning one's own property for an improper purpose (e.g., insurance fraud). In general, the motivation of an arsonist is for

some type of gain, whether it revenge for a wrongdoing, insurance fraud, or for sociopolitical or religious reasons. Pyromania, on the other hand, is an impulse- or urge-driven behavior, which affects, among other things, the social and occupational lives of the individuals suffering from the illness. These urges have been described as addictive, and the act of setting a fire produces a sense of calm for the individual.

Unlike true pyromania, arson appears to be a common occurrence that affects many people, both financially and emotionally. In 2004, there were 68 245 reports of arson offenses in the United States, according to statistics from the Federal Bureau of Investigations, with an average dollar loss per incident of \$12 017. Recent data provided through the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC) found that 1.13% of a large community sample acknowledged having started a fire, in their lifetimes, for the purpose of destroying someone else's property or just to see it burn. Very few arsonists, however, suffer from pyromania. One study found that only three (3.3%) of 90 arson recidivists had pure pyromania and that an additional nine subjects met DSM criteria for pyromania only when intoxicated at the time of the fire setting. In addition, not everyone who suffers from pyromania is an arsonist. A person meeting DSM-IV criteria for pyromania may also meet the legal definition of arson, but the DSM-IV criteria for pyromania do not require that a person ever set fire to another person's property.

Fire setting may also be associated with a variety of psychiatric disorders even when the diagnostic criteria for pyromania are not fulfilled or when the legal definition of arson is not met. Previous studies have suggested that the lifetime prevalence of fire setting may be approximately 26% in psychiatric patients. In these patients, fire setting has been linked to other self-injurious behaviors. Fire setting may also result from command hallucinations, delirium related to drug use, and manic grandiosity. In these patients, it has also been hypothesized that fire setting may be a manifestation of impulsivity, psychopathy, or affective dysregulation.

Child and Adolescent Fire Setting

A fascination with fire is an occurrence that has been documented for centuries. Usually beginning about the age of 2 or 3 years, attraction for fire may remain constant throughout an individual's life. In fact, behaviors such as playing with matches have been noted to occur in 24.4% of child psychiatric outpatients while fire-setting rates of 19.4% have been found in this sample. Although it is presently unknown how common this behavior is for children in the general population, the consequences of this behavior can be serious. Burn injuries account for

40% of accidental deaths in children under the age of 5, making these injuries the second leading cause of death for this age group.

While child and adolescent fire setting is serious and fairly common, true pyromania is quite rare in this age group. Children generally set fires out of vengeance, peer pressure, or impulse, and not out of an urge to relieve a building tension or as a response to an urge. For example, when 17 young fire setters were asked to identify their respective reasons for starting fires, their responses included revenge, to conceal a crime, for self-harm, due to group peer pressure, denial of the act or accidental, and a fascination with fire. In this sample, the individual endorsing a fascination for fire would be a possible candidate for a diagnosis of pyromania.

As noted above, fire setting itself is quite common in the child and adolescent age groups. Statistics have shown that nearly 50% of arson arrests are juvenile offenders. Common risk factors for this behavior have been identified within the juvenile population. Males have been shown to set fires 10 times more frequently than females. A study of 205 juvenile fire setters revealed that children who had experienced some form of maltreatment (i.e., physical abuse, neglect, or sexual abuse) were more likely to set fires in response to anger and be motivated by a family stressor than those without a history of maltreatment. Although associated with lower socioeconomic income, research has revealed that a variety of socioeconomic backgrounds is prevalent in the juvenile fire-setting population. Research has also shown that many fire-setting individuals in the juvenile population have experienced parental neglect and both emotional and physical abuse. Consistently, juvenile fire setting has been associated with adjustment and conduct disorder.

Etiology of Pyromania

Although abusive childhoods have been associated with fire setting in children and adolescents, little research has investigated possible neurobiological correlates of pyromania. In a study of a single individual with pyromania, neuroimaging using single photon emission computed tomography (SPECT) found left inferior perfusion deficits in the frontal lobe. New-onset fire setting has also been described in an individual with an arachnoid cyst of the cerebellar vermis.

The underlying biological mechanism of urge-based disorders may involve the processing of incoming reward inputs by the ventral tegmental area–nucleus accumbens–orbital frontal cortex circuit. This circuit influences motivations (e.g., urges and cravings) and related behaviors. Dopamine may also play a major role in the regulation of this region's functioning. One hypothesis is that differences in these regions may result in urges seen in

pyromania and other impulse control disorders. The efficacy of topiramate (see below) lends further support to this hypothesis. Topiramate is thought to modulate dopaminergic function neurons in this area.

Because serotonergic systems have been implicated in impaired impulse regulation, serotonin dysregulation may also contribute to the pathophysiology of pyromania (as well as other impulse control disorders). Selective serotonin reuptake inhibitors (SSRIs) have shown preliminary promise in the treatment of pyromania. The use of SSRIs is based on the hypothesis that the etiology of impulsive behaviors and disorders may relate to low levels of serotonin in selected brain regions.

Treatment

There are no randomized, controlled, clinical trials examining either pharmacotherapy or psychotherapy for the treatment of pyromania. No medications have been approved by the Food and Drug Administration (FDA) for the treatment of pyromania.

Medications that have been described in case reports and which may show benefit in the treatment of pyromania include topiramate, escitalopram, sertraline, fluoxetine, lithium, and a combination of olanzapine and sodium valproate. An equal number of medications have also shown no benefit in the treatment of pyromania in case reports: fluoxetine, valproic acid, lithium, sertraline, olanzapine, escitalopram, citalopram, and clonazepam.

Another case report, illustrating the treatment of an 18-year-old male with pyromania, described the use of a combination of topiramate with 3 weeks of daily cognitive behavioral therapy (CBT), which included imaginal exposure, relaxation training, response prevention, and cognitive restructuring of fire-setting urges. Other studies describing behavioral treatments of pyromania include methods such as fire safety education, aversive therapy, positive reinforcement, stimulus satiation, and operant structured fantasies and prevention programs designed for pyromania.

There is no standard treatment for pyromania at this time. Given existing data, an approach using both psychotherapy and pharmacological treatment may be most beneficial.

Pyromania often may go undiagnosed. Many reasons exist for why such severely distressing behaviors may not be diagnosed. Shame and secrecy are often associated with pyromania, largely due to the illegal or perceived immoral nature of the behavior. Many people are also embarrassed because of the diminished control they exhibit over the fire-setting behavior. Such embarrassment and shame may explain, in part, why so few patients may volunteer information regarding this behavior unless specifically asked. Often related to the shame and secrecy may be a patient's

misunderstanding of what a mental health clinician is required by law to report. Patients suffering from pyromania may believe that the clinician is required to report their illegal behaviors. Clinicians therefore may want to inform patients at the outset of the evaluation concerning what they do and do not have to report.

Conclusions

Pyromania is an impulse control disorder that has received relatively little attention from the psychiatric community. Nonetheless, pyromania may cause significant psychological, social and legal repercussions. Because few individuals volunteer information regarding their fire-setting, it is important that clinicians recognize the disorder and screen patients appropriately. Various treatments have been helpful in case studies but more research examining etiology and treatment is needed.

See also: Compulsive Buying; Impulsive–Compulsive Sexual Behavior; Intermittent Explosive Disorder; Kleptomania; Obesity and Binge Eating Disorder; Pathological Gambling; Problematic Internet Use.

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Relevant Websites

- <http://www.impulsecontroldisorders.org> – University of Minnesota Impulse-Control disorders Clinic.

Trichotillomania (Compulsive Hair Pulling) and Compulsive Skin Picking

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Glossary

Habit-reversal therapy – A form of psychotherapy with evidence for efficacy in the treatment of trichotillomania, initially developed by Azrin and Nunn for the treatment of nervous habits. This type of therapy involves training patients to recognize situations where they are likely to undertake hair pulling and to perform competing response strategies, such as clenching fists.

Impulsivity – A multifaceted term in psychiatry referring to behaviors that are premature, risky, inappropriate, and which lead to negative long-term outcomes or harm. In the neurosciences, several dissociable manifestations of impulsivity have been described, including notably impaired response inhibition – difficulty suppressing simple motor responses when instructed to stop by a cue. Impaired response inhibition has been identified in trichotillomania, obsessive-compulsive disorder (OCD), attention-deficit hyperactivity disorder (ADHD), and other conditions.

Noradrenaline – Noradrenaline is a molecule that, within the central nervous system, is involved in neurotransmission. Noradrenaline has been implicated in processes such as arousal, attention, and impulse control.

Obsessive-compulsive (OC) spectrum disorders – Disorders characterized by repetitive habits that are difficult to suppress, purportedly linked to OC disorder (OCD) in terms of etiology and comorbid expression.

Putamen – Part of the basal ganglia that has been implicated in the generation of motor habits, such as tics in Tourette's syndrome or hair pulling in trichotillomania. Patients with trichotillomania show abnormally increased gray matter density in this region compared to healthy volunteer controls.

Trichophagia – Referring to the ingestion of hair, it is often observed in association with trichotillomania and can lead to significant medical complications.

Case Example

Mrs. G, a 24-year-old taxi driver, presented to an academic research unit with a history of repetitive hair pulling since the age of 13. She could not identify any obvious triggering factors during adolescence, and had thus far managed to conceal eyebrow loss successfully using makeup. More recently, the hair pulling had escalated, now affecting not only her eyebrows but also her scalp, and occupying 3–4 h per day. Consequentially, hair loss had become noticeable to her partner and friends, and was now associated with functional impairment, social avoidance, and comorbid depression.

Introduction

Trichotillomania (compulsive hair pulling) is a common, chronic, poorly recognized, and ill-understood mental disorder. Responsible for considerable shame and distress, its sufferers expend great energy concealing its effects. Trichotillomania is the most researched of several phenomenologically and possibly psychobiologically related grooming behaviors, along with nail biting and skin picking – which in milder forms are common in the background population. Their psychopathology exists in the focus, duration and extent of the behavior, as well as the resulting impairment (e.g., noticeable hair loss or tissue damage, distress, and functional disability). There is increasing awareness of the morbidity associated with these habit disorders, but to date few research trials into neurobiology, pharmacotherapy, or psychotherapeutic strategies have been performed. Patients diagnosed with trichotillomania show similarities to those with compulsive skin picking with respect to demographic characteristics, psychiatric comorbidity, and personality dimensions. In this article, we focus on the

psychopathology of trichotillomania, comparing, where possible, data on compulsive skin picking and other grooming disorders. Improved understanding of the underlying neuropsychological mechanisms, including affective and cognitive components, may shed light on the nosological relationships between these conditions and with other impulsive/compulsive mental disorders, paving the pathway for more effective targeted treatments.

Clinical Characteristics and Co-Occurring Disorders

The phenomenon of hair pulling has been recognized for centuries. Accounts are found in the *Old Testament (Book of Ezra)*, Homer's *The Iliad*, and the plays of William Shakespeare (e.g., *Romeo and Juliet*). The earliest references to hair pulling in the medical literature are attributed to the Greek physician, Hippocrates. In his work, *Epidemics I*, Hippocrates recommended that physicians examine whether a person 'plucks his hair' as part of their general examination to determine if disease is present. In modern medical texts, accounts of trichophagia (hair eating) appeared before hair pulling. In 1889, the French dermatologist Francois Hallopeau coined the term 'trichotillomania' in describing a young man who pulled out his body hair.

Trichotillomania was not formally incorporated into official psychiatric nosology until 1987, revised, third edition of the *Diagnostic and Statistical Manual (DSM-III-R)*. The DSM-III-R diagnostic criteria were essentially the same as those currently in DSM-IV, except that the phrase "recurrent failure to resist impulses to pull" was removed, one of the criteria was expanded to include tension experienced when attempting to resist hair pulling, and distress or impairment due to hair pulling was added as a diagnostic requirement (**Figure 1**). During the drafting of DSM-IV, trichotillomania was considered for inclusion both in the anxiety disorders (because of presumed similarities to obsessive-compulsive disorder (OCD)) and the disorders first presenting in childhood or adolescence. As in the case of DSM-III-R, the DSM-IV included trichotillomania in the general

category of impulse-control disorders not elsewhere classified, together with pathological gambling, pyromania, intermittent explosive disorder, and kleptomania.

Repetitive hair pulling in trichotillomania is usually from the scalp and/or eyebrows, but any body site with hair can be affected. Although rising tension and subsequent pleasure, gratification, or relief are integral to the current diagnostic criteria, in many cases, clinically distressing and noticeable hair pulling exists in people who do not endorse these strict criteria. These individuals should not be overlooked when it comes to providing treatment, and the DSM allows for some clinician discretion in the application of the criteria more generally. Trichotillomania has, in surveys, been linked with reduced work productivity, disruption of family life, and avoidance of sports/social activities. Comorbidity with other mental disorders occurs in the majority (82%) of cases presenting to psychiatrists, of which depression, OCD, other anxiety disorders and substance abuse are the most common co-occurring Axis I disorders. Medical complications can also arise, including repetitive strain injury, dermatological scarring, and gastrointestinal obstruction following hair consumption (trichophagia). A recent review of 68 trichotillomania patients found that trichophagia was common (16.2%) and most patients with this condition had not received an evaluation for the possibility of medical consequences resulting from the ingestion of hair. Like trichotillomania, compulsive skin picking is also associated with psychosocial distress. For example, researchers found that 12% ($n = 4$) of 34 outpatients with skin picking reported suicidal ideation attributable to their disorder.

In contrast to trichotillomania, there is as yet no specific diagnostic category for other body-focused habit disorders such as skin picking, knuckle cracking, and nail biting. According to the DSM-IV, these may be diagnosed either under the category of stereotypic movement disorder usually first diagnosed in infancy or adolescence (DSM 307.3), or alternatively as impulse-control disorders not otherwise specified (DSM 312.30). Indeed, emerging evidence argues for the inclusion of habit disorders such as trichotillomania and compulsive skin picking in a separate section of OCDs in a revised nosology.

- A. Recurrent hair pulling
- B. Increasing tension immediately before pulling or when resisting the behavior
- C. Pleasure, gratification or relief when pulling out the hair
- D. Not better accounted for by another mental disorder or dermatological condition
- E. Causes clinically significant distress or impairment in social, occupational, or other important areas of functioning

Figure 1 DSM-IV Criteria for trichotillomania (312.39)(APA 1994), abridged by the authors.

Trichotillomania, Habit Disorders, and the Obsessive–Compulsive Spectrum Disorders

The repetitive motor symptoms of trichotillomania and other body-focused habit disorders appear similar to the repetitive motor tics seen in Tourette's syndrome or even compulsive rituals in OCD. These conditions are thought to share overlapping elements of phenomenology, etiology, and neurobiology and to constitute a putative OC spectrum. Such repetitive, unwanted behaviors may persist due to underlying problems with inhibitory executive neurocognitive control governing habits. Comorbidity between different candidate spectrum disorders is common. For example, whereas compulsive skin picking was found most commonly to present in the context of OCD, it also frequently accompanied body dysmorphic disorder, obsessive–compulsive personality disorder (OCPD), and borderline personality disorder. One other study examined OC-spectrum disorders in OCD subjects and found that trichotillomania clustered with pathological gambling and hypersexual disorder within a proposed reward deficiency group. In contrast, disorders such as self-injurious behavior and body dysmorphic disorder clustered within impulsivity and somatic groups, respectively. The finding that relatives of individuals with OCD showed higher-than-expected rates of grooming disorders (hair pulling, nail biting, and skin picking) lends stronger support to the spectrum theory, although further research is clearly needed.

To further characterize the validity of the OC-spectrum approach, large-scale studies are needed to explore whether rates of habit disorder in OCD-patient relatives (and OCD in habit-disorder-patient relatives) are disproportionately elevated relative to other Axis I conditions. Important differences between trichotillomania and other putative OC-spectrum conditions should not be overlooked. For example, hair pulling in trichotillomania is seldom driven by (or associated with) intrusive thoughts, as distinct from the compulsions seen in OCD. Further research examining impulsive–compulsive neurocircuitry may identify a subtype of trichotillomania more like OCD, and another more like other impulse-control disorders or addictions.

Epidemiology

There have been no population-wide epidemiological studies of trichotillomania, compulsive skin picking, or other body-focused habit disorders. Studies of college students have reported prevalence rates ranging from 0.6% to 3.9% for strictly defined trichotillomania. In addition, hair pulling resulting in noticeable hair-loss

(although not meeting diagnostic criteria for trichotillomania) was reported by as many as 1.5% of males and 3.4% of females. Interestingly, one study found that, when the results were analyzed by gender, 4.1% of females and 3.5% of males had trichotillomania. In treatment trials, usually threefold or greater proportions of women than men participated. It remains to be seen whether this reflects genuine female preponderance or selection bias against males in seeking treatment. Archetypal trichotillomania begins in early puberty (age range: 11–13 years) and follows a relapsing-remitting course into adulthood. The majority of sufferers have never received treatment, and patients report disappointing outcomes from the available applied interventions. By contrast, hair pulling in very young children may be regarded as a distinct clinical entity that can resolve before adolescence without the need for medical intervention.

Studies investigating smaller numbers of US and European college students have identified rates of severe skin picking, resulting in significant distress and tissue damage, ranging from 3.8% to 4.6%, of whom the majority (>80%) were female. However, as many as 90% of a cohort of 133 German students reported occasional skin picking. Like trichotillomania, the onset of picking was reported to occur around early puberty. The students primarily squeezed (85%) and scratched (77.4%) the skin, with a primary focus on the face (94.7%) and cuticles (52.6%). About 20% ate the picked tissue afterward. These preliminary findings suggest compulsive skin picking is an under-recognized problem that occurs on a continuum ranging from mild to severe and may be associated with significant medical consequences.

Neuropsychological Models

Affect-Regulation and Reward

Hair pulling is thought to increase during times of both boredom and stress. It has, therefore, been hypothesized that the behavior may, at least in its initial phases, be used to regulate arousal – either by stimulating or soothing. In one study, 39% of individuals with trichotillomania reported pleasure or a sense of accomplishment from the act of hair pulling which may, therefore, contribute to its repetition. Skin picking and nail biting, likewise, may induce a sense of satisfaction or relief, although for many these symptoms appear to run automatically and to cause distress rather than relief. Mechanisms involved in reinforcement are likely to overlap with those involved in addictive behaviors including substance addiction, and may involve dopaminergic projections within the ventral striatal reward neurocircuitry.

Individuals with trichotillomania and other body-focused repetitive behaviors often exhibit comorbid anxiety and depression. In such cases, individuals may engage

in hair pulling to distract themselves from life stressors and unpleasant cognitions. The risk of developing bald spots may be viewed as a relatively minor setback, at least initially. Unfortunately, the development of bald spots can, in turn, exacerbate depression and anxiety, leading to even more pulling as a misguided attempt at symptom management. These findings are consistent with a study that identified negative affective states and poor self-esteem as the primary triggers for trichotillomania. In another more recent study, individuals with trichotillomania were compared to a control group and reported lower life satisfaction, higher levels of distress, and lower self-esteem. Poor self-esteem was related to concerns about appearance, embarrassment, the need to avoid certain activities due to hair loss, and frustration with the inability to control hair pulling.

Nervous habits may begin as a reaction to physical injury or psychological trauma, and there has been speculation over the years as to whether trichotillomania is associated with childhood trauma. Cases of trichotillomania have been described in association with early physical and/or sexual abuse. One study of females with trichotillomania ($n=60$) found that 18% had histories of childhood sexual abuse. However, it remains to be clearly demonstrated in an appropriate study design whether individuals with trichotillomania experience increased rates of childhood abuse versus people with no history of DSM disorders, or those with other Axis I disorders.

Neurocognitive Impairment

Neurocognitive deficits have been hypothesized as potential contributors to the etiology of habit disorders, either representing vulnerability markers or contributing to the manifestation of symptoms themselves. Such a proposal is based mainly on evidence from research into OCD, where impaired inhibitory control and linked frontostriatal abnormalities have been identified in patients and their unaffected first-degree relatives. Unfortunately, there have been no published studies of cognition in patients with skin picking or nail biting to date. The main studies in trichotillomania are discussed below.

The first neurocognitive investigation in trichotillomania used the Stylus maze test, in which individuals attempt to learn the correct path for navigating across a peg-board, using a stylus. Patients with trichotillomania showed problems on several indices of the test involving a range of abilities including memory, planning, motor execution, and error learning. However, a subsequent study using a similar test (the Austin maze task) found no evidence for deficits in trichotillomania patients who were free from major depression and psychosis.

Impulsivity is a multifaceted term in the neurosciences, and represents a focus of attention in body-focused repetitive behavior research. One form of impulsivity is referred

to as ‘motor impulsivity’ – a diminished ability to suppress motor responses when appropriate to the situation at hand. This ability has classically been measured using tasks that require volunteers to make simple motor responses (e.g., pressing a button) on some computer trials but not on others. Translational research suggests that motor inhibition is dependent on the right inferior frontal gyrus and noradrenergic neurotransmission across species. Using a Go/No-Go task where participants responded to the letter ‘X’ but not the letter ‘O’ on-screen (or vice versa) one study reported intact performance in patients with trichotillomania. Another study used a stop-signal task previously validated in ADHD and brain-lesion studies. Using an individually tailored tracking algorithm, the time taken by the brain to suppress an already initiated response – referred to as the stop-signal reaction time (SSRT) – is estimated. In this later study, patients with trichotillomania exhibited impaired inhibitory control compared to healthy controls and patients with OCD. The degree of deficit was similar to that previously reported in studies of adult patients ADHD.

The Wisconsin Card Sorting Test (WCST), which measures rule learning and cognitive flexibility, is a classic test of frontal lobe integrity. Participants attempt to learn a rule governing which of two cards is correct on the basis of trial and error (feedback). The rule is then altered and individuals attempt to show flexibility and acquire the new rule. Two studies have employed this task in patients with trichotillomania, and both reported performance to be intact. A more recent study has used a computerized variant of the WCST called the extradimensional set-shift task (ED-shift task) from the Cambridge Neuropsychological Test Automated Battery (CANTAB). Consistent with these previous WCST studies, patients with trichotillomania exhibited normal performance on all stages of the ED-shift task. OCD patients exhibited cognitive inflexibility on the task, suggesting that there are important differences (as well as some overlap) between the neuropsychological profiles of trichotillomania and OCD. Future work should measure similar functions in skin picking and nail biting in order to clarify the extent of overlap in the cognitive profiles of the body-focused habit disorders.

Neuroanatomical Models

It is important to question whether trichotillomania, skin picking, and nail biting are associated with similar changes in brain structure or function since this would inform neurobiological models, enhance our understanding of the relationships between conditions, and potentially lead to advances in treatment. For example, it is well established that OCD is associated with abnormalities in distributed brain circuitry, including the

orbitofrontal cortices and basal ganglia (especially caudate). To the authors' knowledge, there have been no imaging investigations of nail biting or skin picking at this time. The main results from trichotillomania studies are as follows.

Most imaging studies in trichotillomania have used region of interest (ROI) approaches; that is, they have set out to measure whether specific structures of the brain show differences in patients versus controls. This minimizes multiple comparisons but could lead to important regions being overlooked. Based on a possible link between OCD and trichotillomania, one study measured caudate volumes in trichotillomania but detected no abnormalities. Another study reported reduced left inferior frontal gyrus and increased right cuneal cortex volumes in patients compared to controls, while another study found smaller left putamen volumes. Using parcelation techniques, one other study identified reduced cerebellar volumes in trichotillomania patients compared to controls.

A single study has investigated whether trichotillomania is associated with brain changes without restriction to particular ROIs. This study took advantage of recently developed methods of neuroimaging analysis thought to provide greater power to detect group differences than conventional parametric techniques. Patients with trichotillomania showed increased gray matter density in distributed circuitry including the cingulate cortex, amygdala–hippocampal formation, and putamen. These regions have been implicated in action monitoring, affective processing, and motor habit generation, respectively.

In terms of brain function (as opposed to structure), positron emission tomography (PET), single photon emission computed tomography (SPECT), and functional MRI (fMRI) have been used in some trichotillomania studies. In one PET study, normalized resting cerebral glucose metabolic rates were shown to be abnormally increased in the bilateral cerebellum and right parietal cortex in patients with trichotillomania. Another study used SPECT to explore the effects of 12-week pharmacotherapy with the selective serotonin-reuptake inhibitor (SSRI) citalopram on brain circuitry in patients with trichotillomania. Treatment was associated with reduced activity in frontal cortical regions, the left putamen, and the right anterior-temporal lobe. Using a sequence learning task in conjunction with fMRI, another study found no evidence for abnormal brain activation in trichotillomania patients versus controls.

Animal Models

Comprehensive investigation of the neurobiology of any psychiatric condition, or group of conditions, is enhanced by the development of animal models. Etiological models

focus on spontaneously arising repetitive or stereotypic behaviors, such as tail chasing, fur chewing and weaving, and motor behaviors driven by conflict, frustration, or stress – such as grooming, cleaning, and pecking. Laboratory-study behaviors that are induced and not spontaneous (e.g., as resulting from pharmacological or genetic manipulations) may limit the generalizability of results. Barbering (abnormal whisker and fur trimming) is limited to a subgroup of laboratory mice and has been proposed as a mouse model of trichotillomania and possibly other body-focused behaviors. Mouse barbering appears to parallel trichotillomania in terms of phenomenology (hair plucking from the scalp and around the eyes/genitals), demography (female biased, onset during puberty), and etiology (genetic background). The barbering mouse model might provide useful information on genetic and environmental etiological factors in trichotillomania.

Another key animal model, to date, is based upon the observation that mice with mutations of the Hoxb8 gene groom excessively. This model is potentially useful since the excessive grooming of Hoxb8 mutants is similar to the excessive grooming seen in trichotillomania. Critically, the Hoxb8 gene is expressed in the orbital cortex, the anterior cingulate, the striatum, and the limbic system – circuitry which has been recently implicated in the pathophysiology of trichotillomania based on the whole-brain-permutation brain analysis described above.

Treatment

Multiple psychometrically sound instruments for the measurement of trichotillomania have been developed. Assessments of habits in subjects with intellectual disability are well established and rating scales for skin picking now exist. No formal treatment guidelines exist for these disorders since the evidence base is limited by the lack of satisfactory clinical trials. Treatment-development has been further hampered by a failure to take account of overlap with other Axis I conditions.

Psychotherapy

There is some evidence supporting psychotherapy for trichotillomania and compulsive skin picking, albeit the studies have had limitations. In the first formal treatment study for trichotillomania, 34 subjects were randomized to either habit reversal therapy – a form of cognitive behavioral therapy involving attention to triggering situations and practiced resistance to pulling urges – or negative practice where subjects stand in front of a mirror and act out motions of hair pulling without actually pulling. Habit reversal reduced hair pulling by more than 90% after 4 months, compared to 52–68% reduction for negative

practice at 3 months. Another study examined 25 subjects randomized to 12 weeks (10 sessions) of either acceptance and commitment therapy/habit reversal or wait list. Subjects assigned to active therapy experienced significant reductions in hair-pulling severity and impairment compared to those assigned to the wait-list, and improvement was maintained at the 3-month follow-up. However, the wait-list lacks credibility as a realistic control treatment.

Recent case studies have also described a positive effect for the use of cognitive behavioral therapies involving habit-reversal elements for the treatment of skin picking. Elsewhere, 372 individuals were consecutively enrolled in an uncontrolled, Internet-based, self-help treatment for self-injurious skin picking. Results revealed significant reductions in frequency of picking episodes and symptom-severity ratings from baseline over a 5-month period, suggesting a role for self-help, computer-based treatments in this field.

Pharmacotherapy

Several controlled pharmacological trials have been performed in trichotillomania. Four of the six published double-blind pharmacological studies examined antidepressants, mainly SRIs. One study compared the serotonergic tricyclic clomipramine to the noradrenergic tricyclic desipramine in a 10-week double-blind, crossover design (5 weeks for each agent). Clomipramine significantly outperformed desipramine, and 12 of the 13 subjects showed significant improvement on clomipramine. A preliminary study reported in poster form, using a randomized, double-blind, nonplacebo, crossover design, compared 10 weeks of fluoxetine with 10 weeks of clomipramine treatment. Both agents demonstrated a similar positive treatment effect.

Fluoxetine has been studied in two small randomized trials with negative results in patients with trichotillomania. In one study, fluoxetine was compared with placebo in a 6-week double-blind crossover study with a 5-week washout period between treatment arms (15 completers). No significant differences were found between fluoxetine and placebo on measures of hair-pulling urges, frequency, or severity. Another crossover study compared 16 subjects on 12 weeks of fluoxetine or placebo, with each agent separated by a 5-week washout period. Fluoxetine again failed to show significant improvement compared to placebo.

The opioid antagonist naltrexone (50 mg per day) was examined in a placebo-controlled, 6-week, randomized, double-blind parallel-arm study in trichotillomania. Of a total of 17 subjects completing the study, significantly greater improvement was noted for the naltrexone

group ($n=7$) than placebo ($n=10$) on one out of three trichotillomania measures.

A recent double-blind, placebo-controlled study – presented thus far in poster form only – examined olanzapine (mean dose: 10.8 mg per day) for 12 weeks in 25 trichotillomania subjects. Preliminary data reported in conference poster form revealed that 85% of those assigned to olanzapine, compared to 17% of those on placebo, improved during the trial.

Pharmacological agents have also been the most frequently studied treatment approach for compulsive skin picking, with nearly all studies examining the efficacy of SRIs. Twenty-five adult subjects with severe morbid nail biting and no history of OCD participated in a 10-week, double-blind, crossover trial of clomipramine and desipramine. A high dropout rate was observed. In the 14 subjects who completed the study, clomipramine was superior to desipramine hydrochloride in decreasing measures of impairment associated with nail biting. In another study, 21 skin-pickers were randomized to 10 weeks of either placebo or fluoxetine. Eight of the 10 participants in the fluoxetine condition were classified as much improved or very much improved, compared with only three of 11 participants in the placebo condition. In a subsequent study, eight responders, following 6 weeks of open-label treatment with fluoxetine, were then randomized to 6 weeks of double-blind fluoxetine or placebo. The four patients randomized to double-blind fluoxetine maintained clinically significant improvement, whereas the four randomized to placebo returned to their baseline symptom level.

Psychotherapy versus Pharmacotherapy, and Combination Treatment

Two partially controlled studies have compared pharmacological and psychological treatment interventions in trichotillomania. A placebo-controlled, randomized, parallel-treatment design was used to compare cognitive behavioral therapy and clomipramine. Twenty-three subjects entered the 9-week study. The cognitive behavioral therapy was a modified manualized treatment based on habit-reversal therapy. There was no psychological control treatment. Cognitive behavioral therapy was significantly more effective than either clomipramine or pill-placebo. Although clomipramine resulted in greater symptom reduction than placebo, the difference was not statistically significant, possibly owing to lack of power ($n=6$, in the clomipramine group). In a second comparison study lacking pill-placebo, behavioral therapy was compared to fluoxetine in a 12-week randomized trial using a wait-list control. Forty-three subjects were enrolled and 40 completed the trial (14 in behavior therapy, 11 in the fluoxetine group, and 15 in the wait list).

Behavior therapy resulted in statistically significant reductions in trichotillomania symptoms compared to either fluoxetine or wait-list in the completers. One other uncontrolled study investigated the effects of adding habit-reversal therapy for trichotillomania patients who had received 12-week sertraline but had not responded ($n=24$ completers total). Combination treatment appeared more effective than continuing with sertraline alone.

Treatment: Summary and Future Directions

It is unwise to generate strong recommendations for treating trichotillomania and body-focused habit disorders such as compulsive skin picking on the basis of such limited trial data. Although habit reversal appears promising for trichotillomania, the effectiveness of this treatment requires testing in properly constituted randomized controlled trials. Pharmacotherapy has been studied with more rigor, but trials have been too small and short in duration to be conclusive. For trichotillomania and nail biting, clomipramine demonstrated greater efficacy than did the tricyclic desipramine, but this finding has not been verified against placebo. For trichotillomania, SSRIs have not shown greater benefit than placebo so far, although two small positive studies suggest efficacy for fluoxetine as a treatment for skin picking. Initial promising findings involving olanzapine and naltrexone merit further evaluation. A recent meta-analysis of trichotillomania studies was unable to extend recommendations much further. There exists a need for further double-blind controlled trials to examine potentially beneficial pharmacological treatments in the short and, arguably more importantly, the longer term.

Conclusions

From a phenomenological perspective, trichotillomania and body-focused repetitive behaviors can be considered candidate members of the OC spectrum of disorders. The body-focused disorders may result from the progression of initially mild hair pulling and other grooming behaviors into a repetitive ingrained pathological behavior. Vulnerable individuals may be distinguishable by deficient top-down cortical inhibitory control governing motor habits, or overactive habit-forming circuitry. Brain abnormalities in neural regions involved in cognition and action monitoring (frontal lobes, cingulate cortices, etc.), affect regulation (amygdalo-hippocampal formation), and habit learning (putamen) have been tentatively implicated

in trichotillomania and may also be involved in other grooming disorders. Treatment algorithms are lacking, with habit-reversal therapy showing suggestive efficacy but limited availability. SSRIs show promise for treating compulsive skin picking but not for trichotillomania. Clomipramine produced benefit in trichotillomania and comorbid nail biting. Alternative pharmacotherapies merit exploration.

Acknowledgments

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See also: Basal Ganglia; Cognition: Attention and Impulsivity; Compulsive Buying; Genes and Behavior: Animal Models; Impulsive–Compulsive Sexual Behavior; Intermittent Explosive Disorder; Kleptomania; Neural Basis of Attention-Deficit/Hyperactivity Disorder; Obesity and Binge Eating Disorder; Pathological Gambling; Problematic Internet Use; Pyromania.

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Analysis of Learning in Invertebrates

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Glossary

cAMP – An intracellular signaling molecule, or second messenger that usually works by activating protein kinase A and can also have effects independent of such activation.

Gastropod – One of the classes of animals from the phylum Mollusca.

Operant conditioning – A training procedure in which an animal is reinforced contingent on some action of its own. It is also called instrumental conditioning or type II conditioning.

Pavlovian conditioning – A synonym for classical conditioning named after Pavlov, the originator of this training scheme.

Sensitization – This term has several slightly different usages. In the invertebrate learning literature, it usually refers to a nonassociative enhancement of an innate response due to application of some sort of strong stimulus to an animal.

Introduction

The learning abilities of invertebrates have attracted attention since the days of Darwin. Romanes, the father of comparative psychology, collected anecdotes about the learning abilities of all types of animals because he felt that the flexibility that learning imparted to behavior provided a behavioral criterion for the presence of mind, whose evolution he wished to trace. The experimental study of learning in invertebrates derived from Romanes' efforts. Of course, human behavior and culture are largely the products of learning; thus, the question of just what various kinds of animals can learn has been a topic of continuing interest. However, starting in the 1950s, there was a growing recognition that various features of invertebrates made them valuable material for gaining insight into neural mechanisms of learning. It was by that time clearly appreciated that all of the basic mechanisms of neural function that had by then been studied were highly conserved, being almost the same from the very lowest animals to humans, and there was no reason to suppose that this would not continue to be true for the mechanisms that

underlay learning. Since neuron numbers are much lower (invertebrates commonly utilize single neurons where vertebrates would utilize multiple neurons in parallel) and neural circuitry was thought to be simpler in invertebrates, and since invertebrates often had special features offering experimental advantages, such as extraordinarily large cells or rapid life cycles favoring genetic analysis, it seemed likely that invertebrates might be able to provide information about mechanisms of learning, which up to that time had been refractory to fruitful analysis in higher animals.

The first influential work taking advantage of invertebrates was on simple forms of nonassociative learning, such as habituation and sensitization, in the marine gastropod mollusk *Aplysia* ([Figures 1\(a\)](#) and [1\(d\)](#)) and, to a lesser extent, on the freshwater crayfish. Associative learning, in the form of Pavlovian conditioning, also began to be studied with some success. Early papers provided a number of valuable insights that have proved to be general. They confirmed the long-conjectured hypothesis that learning would involve synaptic change. They provided evidence that neuromodulators played important roles in establishing the neural changes that underlie learning. In addition, rather surprisingly, they suggested the possibility that learning may be more a matter of adjusting preexisting neural connections than of forming entirely new ones.

Not long after this pioneering work on invertebrates, long-term potentiation (LTP) was discovered in the mammalian hippocampus. This and other developments led to rapid advances in the analysis of learning in mammals. Vertebrate (mostly mammalian) and invertebrate work then became a two-way street. Findings on mammals have usually been found to apply, at least in a general sort of way, to invertebrates, and conversely. In some cases, some of the experimental advantages of invertebrates for studying certain kinds of issues have led to discoveries there, which have been confirmed in vertebrates. Sometimes, findings on vertebrates have spawned research on invertebrates, both to probe generality and to take advantage of special features of invertebrates that could facilitate analysis.

The cellular analysis of classical conditioning, which began in the sea slugs *Hermisenda* ([Figure 1\(b\)](#)) and *Aplysia* ([Figure 1\(a\)](#)), provides a good example of how related work on invertebrate and on higher vertebrate systems can be mutually facilitatory. Work on these

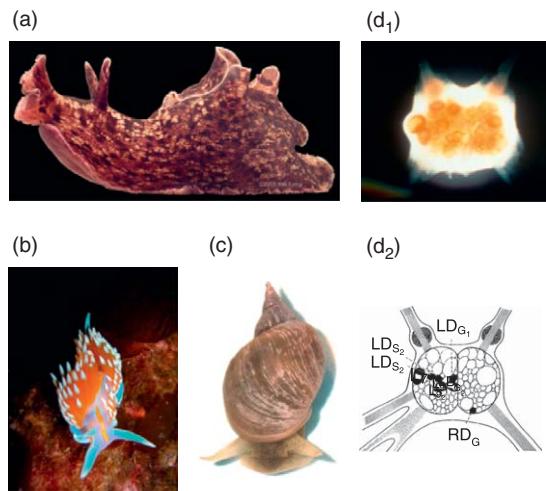


Figure 1 Gastropod mollusks discussed. (a) *Aplysia*. (b) *Hermissenda*. (c) *Lymnaea*. (d₁) The abdominal ganglion of *Aplysia*. (d₂) Diagram of *Aplysia* abdominal ganglion with some neurons labeled. (a) Compliments of Tim Kang. (b) Compliments of David McNair. (d₁) Reproduced with permission from Squire LR and Kandel ER (2009) *Memory: From Mind to Molecules*, 2nd edn. Greenwood Village, CO: Roberts and Company. (d₂) Reproduced with permission from Kupfermann I, Carew TJ, and Kandel ER (1974) Local, reflex, and central commands controlling gill and siphon movements in *Aplysia*. *Journal of Neurophysiology* 37: 996–1019.

molluskan models, which took advantage of large neuron size and relatively simple circuitry, led to the first models of how synapses and networks alter during classical conditioning. The *Aplysia* model focused on evidence that Pavlovian training seemed to lead to increased release of transmitter from the neurons representing the conditioned stimulus (CS), and the *Hermissenda* model provided evidence for both synaptic facilitation and enhanced excitability of neurons of the CS pathway. The independent discovery of LTP in mammals eventually led to the recognition that synapses subject to certain kinds of LTP were Hebb synapses, which become enduringly strengthened by paired pre- and postsynaptic activity, and this mechanism provided a seemingly ideal substrate for classical conditioning. However, although indirect evidence was obtained that LTP was somehow involved in certain kinds of associative learning (place and contextual fear learning), it was at that time impossible to prove that LTP actually mediated the Pavlovian conditioning for which it seemed so suited. However, the findings on mammalian LTP motivated further experiments on *Aplysia*, which led to the discovery that, contrary to what had initially been thought, a mechanism with properties very similar to mammalian LTP was, in fact, responsible for the learning, seemingly in combination with the originally proposed mechanism. Thus, *Aplysia* provided the first really clear example of the use of LTP to produce Pavlovian conditioning, and it is still arguably the best such example, though

work on learning mediated by mammalian cerebellum and amygdala may now be regarded as equally convincing. Returning to the two-way street, there is now also some evidence that something like the presynaptic mechanisms originally described in *Aplysia* might also be found in mammalian systems. Likewise, there is increasing evidence that, similar to what was first described in *Hermissenda*, learning-induced changes in neuronal excitability (nonsynaptic plasticity) can also make important contributions to associative memory in mammals.

Although invertebrates are currently mostly exploited for their many technical advantages, comparative study *per se* continues to have great interest. Members of the invertebrate phyla seem as different from us as aliens from another planet, and the evolution of our and their learning abilities must have been largely independent. Thus, any commonalities that are found, and we see below that there are many, must have arisen largely independently. This suggests that primitive conserved features predisposed toward parallel evolution and/or that the inherent logic of what has to be accomplished constrained the learning circuitry and mechanisms to evolve in a particular way.

In this article, we first focus separately on each of several kinds of invertebrate animals that have provided useful information on learning. For each we briefly describe the kinds of learning that have been studied and aspects of the nervous system or other particulars that have been important to the analysis of learning. Having provided this background, we then compare and discuss some of the important findings that have emerged.

Systems Studied

Gastropod Mollusks

A long history of behavioral studies has established the existence of various forms of nonassociative and associative learning in snails such as *Helix* and *Lymnaea* and slugs such as *Aplysia*, *Hermissenda*, *Limax*, *Pleurobranchaea*, and *Tritonia* (see Figure 1). Habituation in mollusks was first described as early as 1910 in the freshwater snails *Physa* and *Lymnaea* and classical conditioning was first described in 1917 in *Physa*.

Starting in the mid-1960s, work on gastropods began to provide some of the first insights into the mechanisms of learning, and these animals continue to provide new and important information that cannot readily be obtained elsewhere. Gastropods have proved favorable for analysis because they have easily accessible central nervous systems with unusually large neurons (up to 800 µm in *Aplysia*; Figure 1(d)), many of which can be recognized from individual to individual; they also have a variety of reflexes and central pattern generator-driven behaviors that are subject to various forms of associative and nonassociative learning and whose circuitry is relatively simple. Analysis of synaptic

plasticity in isolated ganglia as well as at synapses between cultured neurons that have become synaptically connected has uncovered mechanisms that have then been found to be utilized for behavioral learning, and there also has been considerable success in analyzing changes within nervous systems of intact animals that have actually learned. Finally, an ever increasing amount of genomic and proteomic information has become available on mollusks, which has facilitated molecular analyses of learning and memory mechanisms and has provided strong evidence that these mechanisms are highly conserved between invertebrates and vertebrates. Most recently, the sequencing of the *Aplysia* genome has been completed.

We discuss below work on learning affecting defensive withdrawal reflexes of *Aplysia*, learning of a protective response in *Hermisenda*, and an appetitive Pavlovian conditioned reflex in *Lymnaea* as illustrative of a set of analyses too large to recount fully here.

***Aplysia* defensive reflexes**

The first successful cellular analyses of learning addressed mechanisms of habituation and sensitization in *Aplysia*. *Aplysia* has delicate respiratory organs, the gill and siphon, which are reflexively retracted when it is touched. However, when a specific spot is repeatedly touched, retraction wanes (habituation); this habituation persists up to a few hours after one bout of stimulation (short-term habituation) but can last many days after repeated, spaced bouts (long-term habituation). If an animal is given a painful or traumatic stimulus (e.g., electric shock), retraction to touch all across the body becomes exaggerated (sensitization); following one noxious stimulus, sensitization lasts perhaps 30 min or less, but after repeated spaced bouts of noxious stimulation it lasts days or weeks (short- and long-term sensitization, respectively).

Illustrating the simplicity common in invertebrates, it was found that the sensory neurons that react to touch make monosynaptic connections with the motor neurons involved in retraction (see **Figure 2(a)**). Excitatory postsynaptic potentials (EPSPs) produced in the motor neurons by the mechanoreceptors decrease in amplitude when the mechanoreceptors are repeatedly stimulated, at least partly accounting for habituation; and EPSPs increase following traumatic stimulation, accounting for sensitization. It was rapidly discovered that the facilitation is associated with an increased release of transmitter from sensory neuron terminals (the facilitation was therefore referred to as presynaptic) and also discovered that it is release of serotonin (5-HT) and perhaps also peptide neuromodulators from facilitator neurons onto sensory-motor synapses of the gill/siphon retraction circuit that is the proximate cause of presynaptic facilitation. Slightly later work established that direct puffs of 5-HT onto sensory-motor synapses, even in culture, could cause facilitation; furthermore, repeated exposures separated by rests, which mimic the patterns of repeated spaced traumatic events that cause long-term

behavioral sensitization, also cause long-term synaptic facilitation. These seminal discoveries opened the floodgates to a huge amount of experimental work aimed at elucidating the ionic and molecular mechanisms of serotonin-induced facilitation in *Aplysia* (for which Eric Kandel received the Nobel prize in 2000).

The mechanisms whereby 5-HT causes increased spike-evoked transmitter release provided insights into mechanisms of plasticity that had considerable generality. First, it was found that 5-HT exerts its effect through production of cAMP and, in turn, activation of PKA in the sensory neurons. Single-channel recording experiments then led to the discovery that 5-HT-induced activation of PKA causes phosphorylation of certain species of K^+ channels, which are thereby made more prone to close. As a result, durations of presynaptic terminal calcium elevations increase, providing a possible explanation for release of more quanta per action potential. More recent work has shown that this is far from the whole story, but at the time the idea that behavioral sensitization could be explained in terms of altered channel function attendant on phosphorylation of the channel molecule itself, seemed like Lashley's dream of identifying the physical-chemical change responsible for learning, the engram, come true. Although the modern study of LTP and LTD has tended to emphasize the role of insertion and deletion of postsynaptic receptor molecules in synaptic plasticity, the phosphorylation of existing receptors generally occurs first and works to modulate their actions, much as proposed for the K^+ channels of *Aplysia* sensory neurons. Moreover, whereas other kinases (PKC, CaMKs, MAPKs, etc.) have subsequently also been found to have crucial roles in plasticity at both mammalian and *Aplysia* synapses, the importance of kinases as such was established by this early work.

By the 1980s, it had been known for several decades that long-term as opposed to short-term behavioral learning requires new translation and often gene transcription. Transcription and translation were also found to be needed for long-term serotonergic, PKA-mediated, facilitation in *Aplysia*. This led to experiments establishing that the cAMP-responsive element (CRE) of the genome initiated transcription needed for establishment of long-term facilitation. These findings were the direct motivation for mammalian experiments showing the role of the CRE-binding (CREB) protein in late LTP and in long-term learning in mammals. Subsequent experiments showed that one of the products of the protein synthesis needed to establish long-term facilitation is a molecule that breaks down the regulatory subunit of PKA, rendering existing PKA molecules constitutively active. This presaged recent findings on PKMzeta, which appears to play a crucial role in the persistence of late LTP. These findings constitute examples of the two-way street mentioned in the introduction of this article.

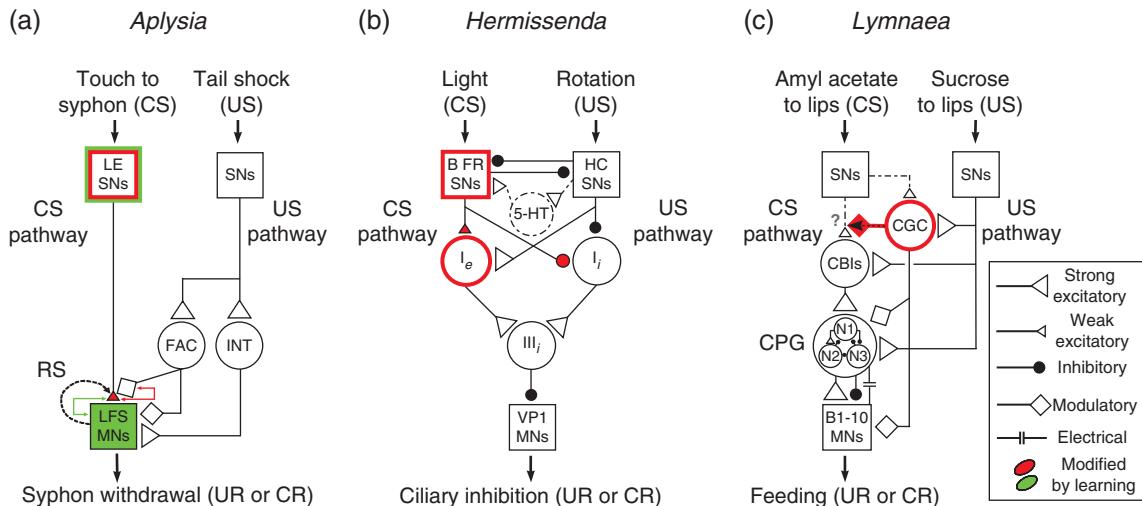


Figure 2 Cellular mechanisms of classical conditioning in mollusks. The location of learning-induced alterations is indicated in color. (a) Classical conditioning of the syphon withdrawal reflex in *Aplysia*. During conditioning, the conditioned stimulus (CS, touch to the syphon) weakly activates LE sensory neurons (SNs), which make monosynaptic connections onto LFS motor neurons. The unconditioned stimulus (US, electric shock to the tail) strongly activates tail sensory neurons that excite both facilitatory interneurons (FAC) that release 5-HT and other neuromodulators to produce presynaptic facilitation at the LE to LFS synapse, and other types of interneurons (INT) that postsynaptically excite the LFS motor neurons leading to syphon withdrawal, the unconditioned response (UR). A retrograde signaling (RS) mechanism (dashed arrow) has been suggested to operate between the postsynaptic motor neuron and the presynaptic sensory neuron. Red and green double-ended arrows indicate neuronal elements that must be activated conjointly for the induction of either activity-dependent presynaptic facilitation (red) or Hebbian LTP (green) at the LE-LFS synapses. After conditioning, the synaptic connections of the LE sensory neurons with the LFS motor neurons are strengthened (red synaptic terminal). The postsynaptic response to glutamate is also increased (green LFS motor neurons). The excitability of the LE sensory neurons is also increased both by classical conditioning and in simple sensitization (nonsynaptic plasticity, bold red and green outlines). These changes lead to an increased syphon withdrawal response to the touch CS, the conditioned response (CR). (b) Classical conditioning of the phototactic response in *Hermissenda*. I_e and I_i interneurons are sites of convergence between identified components of the CS (light) and US (rotation) pathways. Statocyst hair cells (HCs) have indirect excitatory synaptic connections with B-type photoreceptors (B FR) through a proposed 5-HT-mediated interneuronal pathway (dashed lines). Hair cells and photoreceptors also have reciprocal inhibitory monosynaptic connections. After classical conditioning, components of the CS pathway become involved in ciliary inhibition. Changes in both cellular excitability (bold red outlines) and synaptic efficacy (red synaptic terminals) contribute to CS-elicited inhibition of ciliary locomotion. The net effect of cellular and synaptic plasticity is to increase the spike activity of type III $_i$ inhibitory interneurons during light, which produces an inhibition of VP1 ciliary activating motor neurons in conditioned animals. (c) Single-trial chemical classical conditioning of feeding responses in *Lymnaea*. In naive animals, the proposed excitatory connections (dashed line) between the CS chemosensory neurons (SNs) and the command-like cerebral-buccal interneurons (CBIs) are weak; the CGCs are at their normal membrane potential (~ -65 mV), and the presynaptic modulatory input from the CGCs to the SNs is inactive or weak. During training, the food unconditioned stimulus (US) activates the feeding CPG through direct and indirect excitatory inputs (through the CBIs) to produce an unconditioned feeding response. There is already associative memory as early as 2 h after training, and this is assumed to be supported by some form of presynaptic plasticity (red question mark) in the SN to CBI pathway, lasting for up to 20 h after training. In conditioned animals, >20 h after training, the CGC soma and proximal axon segments are depolarized compared with naive and unpaired control animals; this leads to an enhancement of the CGC presynaptic modulatory inputs to SNs and a consequent strengthening of the SN to CBI excitatory synapse, which enables a conditioned feeding response to the CS. The bold red outlines of the CGC soma and axon indicate learning-induced nonsynaptic plasticity (membrane depolarization), which increases the strength of CGC to SN and SN to CBI synaptic connections in a remote-controlled manner. This particular type of extrinsic modulation may be crucial (black dashed arrow) for the memory trace to be expressed >20 h after training. The effect of somal depolarization does not spread onto the more distal modulatory connections of the CGC with the CPG or motor neurons leaving the normal modulatory function of the CGC unaffected by learning. (a) Modified from Antonov I, Antonova I, Kandel ER, and Hawkins RD (2003) Activity-dependent presynaptic facilitation and Hebbian LTP are both required and interact during classical conditioning in *Aplysia*. *Neuron* 37: 135–147. (b) Modified from Crow T and Tian L-M (2006) Pavlovian conditioning in *Hermissenda*: A circuit analysis. *Biological Bulletin* 210: 289–297. (c) Modified from Benjamin PR, Kemenes G, and Kemenes I (2008) Non-synaptic neuronal mechanisms of learning and memory in gastropod molluscs. *Frontiers in Bioscience* 13: 4051–4057.

When the early experiments on nonassociative learning were done, it was not entirely clear to what extent gastropods like *Aplysia* were capable of associative learning. However, behavioral demonstrations of classical conditioning in *Aplysia*, *Hermissenda*, and other mollusks

were soon forthcoming, and it was then rapidly found that the neural changes responsible for classical conditioning in *Aplysia* seemed to be similar to those seen in simple nonassociative sensitization (see Figure 2(a)). The conclusion at the time was that 5-HT, which by itself was

thought to cause facilitation of the synapses between the sensory and motor neurons of the gill and siphon reflex by enhancing the amount of transmitter released per presynaptic spike, produced similar changes in exaggerated form when 5-HT was applied to a sensory-neuron terminal whose free calcium was elevated due to the just-prior arrival of a presynaptic spike. Classical conditioning was thus said to be due to activity-dependent enhancement of heterosynaptic (serotonergic) facilitation.

More recent work has changed, or elaborated, this picture substantially. It is now clear that both sensitization and classical conditioning involve increased reactivity of the motor neurons to glutamate as well as increased pre-synaptic release. 5-HT acts postsynaptically as well as presynaptically. The postsynaptic effect of 5-HT is to increase the number or efficacy of postsynaptic AMPA-type receptors. In addition, activation of postsynaptic N-methyl-D-aspartic acid (NMDA) receptors and depolarization caused by the unconditioned stimulus (US) are crucial to the production of conditioning. Moreover, retrograde signaling from the motor neurons back to the sensory-neuron terminals innervating them probably plays a crucial role in enhancing transmission.

Operant conditioning in Aplysia

Most work on the cellular basis of learning has utilized classical conditioning because of its experimental convenience. However, there have been some interesting findings on operant conditioning in which an animal is reinforced when it spontaneously carries out some particular action. Withdrawal of *Aplysia*'s gill, the classical conditioning of which has been so extensively studied, can also be controlled by shocking an animal whenever the gill relaxes by more than a certain amount. These experiments, similar to ones first done on cockroach leg position, suggest the occurrence of operant conditioning. The frequency of spontaneous swallowing movements in *Aplysia* can also be greatly increased if reinforced by food, and/or by the electrical stimulation of nerves that normally sense the presence of food. Command-like neurons whose activity correlates with feeding movements and whose stimulation increases the likelihood of such movements have been identified. After operant conditioning (or its *in vitro* equivalent), these neurons have an elevated input resistance (which amplifies incoming EPSPs), and their membranes become much more prone to burst in response to externally imposed depolarizations. As would be the case for similar kinds of learning in mammals, these changes depend on the integrity of dopamine transmission, and it has been found that the same changes can be produced by briefly squirting dopamine on these cells at the end of a burst of activity that is produced by direct depolarization.

Classical conditioning of a protective response in *Hermisenda*

In the untrained sea slug *Hermisenda*, light evokes positive phototaxis. However, if light is repeatedly paired with turbulent conditions that cause the animal to stop forward progression and hold fast to the substrate, simulated in the laboratory by vigorous rotation, the normal positive taxis to light is replaced by a cessation of locomotion; this is caused by an inhibition of the ciliary movements that underlie forward locomotion.

Figure 2(b) schematizes the circuit underlying the neural control of cilia by the CS and the US, as currently understood. Ciliary activity is promoted by VP1 motor neurons, which are subject to inhibition by type III_s inhibitory neurons. The III_s are excited by two classes of interneurons, the I_es and I_is, one of which receives excitatory input from both the CS- and US-responsive sensory neurons. The interneurons and motor neurons are all spontaneously active. As indicated by the connectivity as shown in the figure, USs cause excitation of the I_es, which promotes cessation of ciliary activity by exciting the type III_i inhibitory interneurons. The US also causes inhibition of the I_is, which reduces their spontaneous activity and thus promotes continued ciliary activity; however, the former effect dominates the latter, and thus USs stop the cilia. As also seen from the circuit's connectivity, light tends to increase activity of the I_es, which promotes stoppage of the cilia, but it decreases activity of the I_is, which promotes ciliary beating. In untrained animals, the former effect dominates. However, pairing of light (the CS) and rotation (the US) causes three changes: (1) excitability of the CS-representing B photoreceptors increases, (2) the strength of the excitatory photoreceptor–I_e synapse increases, and (3) the strength of the inhibitory photoreceptor–I_i synapse increases. The first two of these changes favors stoppage of ciliary activity in response to light; the third does the opposite, but the factors favoring stoppage dominate, leading to the observed conditioned response.

All three changes occur at points in the circuit where CS- and US-evoked activity converges, consistent with the fact that these changes only occur if CS and US are paired together, not if they are separated in time. It is noteworthy that at least some of these changes seem to depend on activity of the to-be-modified cell occurring in combination with serotonergic input, as in the conditioning of *Aplysia*'s protective withdrawal reflex.

Appetitive classical conditioning in *Lymnaea*

Paired application of food as a US and a tactile or suitable chemical stimulus as a CS to the lips of the pond snail *Lymnaea* produces a conditioned appetitive response in which the CS comes to evoke rhythmic feeding movements. Learned feeding responses can be seen as fictive feeding in semi-intact preparations and recorded in

various parts of the neural circuitry that mediates unlearned and learned feeding behavior. During feeding, a population of cerebral–buccal command interneurons (CBIs) fire at the feeding frequency and drive feeding movements that are orchestrated by a central pattern generator circuit (CPG) (**Figure 2(c)**). USs provide strong excitation to both the CBIs and CPG neurons and also increase the tonic firing of the cerebral giant cell (CGC), a modulatory neuron that releases 5-HT to most elements of the feeding circuit. In untrained animals, the CBIs are weakly excited by the CS through a pathway that is not yet fully characterized. However, soon after a single pairing of amyl acetate as a CS and sugar as a US, which results in very fast learning, amyl acetate alone causes fictive feeding initiated by CBI activity. This conditioned reflex is maintained for many weeks. It seems likely that early postpairing responsiveness is due to changes intrinsic to the still uncharted CS–CBI pathway, but work on this is still in progress. However, about 20 h after pairing the CGCs become depolarized due to upregulation of persistent sodium current. This depolarization, though it does not alter the tonic-firing rate of the CGCs, does increase the amount of transmitter released from the CGCs by ongoing tonic firing, and increases of a comparable magnitude imposed on an untrained animal allow the CS to produce feeding. So far technical issues have made it impossible to determine whether this change in the CBCs merely supports a response that would still occur without it or is actually necessary for the maintenance of responding at long times after pairing. In either case this extrinsic modulation, which is capable of causing conditioned responding, is of considerable interest (see further below).

Insects

The modern study of learning in insects derives from two lines of endeavor. One is the pioneering work of von Frisch, Tinbergen, and other ethologists, who established that learning could play important roles in the navigation, food finding, and reproductive activities of insects. The other stemmed from attempts to take advantage of the techniques of fruit fly genetics to look for and study mutants with altered learning and memory capabilities. The small size of insect nervous systems has rendered difficult electrophysiological approaches used in other kinds of animals. However, small size is an advantage for modern optical recording and stimulation techniques, because in small brains and ganglia individual neurons can be directly imaged *in situ* without disruption. Thus, the kinds of experiments that in larger-brained animals have had to be done on tissue slices, where probably crucial neuromodulatory and other inputs are lost, can be done in insects with the nervous system entirely intact and the animal, though restrained, still behaving.

Kinds of learning analyzed in insects

Insects naturally make use of learning capacities in courtship and mating, food finding, navigation, defense, and many other arenas. We focus here on honeybees and fruit flies (*Drosophila*) (**Figure 3a, c**). Using behavioral tests that take account of insects' natural way of life, rapid and thorough learning and long retention can be demonstrated.

In honeybees, many experiments have been done in which bees are allowed to settle and feed on visual discriminanda. The bees remember and will later fly to those stimuli if given the opportunity; this has allowed extensive analysis of their visual processing and visual memory abilities. A large body of work has also been done on Pavlovian olfactory conditioning in bees. Touching a bee's antenna with sugar water leads to proboscis extension. If an odorant is presented just before sugar water, the odorant comes to elicit a conditioned proboscis extension response (**Figure 3(a)**). Experiments utilizing this Pavlovian conditioning paradigm have shown that bees learn, generalize, acquire second-order conditioned responses, extinguish, etc., in much the same way as rats and other mammals.

If *Drosophila* are exposed to one odorant in the presence of food and another with food absent, and then given a choice between the two odorants in a T-maze, they choose the previously rewarded odor. If, on the other hand, they are subjected to electric shock in the presence of one odor and not another, they learn to avoid the shocked odor. More work has used the aversive than the appetitive situation; findings are similar, but there are also some interesting differences (discussed below).

Mutants and stages of learning

As had been hoped, screening of randomly mutated flies led to the discovery of a number of mutants with abnormal learning or memory. Analysis and comparison of these mutants with each other and with wild-type flies led to the recognition that following training, memory goes through a number of stages that require products of different genes. At least four stages are generally recognized. Training leads initially to a briefly retained or short-term memory (stage 1, operative for up to about an hour after training) and then to an intermediate or middle-term memory (stage 2, operative from about 15 min to 3 h post training). Middle-term memory generates two independent forms of longer-term memory, anesthesia resistant memory (stage 3, lasting 1–2 days), which unlike its precursors is not lost if a fly undergoes a period of anesthesia, and also long-term memory (stage 4, starting about 5 h posttraining and continuing for days or weeks), which requires multiple, spaced training trials for development and is dependent on new genetic transcription. Initial learning and short-term memory are greatly disrupted by mutations affecting a gene called *rutabaga*

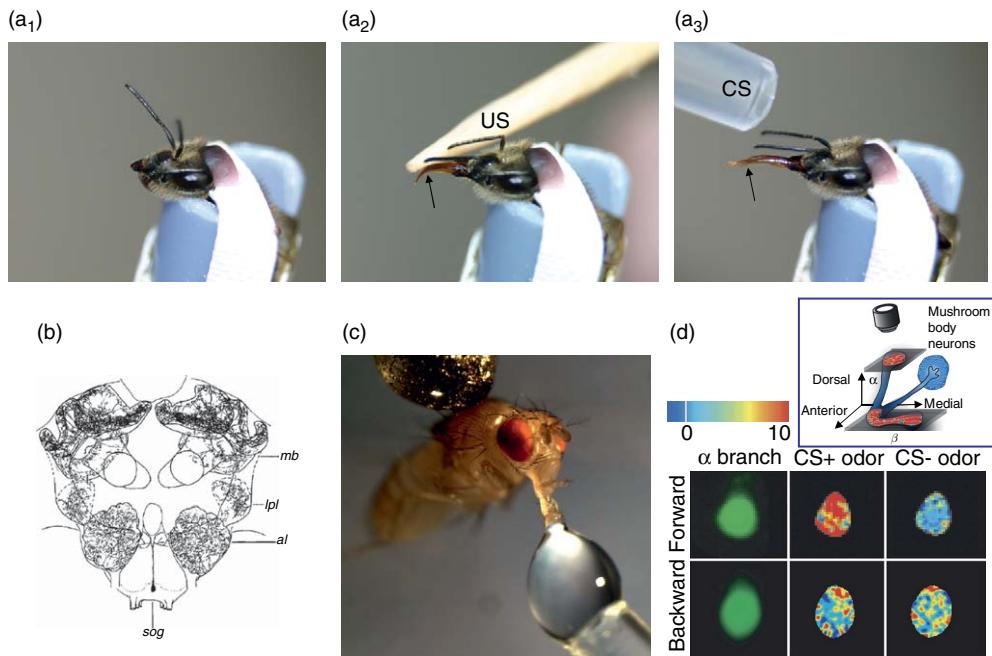


Figure 3 Insects discussed. (a) Honeybee restrained on its back in a classical conditioning experiment (mainly the head can be seen). (a₁) Bee at rest. (a₂) An unconditioned proboscis (arrow) extension evoked by sucrose on a toothpick (US). (a₃) A conditioned response evoked by an odor CS after pairing of US and CS. The tube delivering the odor is marked CS. (b) The VUMmx1, octopaminergic reinforcement neuron. VUMmx1 arborizes in the subesophageal ganglion (sog) and innervates glomeruli of antennal lobe (al), lateral protocerebral lobe (ipl), and mushroom body (mb). (c) A tethered *Drosophila*, drinking. (d) Optically recorded calcium responses of *Drosophila* mushroom body to stimuli 24 h after a series of pairings of CS+ (octonol) with shock and CS- (benzaldehyde) with no-shock. Left column is basal fluorescence and remaining columns are elevations relative to that, color coded as indicated above. Only the CS+ in the forward pairing condition has strong elevation of response. (a) Series compliments of U. Mueller. (b) With permission from Hammer M and Menzel R (1995), Journal of Neuroscience, 15, 1617-1630 with permission of Society for Neuroscience]. (c) Picture compliments of James Waters. (d) Modified with permission from Yu D, Akalal DB, and Davis RL (2006) Drosophila alpha/beta mushroom body neurons form a branch-specific, long-term cellular memory trace after spaced olfactory conditioning. *Neuron* 52: 845-855.

(the gene is commonly referred to by the name of the mutant) that codes for a calcium-dependent adenylate cyclase and, like the calcium-stimulated adenylate cyclase of *Aplysia* sensory neurons, is envisaged to be a detector of the coincidence of neural activity and reinforcing neuromodulatory input. Middle-term memory is dependent on a gene (*amnesiac*) whose product is an adenylate-cyclase-stimulating peptide that is released as a neuromodulator and also is dependent on genes for NMDA receptor subunits. Anesthesia-resistant memory is dependent on a gene (*radish*) which makes a protein whose function is as yet little understood, and long-term, protein-synthesis-dependent memory depends on genes that produce CREB and is prevented by mutants that produce excessive amounts of isoforms of CREB that prevent transcription (e.g., CREB2). These and other mutants have played key roles in further analysis.

Anatomy of olfactory learning

The above findings, used in conjunction with anatomical information and modern genetic/molecular technology,

have led to localization of at least parts of the engram underlying olfactory conditioned responses.

As seen in Figure 4, olfactory receptors terminate in the antennal lobes where they excite projection neurons directly and also inhibit them through interneurons. The projection neurons project to both the cerebral mushroom bodies and the lateral horn. In the mushroom bodies, they form synapses on the principal intrinsic cell type, the Kenyon cells, which, in turn, make synaptic contact within the mushroom bodies on extrinsic neurons that, along with the antennal lobe projection neurons, send axons to the lateral horn. The lateral horn output is, in turn, thought to provide access to motor circuitry. The mushroom bodies also receive other important inputs: (1) they receive processed sensory input from other parts of the brain; (2) they receive major innervation from peptidergic/cholinergic DPM cells, which themselves project back to the mushroom bodies (see further below); and (3) they, and also the antennal lobes and lateral horn, receive octopaminergic and dopaminergic innervation that conveys information about rewarding and aversive events, respectively. In the honeybee, the octopaminergic

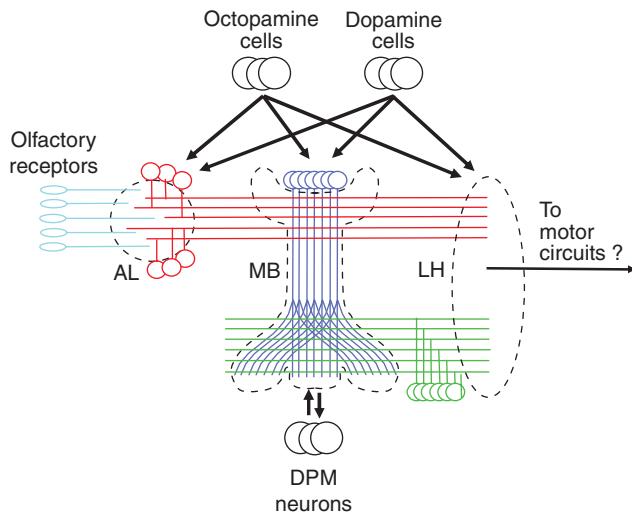


Figure 4 Brain circuitry involved in appetitive and aversive olfactory learning in insects, as discussed in text. Olfactory receptors (cyan) excite projection neurons (red) of the antennal lobes (AL) and also interneurons (not shown) that inhibit the projection neurons. The antennal lobe projection neurons innervate both the intrinsic neurons of the mushroom bodies (MB), the Kenyon cells (blue), and neurons of the lateral horn (LH). The Kenyon cells make synapses extrinsic neurons (green) that also innervate the lateral horn. Octopaminergic and dopaminergic neurons richly innervate all three of the illustrated regions. Dorsal paired median (DPM) neurons receive input from and project back to the mushroom bodies.

innervation is, in part, through a type of unpaired VUMmx1 neuron that has a remarkable and elegant branching pattern that innervates all three of the regions diagrammed in **Figure 4** and **Figure 3(b)**.

Most of the proteins coded by genes whose mutation diminishes learning and memory abilities have enriched or sole expression in the mushroom bodies or in neurons that project strongly to them, and damage to or developmental reduction of the mushroom bodies has major effects on learning ability. Thus, these structures have long been thought to be major players in learning. This belief has recently been reinforced by experiments in which the learning abilities of mutants have been rescued by Kenyon cell-specific expression of the wild-type form of the mutant genes.

Such targeted expression experiments rely on lines of flies into whose genome effector genes of interest have been inserted, linked to an upstream sequence that causes transcription only when bound to regulatory protein made by the yeast GAL4 gene. Such lines are crossed to other lines constructed so that GAL4 expression occurs only in specific cell types (and/or at certain temperatures). One can use the GAL4 system to control expression of genes coding for most any protein. Proteins that allow imaging of calcium levels, or synaptic vesicle release, proteins that block transmitter release, and proteins that cause light-evoked depolarization, and thus cell firing, have all been used.

This technology has been used to show that for a short time after forward odor CS-US pairings, odor-evoked calcium elevations and transmitter release of antennal

lobe projection neurons increases specifically for the paired CS. CS odor-specific calcium elevations also occur in Kenyon cells with a time course and under induction conditions that correspond to those of long-term memory (**Figure 3(d)**). It is tempting to think that these responses are expressions of the memory traces that cause conditioned responses. In fact, these responses certainly must contribute to effective conditioned responding. However, it is important to keep in mind that it is not possible for the antennal lobe to control what kind of response an animal makes, because the antennal lobes are purely sensory structures that have no way of controlling particular responses. The situation in the mushroom bodies is more interesting. The aminergic neurons that innervate olfactory processing structures respond to USs; their activity is required for conditioning, and, when they are stimulated directly, their activity can substitute for an actual US. Moreover, the octopamine cells are specific for appetitive, and the dopaminergic cells for aversive conditioning. Thus, if the sets of Kenyon cells innervated by the octopamine and dopamine neurons were separate, and if they made differential downstream connections to motor circuitry for approach versus avoidance responses, they could perhaps be responsible for CS-specific response selection. Whether this is so is not known. However, this concept cannot be carried too far; insects can presumably learn a greater variety of responses to odors than just approach and avoid, and it seems unlikely that one could have separate classes of Kenyon cells for each type of response that can be selectively associated with a CS.

Consequently, response selection at the level of the Kenyon cells seems improbable. It appears more likely that the crucial cellular changes responsible for associating specific odors with particular responses reside at the synapses between the Kenyon cells and downstream response-evoking neurons. In flies, the recorded calcium responses are in fact in the region of Kenyon cell output synapses; thus, they could perhaps reflect enhanced release from cells responsive to the CS odor onto neurons producing the conditioned response. In bees, there is some direct evidence that synapses between Kenyon cells and the extrinsic output neurons are subject to LTP and LTD.

Nematode worms

The nematode worm, *Caenorhabditis elegans* (Figure 5), was selected for neurobiological analysis because it has an extraordinarily simple nervous system and, like *Drosophila*, it is highly suitable for genetic analysis. The total central nervous system is comprised of about 300 neurons that interact through some 5000 chemical and 600 electrical synapses! Because of the worm's small size (~ 1 mm) it has been possible to make serial electron microscope sections of the entire animal, and from these to generate a complete wiring diagram of the nervous system. The identity of the transmitters found in many of the neurons has been determined, and the transmitters and neuromodulators are the same as those common in vertebrate and other invertebrate nervous systems, including ACh, GABA, glutamate, serotonin, dopamine, and others. Transmission at the electrical junctions is believed to be generally excitatory, but it cannot be determined from anatomy which chemical junctions, most of which are glutamatergic, are excitatory and which inhibitory (glutamatergic synapses can be of either sign). Neurohumoral release also occurs.

Laser ablations of individual neurons has led to the delineation of circuits that appear to be involved in particular behaviors, and remarkably, given the simplicity of

the nervous system, several of these circuits seem to mediate behavior that is subject to forms of learning.

In nature, *C. elegans* lives in soil where it feeds on bacteria. Undisturbed on a moist surface it moves forward in a snake-like sinusoidal wave pattern. Worms (i.e., *C. elegans*) have rather complex reproductive behavior and certain kinds of social behavior. Most relevant to learning paradigms that have been studied, worms congregate in regions rich in suitable food and leave regions lacking it, while they innately avoid or escape certain kinds of chemical and mechanical stimuli.

Neural mechanisms of retraction-response habituation

All animals seem to be subject to habituation when stimuli that innately cause responses are repeated. In worms, anterior mechanical stimulation causes a retraction and rapid backward movement, while posterior stimulation causes forward acceleration. Diffuse vibrations that stimulate both anterior and posterior mechanoreceptors produce retraction, but this is thought to be attenuated somewhat at the level of behavior by the competing forward response. Responses to repeated vibrations gradually wane (habituate), while a brief harsh stimulus causes a temporary reinstatement of reactivity (dishabituation). Habituation produced by stimulation occurring a few times per minute abates within hours (short-term habituation), but habituation induced by spaced training consisting of repeated bouts of training separated by rests of about an hour lasts for more than 24 h (long-term habituation; e.g., responsiveness about 50% down after a 24-h rest). Persistence of habituation for a day or more in worms, as with all kinds of learning in most animals, requires new protein synthesis. Certain training regimens can also cause a form of protein synthesis-independent habituation with longevity in-between that of short- and long-term habituation, which could correspond to the middle- or intermediate-term forms of memory seen in mollusks and insects.

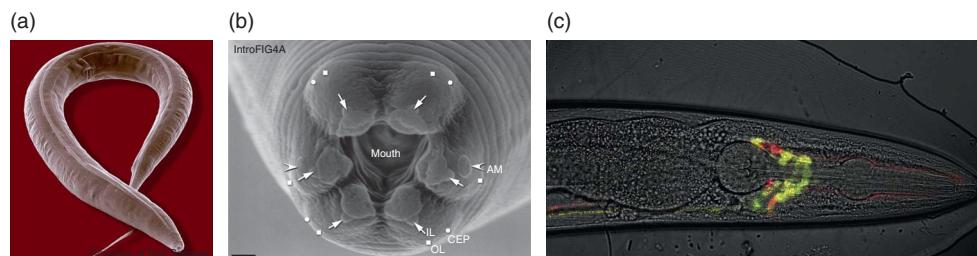


Figure 5 *Caenorhabditis elegans*. (a) The entire worm, anterior end at lower right. (b) Detail of mouth region with location of sensilla marked by arrows (c) Micrograph of head region of *C. elegans* showing neurons expressing green or red fluorescent protein conjugated to neuron-specific proteins. The somata of these neurons are located in the peri-esophageal nerve ring that comprises the worm's largest congregation of neurons. Axons run rostrally to the mouth (right) and caudally in the ventral nerve cord (left) (some faint colors enhanced for clarity by the authors; from a micrograph kindly provided by Cathy Rankin). (a) Picture compliments of Ralf Sommer. (b) Reproduced with permission from Hall DH (2008). *C. elegans* Atlas, Cold Spring Harbor Press.

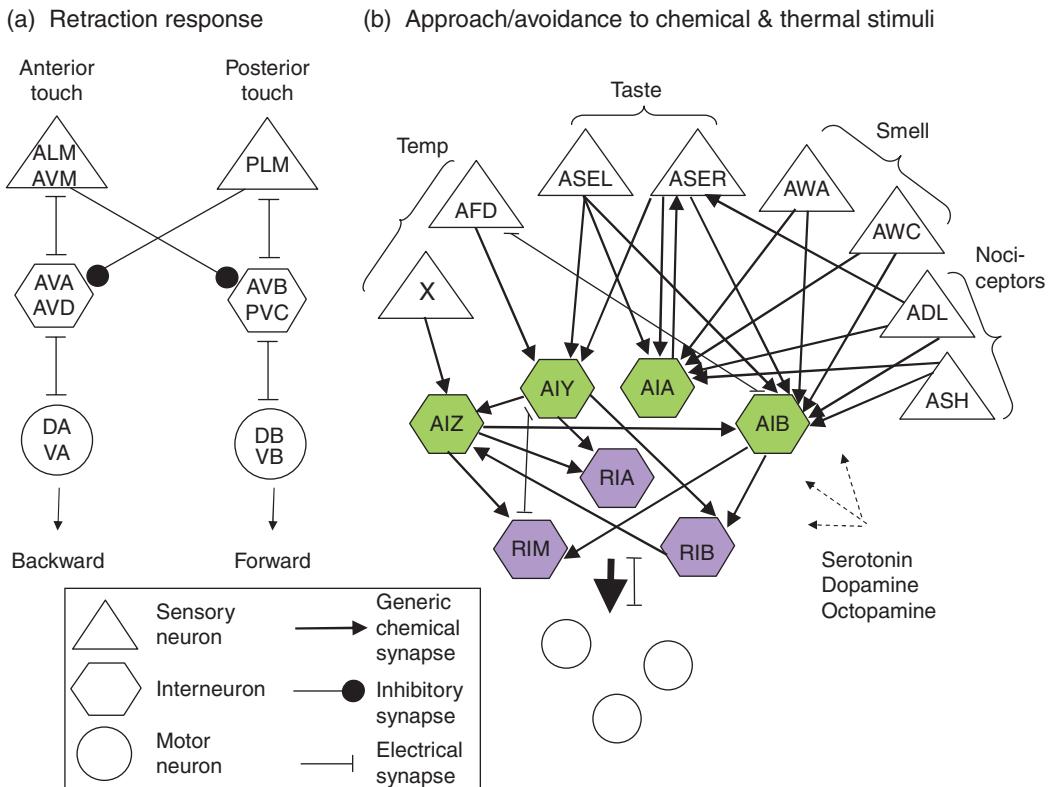


Figure 6 Neural circuitry for retraction responses to vibration (a) and for approach and avoidance of thermal and chemical stimuli (b) of *C. elegans*, as discussed in text. Based mainly on serial electron microscopy and observing behavior following laser ablations of individual neurons. Sensory neurons (triangles), interneurons (hexagons), and motor neurons (circles) with their names included are indicated. In (b) first-order interneurons are green and premotor interneurons are lavender.

The neurons that, based on laser ablations and connectivity, seem to be involved in the retraction and forward acceleration responses are shown in **Figure 6(a)**. Anterior and posterior mechanoreceptors make electrical (excitatory) synapses on pre-motor interneurons that drive retractions and forward accelerations, respectively. Each pathway also sends glutamatergic chemical inputs that are thought to be inhibitory to the other pathway (glutamatergic transmission in *C. elegans* can be either excitatory or inhibitory depending on properties of the postsynaptic receptors). The inhibitory connections would be expected to attenuate the forward accelerations that compete with retraction; therefore, any decrease in the activation of the anterior sensory neurons or in the potency of their synapses would be expected to increase competing forward acceleration, decrease net retraction, and thus support habituation of retraction (see further below).

Optical recording of calcium responses in sensory neurons of this circuit during habituation training show response decreases in the sensory neurons mediating the backward but not the forward response. Moreover, the extent of this decrease co-varies with several manipulations that influence development rate of short-term

habituation. This suggests that the decreased sensory responses are at least part of the mechanism of habituation. If the reduced activation of sensory neurons causes reduced inhibition of the interneurons mediating forward acceleration, the increased activity of these neurons would, as explained above, be expected to increase behavioral habituation of retraction.

It has also been found that mutations which disrupt either glutamate vesicle recycling in ALM/AVM (named neurons are labeled in **Figure 6**) or disrupt non-NMDA glutamate receptor subunits (found on all four interneurons) prevent long-term though not short-term habituation. Moreover, green-fluorescent protein (GFP)-labeled glutamate receptor subunit is reduced at synapses on interneurons following long-term but not short-term training. It seems clear that the crossed inhibitory pathways of **Figure 6(a)** are glutamatergic, but the excitatory synapses between the anterior sensory neurons and the interneurons that mediate backward responding appear to be electrical, not glutamatergic. The exact synapses at which glutamate transmission is decreased in long-term habituated worms has not yet been reported. If the inhibition of the forward-acceleration interneurons AVB/PVC were decreased by LTD, the forward acceleration that

normally competes with retraction would be increased, and this would presumably be seen as habituation of the retraction response. Furthermore, although we do not usually think of electrical synapses as plastic, electrical synapse-mediated input to fish Mauthner neurons from auditory nerve fibers is subject to LTD. This occurs when auditory input is activated along with inhibitory input, as happens to worm AVA/AVD neurons when vibration stimuli drive both anterior and posterior touch sensory neurons. Thus, LTD of electrical transmission in the retraction circuit is a possibility.

If protein synthesis, which is needed for the production of long-term habituation of retraction, is blocked just following testing of long-term habituation, recovery from habituation occurs by the next day, and the previously established reduction of GFP-conjugated glutamate receptors is reversed. This is consistent with the finding in a variety of animals that protein synthesis is required to maintain long-term memory of associatively learned responses after they are recalled (i.e., reconsolidation is needed after performance of a learned response).

Particularly interesting, given the simplicity of the worm nervous system, are experiments suggesting some sort of context specificity to habituation. The findings are somewhat complex but have been interpreted as providing evidence for the formation of an association between the context and habituation of retraction. The complexities have been interpreted as being consistent with Wagner's comparator theory of habituation and might also seem somewhat parallel to findings from mammals on context specificity of extinction, but interpretations in terms of the properties of the neural circuits known to underlie retraction behavior have yet to be offered. Nevertheless, the surprising suggestion that nematode worms can form associations is upheld by experiments directed specifically to that point.

Associative learning

A number of experimental procedures are thought to provide evidence of associative leaning. All involve accumulation of worms in the vicinity of stimuli (chemical or thermal) that had been associated with presence of food, or away from stimuli that had been associated either with starvation or with innately aversive chemical stimuli. Training might consist, for example, of incubating worms on a substrate with an inherently attractive salt (the CS) paired with either an aversive US (e.g., the absence of food or the presence of garlic) or an attractive one such as food. After such training, worms act as though they have formed an association between the CS and the US. If placed on a gradient with the CS salt at one end and an equally attractive control salt that had not been paired with a US at the other, they move toward the CS salt if it had been paired with something attractive and away if it had been paired with something aversive. Retention of

such learning is often not followed for more than a few hours, but at least under some circumstances retention has been reported to be more than a day. While some experiments seem to provide firm evidence of associative learning, some have been structured in such a way that the appearance of associative learning is in fact due to expression of separate stimulus and/or response biases established by the training.

The neurons that seem, based on laser ablations and connectivity, to be involved in learning to approach stimuli associated with food and to avoid stimuli associated with noxious stimuli or starvation are shown in **Figure 6(b)**. Primary afferents such as AFD, ASEL, and AWC code information about temperature, chemical environment, etc., which are the modalities of the CSs used in learning experiments. These sensory neurons provide input to first-order interneurons such as AIY and AIZ that, in turn, interact with further interneurons that do not receive any direct sensory input and that largely stand between the first-order interneurons and motor neurons.

Only a few sensory neurons serve each modality. In contrast to the situation found in animals that use neurons less parsimoniously, each *C. elegans* chemosensitive neuron has independent receptors for many molecules. This allows the possibility of substance-specific adaptation to many chemical agents. However, any associations formed will necessarily generalize to other agents that are represented by the same sensory neurons.

The first-order interneurons are often categorized with respect to their presumed behavioral effects, but such characterizations tend to be contingent on the particular circumstances under which they were determined. For example, it has been proposed that AIY causes movement from cooler to warmer temperatures and AIZ the opposite (and would perhaps be expected to have similarly antagonistic effects on chemical gradients). However, it has also been proposed that AIZ promotes changes of direction, while AIY suppresses them. These are not incompatible characterizations. One is stated in terms of effects of the neurons on net migrations and the other in terms of the kinds of movements that may lead to such migrations. A generally agreed, comprehensive account is not yet available but work on this is advancing.

Although, as in the insects, optical recording methods are beginning to be employed, the more common approach to finding neurons involved in associative-like learning has been to begin by screening for mutants that fail to learn some specific assay task, and then to try finding the gene's homolog in better known animals and thus identify its product. This done, the wild-type gene can be expressed, one neuron at a time, in cells that normally express it to see what will rescue the lost kind of learning. The cells where the gene product is needed for learning can thereby be identified. In recent work,

molecules allowing imaging of calcium or other signaling molecules have also been expressed in neurons that had been identified by the genetic analysis, and physiological changes associated with learning sought.

At the time of writing, only a few specific learning paradigms have been studied with all these techniques. However, in each of these cases, it appears that learning may involve altered synaptic transmission between sensory neurons known to code the CS and first-order interneurons whose altered activity promotes the particular kind of response that was learned. In some cases, it seems most likely that the alterations are postsynaptic, in others presynaptic. For example, in worms that have learned to avoid a temperature at which they were previously starved, the calcium response of the first-order interneuron AIZ to temperature change, which in untrained worms is substantial, is greatly reduced, suggesting reduced transmission from X to AIZ (see **Figure 6(b)**). The proposal that this response reduction is due to factors operating in AIZ comes from the fact that the learning requires intact signaling by several neuromodulators (e.g., insulin) whose receptors and downstream signaling molecules are expressed in AIZ and that this learning requires normal calcineurin function in AIZ. It should be noted that, in the hippocampus, calcineurin signaling plays a central role in establishing LTD and that insulin modulates LTP and LTD formation. Several possible examples of altered presynaptic release of transmitter are offered by experiments in which worms learned to avoid naturally attractive chemical agents. Avoidance of NaCl appears to be due to altered transmission from ASER to AIA. The learning depends critically on release of an insulin homolog from AIA and on the presence of normal insulin receptors on, as well as insulin signaling pathway molecules in, ASER. Since the calcium responses of ASER to NaCl are not altered by training, the proposed theory is that activity of ASER in the presence of neuromodulatory signals produced by starvation cause release of insulin from AIA, which feeds back and contributes to reducing transmitter release from ASER. Fairly comparable results also suggest that learning to avoid attractive chemicals can involve reduced transmission from AWC to AIB. Although *C. elegans* appears to have homologs of NMDA receptors, these have so far been seen to play only minor roles in associative conditioning. Thus, whereas the mechanisms seen so far in *C. elegans* do not mirror the working hypotheses that are currently based on studying mammals, neither are they without mammalian precedents.

That these changes seen during associative learning appear to be synaptic should be contrasted with findings that sensory adaptation to chemicals and at least short-term habituation to mechanical stimuli are due to changes within receptor cells themselves. These later findings show that sensory responses are potentially plastic, and

one might imagine that this plasticity would also be used during associative learning. However, what worms seem to typically learn is which stimuli to approach and which to avoid, and this can be learned only by altering the effective connectivity between neurons that represent the stimulus and others that represent the desirable response.

However, a kind of exception to this rule occurs when worms learn to avoid or approach a temperature at which they have been starved or fed. There is evidence that synaptic transmission alters between neurons that sense temperature change and neurons that mediate approach or avoidance. However, there are not different sensory neurons to represent different temperatures. Therefore, an animal cannot learn to approach one temperature coded by one set of neurons and avoid another temperature coded by a different sensory neurons. Rather, temperature-sensitive neurons, of which there are only a few, become sensitive to any temperature at which a worm spends several hours, independent of any US that may be present. Approach or avoidance then becomes associated with temperature as a modality but not with a specific temperature. Evidence that this is the case can be seen if a worm is starved at a given temperature long enough for it to accommodate to that temperature and also long enough for it to go into a starved state. The worm will now tend to avoid the temperature at which it was starved if tested in the absence of food. However, if the worm is fed at any temperature before testing, it will rapidly switch into a fed state and when tested will approach the temperature at which it was protractedly starved. Feeding has put it into a state where it will approach whatever temperature is familiar. Thus, there seems to be no association between a specific temperature and food availability.

The neurotransmitters/neuromodulators serotonin, octopamine, and dopamine appear to play essential roles in establishment of associations. For example, serotonin seems to be able to act as a proxy for food availability and octopamine for starvation in food-conditioning experiments. However, these modulators are also involved in other kinds of signaling by other neurons, where their meaning may be quite different. Thus, serotonin, which signals food availability under some circumstances, is released by neurons that detect aversive stimuli and that drive aversive learning in other kinds of learning situations.

Comparisons and Commentary

Are Changes Extrinsic or Intrinsic to Conditioned Response-Mediating Circuitry?

Prior to the period of rapid progress that began with analyses of nonassociative learning in *Aplysia* and crayfish, a crucial unanswered question was whether learning was

due to intrinsic changes within the neural circuitry that mediates learned responses or whether, after learning, these circuits were biased to operate differently by input from parts of the nervous system extrinsic to the mediational circuitry itself. The early work on *Aplysia* and crayfish provided evidence for intrinsic change, and subsequent analyses of simple forms of associative learning in a variety of invertebrate systems, as well as in mammals, has supported the importance of intrinsic change. However, it is widely believed that in mammalian declarative learning, change is initially confined to the hippocampus, which then controls behavioral choices by reconstructing patterns of cortical activity that were operative at the time of learning. Only later do changes, intrinsic to cortical circuits, themselves develop and come to mediate performance. It has also been suggested that new learning may sometimes involve prefrontal cortex modulation of other parts of the brain, and that the latter change only slowly with the aid of the input from prefrontal cortex. These are, of course, examples of advanced learning done by the highest parts of the mammalian brain.

However, many older studies of nonassociative learning even in insects and annelid worms suggested that habituation might be attributable to extrinsic inhibitory control of mediational circuitry. Furthermore, whereas habituation of crayfish escape provided some of the first direct evidence of intrinsic synaptic modification in (non-associative) learning, recent work has found that extrinsic inhibitory input is crucial to producing habituation, at least initially. Interestingly, such extrinsic inhibition becomes dispensable once habituation is well established.

It has also been found that about 20 h after establishment of a classically conditioned feeding response in *Lymnaea*, transmitter output from a tonically active serotonergic cell that modulates the feeding circuitry increases due to development of a persistent depolarization. Although soon after conditioning, conditioned responses occur in response to CS in the absence of the depolarization of the serotonergic neuron, at later time points, and well after protein-synthesis-dependent consolidation has occurred, conditioned responding is caused or at least supported by the persistently enhanced transmitter output of the serotonin cell. In this case, then, a learned extrinsic influence is involved in the performance of established learning.

Varieties of Intrinsic Change

The kind of learning that most interests us, at least in part because it is so central of our own way of life, is associative learning in which animals learn to associate many particular stimuli, each with a particular action or perceptual response (the sets of possible stimuli and responses being very large). Intuitively, the most

straightforward way to achieve such association is to alter transmitter release or postsynaptic receptor responses, specifically at synapses between stimulus-representing and response-producing neurons. There is now a great deal of evidence that this happens. Alterations of synaptic efficacy were first found for nonassociative learning in crayfish and *Aplysia*. Soon afterwards, alterations of transmission were found to mediate learned associations in several mollusks, and at about the same time synapses with Hebbian properties were discovered in mammals. There is now evidence of altered synaptic transmission due to associative training in all of the kinds of animals we have discussed, from nematode worms, to gastropod mollusks, to insects, to mammals.

Although perhaps crucial for establishing stimulus and response-specific associations, alterations of synaptic transmission are not the only kinds of alterations that have been found to be produced by training and to contribute to adaptive learned responses. One of the earliest analyses of classical conditioning, the clamping-down of the gastropod mollusk *Hermissenda* to a light when the light reliably warned of oncoming turbulence, found that training altered the membrane conductance of a cell whose activity promoted the conditioned response. In addition, sensitization of defensive withdrawal in *Aplysia*, which provided the first evidence for enhanced synaptic transmission as the basis of a learned response to a stimulus, is accompanied by altered electrical excitability of the sensory neurons. Changes in excitability, membrane conductance, or resting membrane potential have now also been found after induction of LTP in CA3 of the hippocampus, after induction of conditioned eye-blink responses in the deep nuclei of the cerebellum, and after various kinds of classical and operant conditioning of feeding responses in the gastropods *Aplysia* and *Lymnaea*.

Changes of this kind were unexpected, because they seem to work against stimulus or response specificity. Generalized changes in the electrical excitability of a CS-representing neuron would be expected to enhance any kind of response to the activity of that neuron, not just the desired conditioned response. Cell-wide changes in the excitability or membrane potential or conductance of a response-producing cell would be expected to alter the likelihood of the response to any stimulus, not just the CS. Thus, it may be seen as something of a paradox that such changes often develop. Moreover, they often do so only in response to paired presentation of CS and US, not to unpaired presentation. To some extent, there may be no paradox, because it now appears that excitability changes can develop locally within dendritic trees and need not be cell wide. However, even in the case of cell-wide changes, lack of associative specificity becomes a problem only if an animal has to learn and concurrently hold multiple associations. Thus, an animal in which repeated pairings of CS A and US B lead independently to increased

sensory responsiveness to A and increased tendency to make B will tend to make response B when presented with stimulus A just as it would if there were a true association. However, if C–D training were added, both A and C would each evoke both B and D. This sort of situation may never arise for uses to which learning is put in many invertebrates. However, it commonly arises in mammals. Nevertheless, it does appear to be the case in mammals that associative training causes increased responses to the CS in strictly sensory parts of the brain and comparable alterations in CR-producing motor regions. Presumably, these changes support associative responding that is selective because of strictly associative changes elsewhere in the circuit, and they do not necessarily cause undesirable stimulus or response generalization. Nonassociative changes in invertebrates may be comparable.

Reinforcement Mechanisms

It is commonly the case that USs, or CSs that have come to evoke learned responses, cause the release of chemical transmitters/neuromodulators that start cascades of intracellular signaling.

The early experiments on sensitization and classical conditioning of defensive withdrawal in *Aplysia* implicated US-driven release of serotonin to sensory neuron terminals as a reinforcing signal needed for establishing both simple facilitation and the activity-dependent facilitation of transmission that was thought to be responsible for associative change. Dopamine, serotonin, or octopamine (octopamine is commonly viewed as an invertebrate analog of epinephrine) appear to play reinforcement-like roles in every case of invertebrate learning that has been suitably studied. Dopamine is also widely thought to play an essential reinforcing role in mammalian learning tasks mediated by the striatum, and LTP and LTD in the striatum are profoundly affected by dopamine. The working hypothesis that emerges from such findings is that, at a cellular level, changes responsible for learning of stimulus-driven responses generally require three ingredients: presynaptic activity, postsynaptic activity, and some form of neuromodulatory input that is a response to the occurrence of biologically important stimuli. For operant conditioning, activity of a response-promoting cell and neuromodulatory input seem to be crucial. Perhaps the only clear exception to a role for neuromodulators in learning comes from the study of cerebellar eye-blink conditioning and Purkinje cell LTD, where reinforcement, instead of being mediated by a neuromodulator, is mediated by the overwhelmingly powerful synaptic input provided by the climbing fibers. Thus, in most cases where evidence of neuromodulatory involvement is lacking, one might guess that it is waiting to be found.

Despite the above considerations and a wealth of evidence that acetylcholine, dopamine, serotonin, and

norepinephrine significantly modulate LTP and LTD at hippocampal synapses, at the time of this writing, findings from hippocampal slices are commonly discussed without much acknowledgment that, in the intact brain, neuromodulators probably greatly alter the properties of these synapses.

In mammals, dopamine is widely accepted as crucial for appetitive reinforcement, but its role in aversive reinforcement is less resolved. Whether a separate modulator exists for aversive reactions has not yet been established. However, a precedent is provided by the use in insects of dopamine for reinforcement of aversive reactions and octopamine for appetitive ones. In mammals, opiates and serotonin have been suggested as possible mediators of reinforcement in aversive learning, but to date this is largely speculation. Use of different modulators for reinforcement in different kinds of learning is also suggested by work in both nematodes and mollusks. In worms, there is some evidence that octopamine might signal conditions of starvation while serotonin might signal the availability of food. However, there is also evidence that, in some kinds of worm learning, serotonin may reinforce avoidance rather than approach. In *Aplysia* and *Lymnaea*, both dopamine and serotonin seem to play roles in reinforcement, but what the differences are in the roles of these two agents is not yet clear. Nitric oxide is another extracellular signal that has been found to be crucial for inducing or stabilizing synaptic or other cellular changes in insects, mollusks, and mammals, though its exact role is yet to be understood.

Pavlovian conditioning requires that change occur specifically at synapses that connect CS-representing neurons and US-representing or response-producing neurons that the CS must come to activate. This requires that coincident occurrence of pre- and postsynaptic activity and, in most cases, also of neuromodulatory input, which is itself usually highly distributed, be detected. Such coincidence must then set in motion intracellular signaling events that will lead to altered synaptic function.

The mechanistic scenarios used to make change contingent on coincidence seem to be numerous. Most familiar is that of hippocampal-type Hebbian LTP where coincident pre- and postsynaptic activity causes local postsynaptic Ca^{2+} elevation because glutamate supplied by active presynaptic neurons primes NMDA receptors so that postsynaptic depolarization will cause them to open to calcium ions. In striatal Hebbian LTD, metabotropic glutamate receptor-mediated release of Ca^{2+} from internal stores acts synergistically with dopamine-dependent entry of Ca^{2+} through voltage-dependent calcium channels to cause changes of synaptic efficacy. Both at other mammalian synapses and in invertebrates, there are further variations on these themes. For example, in sensory neurons of *Aplysia*'s gill/siphon withdrawal reflex, serotonin and calcium that enters active

terminals act synergistically to activate calcium-dependent isoforms of adenylate cyclase so that when serotonergic reinforcement occurs, PKA becomes activated to an exaggerated extent in presynaptic terminals that have been active. Something similar is believed to occur in insects. In *Aplysia*, postsynaptic NMDA receptor-dependent changes that depend on the US also occur and, through retrograde signaling, contribute to further presynaptic events that help stabilize both pre- and postsynaptic changes. Both mammalian and invertebrate works are uncovering complex interactions between pre- and postsynaptic cells that act in the initiation and stabilization of change.

Intracellular Signaling Molecules

As described in part above, PKA, PKC, CaMKs, MAPKs, PI3K, and various isoforms of CREB, and their associated signaling pathways consistently play roles in initial learning or the production of persist cellular change. It is of considerable interest that these same molecules should play similar roles in learning in mollusks, arthropods, and mammals, given the diverse evolutionary origins of these animals.

Blocking and Prediction Errors

Modern work on classical conditioning in mammals has led to the conclusion that new learning occurs when and only when an animal receives new information about rewards or punishments. Thus, unexpected, but not expected, USs act as reinforcers (the ineffectiveness of expected reward is called blocking). Unexpected occurrences of previously conditioned CSs also produce new learning (higher-order conditioning; secondary reinforcement). These facts are often summarized by saying that prediction errors are what drive new learning. Because blocking and higher-order conditioning are so prevalent in mammals, either or both might be expected to be a product of the basic cellular mechanisms responsible for conditioning. Alternatively, these phenomena might be consequences of the way that the circuitry that mediates reinforcement is organized.

In bees and *Drosophila*, neurons that release neuromodulators responsible for reinforcement are activated by already trained CSs. This is thought to be the basis for higher-order conditioning in insects and is consistent with findings on midbrain dopamine neurons in mammals, which are also driven by established CSs. Thus, available evidence from both mammals and insects suggests that higher-order conditioning derives from the organization of circuitry rather than being an inherent consequence of cellular plasticity mechanisms.

The same appears to be true of blocking. In mammals, analyses of blocking mechanisms in both amygdala-mediated fear conditioning and cerebellum-mediated

eye-blink conditioning seem to show that blocking occurs because CR-producing neuron activity inhibits neurons that mediate reinforcement. There have been a few reports suggesting that blocking may occur in the slug, *Limax*, and in *Hermisenda*. However, more extensive investigation in bees has yielded results that are either negative or equivocal. Consonant with this, it has been reported that *Drosophila*'s dopaminergic reinforcing neurons continue to be activated by shock even when a well-established CS warns that the shock is about to occur; this is very much in contrast to what is seen in cerebellar climbing fibers or the midbrain dopamine neurons of mammals when expected primary reinforcing events occur. These observations suggest that blocking is a circuit-based phenomenon, is not universal, and is not a product of the way that basic cellular-level associative learning machinery works.

Stages of Memory

In invertebrates that have been studied, as in mammals, learning has been found to be coded initially in one or more unstable forms that later become stabilized by events which depend on new protein synthesis (and often new genetic transcription) occurring during or within a short time after initial learning events. In addition, common to mammals and invertebrates is the use of the CREB protein to initiate transcription. As in mammals, the formation of protein-synthesis-dependent long-term memory in invertebrates is favored by spaced as opposed to massed training; the need for spaced practice for generating persisting memory may be an adaptation ensuring that only those environmental contingencies that are stable over time will be encoded into long-term memory.

It seems to be generally the case that memory on the order of a day or more requires protein synthesis. In a few cases, memory somewhat longer than a day can develop independent of protein synthesis, but even in these exceptional cases, slightly longer-term persistence does require new macromolecular synthesis. In mammals, learning is commonly thought of as passing through two stages of memory, protein synthesis-independent short-term memory and protein synthesis-dependent long-term memory. However, in insects and mollusks, one commonly finds evidence for at least three separate stages, with the second, intermediate-, or middle-term stage being of variable mechanism and in some cases itself requiring *de novo* protein and/or messenger RNA synthesis. The discovery of late LTP in mammals suggests that mammals may in fact conform to the three-stage model. However, as discussed below, it seems likely that the third, so-called long-term, stage will soon be further divided.

Intermediate/middle-term storage (on the order of hours) in invertebrates has been seen to utilize a variety of mechanisms. These include calpain-mediated cleavage of regulatory subunits of PKC to form constitutively active kinase (seen in both honeybees and *Aplysia*, and possibly also in some hippocampal LTP), protein synthesis (but not mRNA synthesis)-dependent persisting activation of PKA, and transcription-dependent hydrolysis of PKA (*Aplysia*) as well as undefined translation-dependent actions in a number of gastropod mollusks. There is also evidence that autophosphorylation of CaMKII, implicated in intermediate-stage LTP in mammals, is implicated in *Drosophila* learning and perhaps also in some forms of synaptic plasticity in *Aplysia*.

The involvement of genetic transcription in production of long-term memory has raised the question of how the proteins then synthesized come to affect only particular synapses of a given cell. An apparent answer was provided for LTP when it was discovered that the stabilization of LTP at specific hippocampal synapses is due to a selective effect of the relevant proteins at synapses at which shorter-term LTP had already been established (synaptic tagging). The same discovery was made at about the same time for long-term facilitation in *Aplysia* using cultured sensory neurons with two branches, each innervating a different motor neuron. One pulse of 5-HT to a synaptic region caused only transient facilitation of that synapse, but following multiple spaced pulses long-term, protein-synthesis-dependent facilitation developed at the stimulated synapse but not the other. However, if a single 5-HT pulse was applied at the synapse onto one of the motor neurons soon enough before or after multiple pulses were applied at the other, both synapses underwent long-term facilitation. As in the hippocampus, establishment of short-term facilitation apparently causes production of a tag that allows stabilizing protein that is distributed cell-wide to have a local effect.

The *Aplysia* bifurcated axon preparation, which allows longer periods of study than is usual in the hippocampal slices used to study mammalian LTP, shows that major changes continue well beyond 24 h. Although facilitation and associated morphological changes are present at 24 h, local protein synthesis, dependent on certain local intracellular signaling events, must operate over several more days if these changes are not to later regress. Of course, still later events may turn out to be required to achieve ultimate stability. Although the evidence is, so far, less complete, somewhat comparable events probably operate during establishment of stable mammalian engrams.

A problem for the establishment of very long-lasting memory is that most cellular constituents turn over continually. Therefore, whatever synaptic changes underlie long-term memory must somehow regenerate themselves if memory is to persist. In the 1980s, Francis Crick and

John Lisman independently proposed that self-regenerating changes at individual synapses could be produced if change were mediated by some species of protein kinase that could be activated locally, not only by its normal signaling molecules, but also by phosphorylation of the kinase itself catalyzed by already active kinase molecules of its own kind. Soon, it was discovered that CaMKII has just this property and plays a crucial role in establishment of LTP. However, protein synthesis plays no role in the autophosphorylation of CaMKII, so this specific mechanism could not be the basis for protein-synthesis-dependent long-term change. Currently, two kinds of potentially self-regenerating synapse-specific changes are under active investigation.

One of these is the translation of preexisting mRNAs local to individual synaptic regions of an atypical, auto-active form of PKC, PKM ζ , which lacks the repressive, signal molecule-controlled regulatory domain of the more common isoforms of PKC. PKM ζ causes potentiation of the synapses where it is present, and there is increasing evidence that it plays an important role in several kinds of learning and/or in establishment of late LTP in both mammals and *Drosophila*. Existing PKM ζ appears to promote its own further synthesis, potentially creating a positive feedback loop that could maintain elevated levels locally once these were established by learning.

The other locally synthesized signaling molecule that is currently receiving attention as the possible basis for a positive feedback loop that could maintain synapse-specific change is a molecule, cytoplasmic polyadenylation element binding protein (CPEB), that plays a role in translation of preexisting synaptic mRNAs somewhat analogous to that played by CREB in nuclear transcription. In *Aplysia*, this protein is synthesized locally during development of long-term facilitation and is crucial in the stabilization of recently established functional and morphologic changes that underlie facilitation. The local elevation of this protein's titer due to local synthesis is thought to lead to a conformational change that both increases the protein's effectiveness in promoting translation and promotes a similar conformational change of nearby CPEB molecules, with the result that the altered, more active form of the molecule is maintained locally despite turnover of the population of CPEB molecules over time. The self-regenerating, contagious changes thought to occur in CPEB are analogous to those occurring in the prions responsible for transmissible spongiform encephalopathies. Evidence is emerging for a possible role of these prion-like mechanisms during learning and synaptic change in mammals and *Drosophila* as well as *Aplysia*.

The evidence for the roles of both of these kinds of locally contagious molecular events in learning is intriguing. It remains to be seen whether either or perhaps both of these mechanisms working together really are part

of the sequence of events leading to stabilization of engrams. Whether they are or not, it seems virtually certain that the simple division of memory into two homogeneous stages, short-term and long-term storage is a thing of the past.

Perhaps also indicative of the continued transformation of memory after protein-synthesis-dependent consolidation has already occurred (or begun), is the observation, both in mammals and invertebrates, that if a memory is recalled, a period of protein synthesis is again necessary if the memory is not to be lost, that is, the memory must be reconsolidated. This reconsolidation process is still poorly understood, but there is some evidence in mammals that sufficiently well consolidated memories might not in fact be degraded upon recall. It has also been found in the pond snail *Lymnaea* that reconsolidation of more freshly consolidated memories requires PKA activity as well as protein synthesis, whereas reconsolidation of longer-established memories requires only protein synthesis, again suggesting that consolidated memories continue to change their properties. In mammals, it is often proposed that reconsolidation is involved in integrating new memories into a complex network of previously existing associations. However, such conjectures may seem less appealing when it is appreciated that reconsolidation also seems to occur in lower animals where the amount of stored information may be limited and postlearning reorganization of memory not required.

Discovery of the role of CREB in establishing long-term memory first arose from experiments on long-term facilitation in *Aplysia* and then rapidly extended to *Drosophila* and mammals. When it was discovered that there are isoforms of CREB that suppress CREB-stimulated transcription, the possibility was recognized that the long-established and phylum-general failure of massed training to cause consolidation might be explained if training trials caused not only an activation of transcription-promoting CREB but also a shorter lasting activation of transcription-suppressing CREB that had to abate before transcription could occur. *Drosophila* mutants, in which exaggerated amounts of either isoform could be induced at will, provided compelling evidence supporting this hypothesis, which was also supported in *Aplysia*. A few years later, evidence for similar mechanisms was obtained for fear learning in mammals. This is yet another instance of the remarkable generality across phyla of the cellular mechanisms of memory and of progress in our understanding being facilitated by taking advantages of the experimental opportunities presented by characteristics of animals in different phyla.

In mammals, a (systems) consolidation process, which is much slower than cellular-level protein-synthesis-dependent consolidation processes, is involved in transforming learning that initially depends on the hippocampus for retention to a form where it is

independent of the hippocampus. Systems type consolidation is thought to involve endogenous neural activity that causes transfer of the hippocampal memory to extra-hippocampal (presumably cortical) circuitry. Reminiscent of this, recent work seems to indicate that the transfer from short-term to middle-term memory in *Drosophila* may depend on mushroom-body-driven activity of dorsal-paired midline (DPM) cells feeding back onto (perhaps other parts of) the mushroom bodies (see **Figure 4**). In mammals, systems type consolidation is thought to be involved in recoding engrams so that new and old information become integrated. The significance of the insect findings has yet to be addressed theoretically.

Concluding Remarks

The facts described above seem to show that learning mechanisms in mammals, mollusks, arthropods, and round worms, animals that segregated from each other a very long time ago in evolution have marked similarities. There is considerable diversity, but it is all diversity of detail in how a seemingly common set of possible mechanisms is utilized. As has been repeatedly suggested by investigators of *Aplysia*, it is as though there were a common alphabet of mechanisms that get combined in different ways in different animals (or different parts of the same one). All of the animals studied have as common distant ancestors the first multicellular animals with nervous systems, the coelenterates. Neural plasticity, particularly interneuronal (i.e., synaptic) facilitation that can last for many hours or possibly even days plays roles in the behavior of these ancient animals. Coelenterates even appear to have NMDA receptors, though there as yet is no evidence that these play any role in their synaptic plasticity. Perhaps the commonalities we have seen are a consequence of evolution of learning from the ancient forms of neural plasticity of our very early progenitors.

See also: Active Avoidance and Escape Learning; Acute Dependence; Animal Models of Behavior: Alcohol Addiction; Amnesia; Animal Models of Learning and Memory; Brain Imaging and Addiction; Cardiovascular Conditioning: Neural Substrates; Cellular Plasticity in Cocaine and Alcohol Addiction; Cerebellum: Associative Learning; Declarative Memory; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Drug Sensitization and Drug Abuse; Episodic and Autobiographical Memory: Psychological and Neural Aspects; Ethanol and Nicotine Interactions; Evolutionary and Developmental Issues in Cognitive Neuroscience; Eyelid Classical Conditioning; Fear Conditioning; Fear: Potentiation and Startle; Genetics of Memory in *Drosophila*; Habituation; Hormones and Memory; Implicit Learning and Memory: Psychological and Neural

Aspects; Learning and Memory: Computational Models; Mechanisms of Memory Formation and Storage in *Hermissenda*; Memory Consolidation; Memory in *Caenorhabditis elegans*; Memory in the Honeybee; Molecular Neurobiology of Addiction; Neural Basis of Classical Conditioning; Neural Basis of Recognition Memory in Nonhuman Primates; Neural Basis of Working Memory; Neuron Excitability and Learning; Neurophysiology of Drug Reward; Neurotransmitters and Neuromodulators Regulating Sleep and Wakefulness; Pain and Addiction; Protein Synthesis and Memory; Rewarding Brain Stimulation; Short-Term Memory; Psychological and Neural Aspects; Sleep: Learning and Memory; Synapse Formation and Memory; Synaptic Mechanisms for Encoding Memory; Transgenic Technologies and Their Application to the Study of Senile Dementia.

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Animal Models of Bipolar Disorder

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Glossary

Construct validity – It refers to the degree to which a model accurately reflects the processes or disorder for which it is intended.

Endophenotypes – They represent the internal characteristics discoverable by biochemical test or microscopic examination.

Etiological validity – It occurs when the biological basis of a disease is recreated in the model.

Face validity – It assumes the usefulness of an animal model or a preclinical test solely on the basis of phenomenological similarities between the model/test and the disorder/domain.

Predictive validity – It constitutes the manipulation of a model whereby the results mirror those observed in the human from the same manipulation.

Receptor tautology – Occurs when using an assay, either *in vivo* or *in vitro*, whereby a ligand challenge to a specific neurotransmitter target is reversed by an oppositely acting ligand to the same neurotransmitter target.

depression, cognitive deficits are apparent in nearly every cognitive domain. While this cognitive disruption has been largely overlooked in the past, recent evidence indicates that cognitive deficits are also apparent during the euthymic phase and that these deficits correlate with functional outcome (reintegration to their everyday lives). To date, the underlying causes of these cognitive deficits as well as the episodes of mania and depression have yet to be elucidated.

Difficulties in Generating Animal Models of Bipolar Disorder

One rate-limiting step in bipolar disorder research has been the paucity of suitable animal models. Attempts have been made to model this disorder in animals, including specific modeling of the manic, depressive, and cyclic aspects of the disorder. Numerous hurdles exist that have increased the difficulty in developing suitable models. These hurdles include the fact that there are limited data with regard to pathophysiological mechanism(s) contributing to the disorder. In addition, despite the 50–80% heritability of the disorder, specific genetic contribution(s) have yet to be identified. Another difficulty arises from the lack of knowledge on the mechanism by which existing treatments exert their effects. Moreover, bipolar disorder does not have clearly defined endophenotypes that would provide the empirical basis for cross-species models. The limited amount of available information with regard to the cause of cycling in the disorder has also proven to be a considerable obstacle to producing suitable animal models of bipolar disorder. In fact, given the heterogeneity of psychiatric symptoms among bipolar disorder patients, identifying an all-encompassing animal model may be impossible. The overlap of cognitive deficits to those of schizophrenia – another major psychiatric illness – is another source of difficulty when attempting to develop animal models of bipolar disorder that are distinct from animal models of other disorders such as schizophrenia. Finally, consistent with schizophrenia, the lack of effective procognitive treatments for the deficits in bipolar disorder means that there are no positive controls with which to validate putative animal models of cognitive deficits in bipolar disorder. Unfortunately, the lack of universally accepted animal models of bipolar disorder has limited the development of specific treatments. Current treatments have been discovered via serendipity or by testing the efficacy of treatments that

Introduction to Bipolar Disorder

Bipolar disorder is a serious and chronic psychiatric illness that is classically associated with alternating episodes of either elevated or depressed mood states. The lifetime prevalence of bipolar disorder is approximately 1%. Heritability estimates between 50% and 80% indicate that there is a strong multigenetic component to bipolar disorder. The *Diagnostic and Statistical Manual*, Version IV (DSM IV) subdivides bipolar disorder into type I and type II disorders. Type I bipolar disorder is characterized by manic behavior of at least 7 days, with or without the occurrence of depression. Type II bipolar disorder is characterized by hypomanic behavior (manic-like behavior occurring for 4 days) with major depression. The time between episodes of mania is referred to as the euthymic phase, where the patient attempts to recover from an episode as well as reintegrate into their everyday life. Symptoms and deficits are still apparent in the euthymic period, however. These symptoms, coupled with increasing episode occurrence, lead to substantial social disruption contributing to a near 15% suicide rate. During episodes of mania and

were originally approved for other disorders, such as antipsychotics. Taken together, there is very limited information available to guide the generation of viable animal models of bipolar disorder having face, predictive, and construct validity. Nevertheless, the attempts undertaken to create animal models have provided some insights into possible neurobiological underpinnings of the disorder, as is discussed below.

Models of Mania

Pharmacological

Psychostimulants are known to precipitate a state of mania or hypomania in the healthy human. Hence, there has been great interest in the psychostimulant effects on behavior in animals. Amphetamine is the psychostimulant used most commonly in animal models of bipolar disorder. Amphetamine acts by blocking the reuptake of norepinephrine and dopamine, although it is sixfold more potent at the norepinephrine transporter than at the dopamine transporter (DAT). The most common behavioral measure of mania in animals is that of locomotor hyperactivity, commonly assessed in an open field test or photocell chamber. Low doses of amphetamine induce hyperactivity in open field tests, putative evidence of face validity for increased activity of patients during manic phases. Amphetamine-induced hyperactivity can be susceptible to reversal by bipolar disorder treatments, exhibiting some predictive validity in terms of known treatment effects. Not all studies demonstrate a reversal of amphetamine-induced hyperactivity, however. In fact, the most consistent reversal effects are observed following antipsychotic treatments that were developed for treatment of schizophrenia, but are now also used to treat mania. Given that antipsychotic-induced reversal of amphetamine-induced hyperactivity is also used as a model for schizophrenia, the specificity of this model for mania remains questionable. In addition, since antipsychotics primarily act as antagonists to the dopamine system while amphetamine acts as an indirect agonist, there exists the limitation of receptor tautology. The lack of specificity of amphetamine-induced hyperactivity as an animal model of mania introduces another confound, because it is also used as a model of drug abuse and tardive dyskinesia, as well as for antipsychotic efficacy. Finally, numerous reports have evaluated the use of amphetamine challenge in the human as a model of bipolar disorder. To date, such clinical amphetamine studies have not supported its use as an appropriate model. One attempt to avoid such receptor tautological/antipsychotic efficacy confounds has been to combine amphetamine and an anxiolytic administration, such as chlordiazepoxide. It has been hypothesized that both mood stabilizer and antipsychotic treatments can reverse

this combined-model-induced hyperactivity, while mood stabilizers alone cannot reverse stimulant-alone-induced hyperactivity. While studies have been limited to date, this combined model may offer another avenue to develop novel therapeutics. Other pharmacological models of bipolar disorder have used other psychostimulants such as methamphetamine and cocaine. As with amphetamine and the combined model described above, most models report only hyperactivity as a measure of mania. Consistency among these studies is also limited since induced hyperactivity has been reversed by acute antimanic treatments, while other studies have reported no effect. In the human, acute administrations of antimanic drugs do not induce a full reversal of manic symptomatology. Thus, the interpretation of these findings in terms of translational validity has proven difficult. Studies on acute-stimulant mania models with chronic antimanic agent administration via repeated injections, osmotic minipumps, food chow, or drinking water have also been conducted. To date, these chronic studies have not provided conclusive evidence for the suitability of these acute-stimulant models.

Genetic

Developing genetic models of bipolar disorder can be accomplished in different ways. Strain comparisons can be made across a range of behaviors such as activity levels. The effects of antimanic agents on these behaviors can then be assessed to identify the strain exhibiting the largest antimanic effects for putative evidence of predictive validity. Retrospective gene analysis is then performed to identify differences that may contribute to that strain being more receptive to the effects of antimanic agents, and thus possibly reflecting a genetic contribution to bipolar disorder. A major benefit to this type of model is that the investigation into putative underlying neurobiological differences is not limited by experimental design and *a priori* hypotheses. The suitability of the strain differences approach can be readily investigated. In fact, numerous mouse strains have been assessed and compared in multiple behavioral assays not necessarily specifically addressing their suitability to psychiatric conditions. In terms of locomotor activity alone, Black Swiss, Friend leukemia Virus B strain (FVB/NJ, Jackson Labs, Bar Harbor, ME), and NOD/LTJ mice may be among the most active strains. Thus, future studies could assess the effects of antimanic agents on the high activity levels of these strains in order to determine their suitability as models for bipolar disorder. Subsequently, the specific genetic contributions to these effects could be investigated.

Prospective genetic models of bipolar disorder can also be generated by utilizing our limited understanding of the neurobiological underpinnings of the disorder. Suspected genetic abnormalities contributing to bipolar disorder can be recreated in mice to investigate the behavioral effects of such genetic modification. These models confer a degree of etiological validity and benefit from testing a specific hypothesis. Moreover, the effects of genetic mutations are theoretically more selective than administering a drug that may act on multiple neurotransmitter systems. To date, genetic mutant models of bipolar disorder have been developed both in response to genetic susceptibility studies in bipolar disorder, as well as to studies on the putative mechanism of action of antimanic treatments. Numerous genetic linkage studies have implicated the DAT in the development of bipolar disorder. Mice with no (knockout mice) or reduced (knockdown mice) DAT function have been created. These DAT-mutant mice exhibit a hyperactivity profile as accompanied by other behavioral abnormalities that are consistent with bipolar disorder. One confound inherent in using such genetic models, however, is encapsulated in the DAT-knockout mice. DAT-knockout mice exhibit a variety of behavioral abnormalities that are linked to their lack of DAT expression from birth. Such abnormalities, combined with the development of remarkable compensatory changes to counter the loss of this major monoamine transporter, may reduce the utility of these mice when assessing putative therapeutics. DAT-knockdown mice exhibit only a reduced expression of the DAT and appear healthy and viable, despite their hyperactivity profile. Thus, DAT-knockdown mice may prove to be a more suitable animal model of bipolar disorder than the full DAT knockout when assessing putative treatments.

Another gene putatively, although not consistently, linked to bipolar disorder is the glycogen synthase kinase-3 (GSK-3). Although GSK-3 acts at multiple levels to regulate over 40 proteins, it is generally accepted that it acts as a proapoptotic agent. While increased apoptosis is consistent with the cellular loss that has been reported in bipolar disorder patients, the main interest in this target derives from the reports suggesting that antimanic treatment effects are mediated by GSK-3. Heterozygous GSK-3-mutant mice have been created and display increased activity levels and other behavioral abnormalities. The behavior of GSK-3 heterozygous mice resemble that of lithium-treated mice, however; thus, this model may be more applicable as a drug screen for lithium-like compounds. Moreover, there appears to be greater evidence linking GSK-3 inhibition with antidepressant-like activity than with mania-reducing effects. Hence, the suitability of the GSK-3 mutant mice as a model of bipolar disorder mania has been questioned.

Models of Depression

Numerous animal models of depression exist, although none have been developed with specificity to modeling the depression that occurs in bipolar disorder.

Cycling

The cycling nature of bipolar disorder represents possibly one of the most difficult aspects of the disorder to model in animals. Only a few groups have attempted to develop animal models of such cycling behavior. Each group acknowledges that their models do not necessarily exhibit homology to the disease states of bipolar disorder. In fact, modeling behavioral symptoms is of secondary consideration as the groups are simply developing potential models of cyclic behavior in order to investigate drug effects on the observed cycling. These models include intermittent cocaine injections and the 'kindling model.'

In the intermittent cocaine model of cycling in bipolar disorder, cocaine is injected in rats intermittently once per week. Fluctuations in cocaine-induced dopamine efflux and freezing behavior are observed, where injections in a week increased such behavior, but the following week the same injection reduced such behavior. While chronic lithium attenuated some of these effects, it has yet to be determined whether the effects observed have relevance to cycling in bipolar disorder.

The kindling-behavioral sensitization model of recurrent affective disorders is based on the hypothesis that initial episodes occur following major psychological stressors, while future episodes can occur following only minor stressors. The model encompasses two neurophysiological phenomena: electrophysiological kindling (progressive vulnerability to seizures) and behavioral sensitization (progressive sensitivity to stimulants). However, these two phenomena make it implicitly difficult to test this model, as experimental results can be interpreted to lend support to the model or argue against it. The kindling phenomenon is generated by repeated electrical stimulation-induced seizures evoking after-discharges from the amygdala which eventually increase in complexity to affect other areas of the brain. These after-discharges eventually result in spontaneous seizures. Thus, spontaneous cycling is observed and attempts can be made to delay or reduce such spontaneous seizure events. Of course, the cycling behavior observed is measured in terms of seizures and not behavior related to bipolar disorder. Hence, its suitability as an animal model of bipolar disorder has yet to be determined.

Mania beyond Hyperactivity

Other Symptoms of Mania

Numerous attempts have, therefore, been made to generate animal models of bipolar disorder. Because mania is the cardinal feature of bipolar disorder (exhibited in type I and type II disorders), most attempts have focused on modeling mania in animals and have focused on observations of hyperactivity. Mania is not limited to hyperactivity, however, with other facets of mania that can also be viewed as endophenotypes. Other endophenotypes of mania include irritability, aggression, reduced sleep, poor judgment, increased sexual arousal, and drug abuse or hedonistic behavior. Each of these endophenotypes can putatively be measured in animal models of mania. For example, irritability defined as hyperreactivity to innocuous stimuli could be measured by increased startle reactivity. Aggressive behavior could be quantified using a resident–intruder paradigm. Circadian rhythms to quantify sleep patterns can be generated using simple telemetry devices, while poor judgment could be assessed by assaying levels of risk-taking behavior. Finally, drug abuse and hedonistic behavior can be assessed using intracranial self-stimulation or progressive ratio breakpoint paradigms.

Cognitive Dysfunction

During periods of mania and depression, bipolar disorder patients exhibit deficits across nearly all cognitive domains. Such deficits contribute to the difficulties faced by patients during such episodes and their subsequent rehabilitation. Cognitive dysfunction is not limited to such episodes however. During the euthymic period, bipolar disorder sufferers also experience cognitive deficits, but in more selective cognitive domains. These domains include attention and vigilance as well as executive functioning. Attention and vigilance deficits have been identified using tests that require the subject to attend to relevant, while ignoring irrelevant, stimuli – such as the continuous performance test. Executive functioning deficits have been identified using the Wisconsin Card-Sorting Task, which requires subjects to learn novel rules and shift their focus from one set of rules (attentional set) to another. There is increasing evidence suggesting that these cognitive deficits most closely correlate to functional outcomes such as reintegration to their everyday lives and vocation opportunities. Thus, the need to develop therapeutics for these cognitive deficits is increasingly apparent. Animal analogs of these attention and executive functioning tests are available. Tests of vigilance include the sustained attention task and the 5-choice continuous performance test, for rats and mice respectively. A direct animal analog of the

executive functioning tasks named above exists and is called the attentional set-shifting task. Therefore, future animal models of bipolar disorder could and should also include a cognitive profile that encompasses these deficits.

Conclusions and Future Directions

At present, there are limited viable animal models of bipolar disorder. The limited knowledge of endophenotypes of bipolar disorder that can be assessed in animals further reduces the chances of developing viable animal models. While the behavioral measure in most animal models of bipolar disorder is that of hyperactivity, it is only recently that the activity levels in bipolar disorder patients have been quantified. Specific exploration and locomotor patterns have also been quantified providing a phenotype of bipolar disorder that can be modeled specifically in animals. Attempts are also being made to model other aspects of mania in animals, such as aggression and irritability. Increased knowledge of the cognitive deficits in bipolar disorder also provides further opportunity to provide directly translatable models. Developing an animal model that encompasses each of these quantified domains may lead to more appropriate animal models of bipolar disorder. Finally, increased knowledge of the genetic contributions to bipolar disorder may allow the creation of a combined genetic and pharmacological model, providing greater insight into the neurobiological underpinnings of bipolar disorder, as well as allowing testing of putative therapeutics.

See also: Attention and Speed of Information Processing; Circadian and Ultradian Clocks/Rhythms; Cognition: Attention and Impulsivity; Cognitive Decline in Laboratory Animals: Models, Measures, and Validity; Comorbidity – Depression; Depression; Emotion–Cognition Interactions; Genes and Behavior: Animal Models; Mouse Genetic Approaches to Psychiatric Disorders; Neural and Pharmacological Substrates of Aggression; Neural Basis of Working Memory; Neurobiology of Offensive Aggression; Pathological Gambling; Psychostimulants; Sleep: Medical Disorders; Value of Animal Models for Predicting CNS Therapeutic Action.

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Animal Models of Learning and Memory

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Glossary

A, B, C, X – The specific cues that initially do not control behavior but ordinarily gain behavioral control when they are paired with an unconditioned stimulus (US; i.e., they become conditioned stimuli [CSs]).

A1 – The high activation state of a specific memory representation in the sometimes opponent process (SOP) model.

A2 – The low activation state of a specific memory representation in SOP.

Conditioned stimulus (CS) – A stimulus that, although it initially has no influence on behavior, comes to control behavior as a result of its being paired with a biologically significant stimulus (US).

I – The inactive state of a specific memory representation in SOP.

Rescorla–Wagner (1972) model – This model of learning assumes that learning a CS-US relationship requires the CS be followed by a surprising US. It posits that learning is driven by a total error reduction algorithm.

Rate expectancy theory (RET) – This model, put forward by Gallistel and Gibbon in 2000, assumes that learning consists of encoding temporal relationships between events (i.e., stimuli and responses), and that subjects respond to CSs to the degree that they signal a decrease or increase in the expected time to the next outcome (usually a US).

Sometimes competing retrieval (SOCR) model – This model, proposed by Stout and Miller in 2007, assumes that learning depends on contiguity of a CS and US, but responding to the CS is directly related to the extent that the CS predicts a change in the likelihood of the outcome relative to background cues (including the context).

Sometimes opponent process (SOP) model – This model, put forward by Wagner in 1981, assumes that there are three different states of activation of the representation of an event (high [A1], low [A2], and null [I]). Learning and performance are both functions of the different memory states that the relevant CS and US representations are in.

Unconditioned stimulus (US) – A stimulus that on first encounter elicits responding; such stimuli are said to be biologically significant.

Acquired changes in stimulus control of behavior (i.e., learning, retention in memory, and behavioral expression of knowledge) can be described at many different levels of analysis ranging from neural to associative to higher-order cognitive. The level of analysis here is associative. Associations are often viewed as analogous to the connections between neurons that are presumably responsible for learning and memory. An association is a link between the mental representations of two events (stimuli or responses), such that activation of one representation by the presentation of its stimulus, hereafter called a conditioned stimulus (CS) or cue (e.g., a tone or light), results in the activation of the associated representation, hereafter called an outcome, even if it is physically absent. If the retrieved representation is of a biologically significant event (i.e., an unconditioned stimulus (US), e.g., food or electric shock), a conditioned response appropriate for the outcome is ordinarily emitted. Here, a summary is provided of fundamental phenomena that are reliably observed in simple situations that engage learning and memory by both human and nonhuman subjects. Similarities in operations (i.e., experimental manipulations) and consequent changes in behavior among these phenomena are used to organize the phenomena. Then the more widely cited contemporary models of associative learning are briefly described. The more successful models provide central principles that call for a search for the underlying neurophysiological mechanisms.

Benchmark Phenomena of Learning and Memory

Contiguity

As early as the ancient Greek philosophers, it has been recognized that stimulus control of behavior indicative of an association between two events (e.g., a cue and an outcome) is most apt to appear when the events occur in close temporal and spatial proximity to one another. Shorter intervals between the cue onset and the outcome onset enhance later responding to the cue. Conditioned responses are stronger when the cue precedes the outcome (i.e., forward pairings) than when the two events are simultaneous or the outcome comes first (i.e., backward pairings), but whether this reflects weaker simultaneous

and backward associations or merely weaker behavioral expression of equally strong associations is currently unclear. Despite the wide recognition of the critical role of contiguity, only a few models (e.g., Gallistel and Gibbon's 2000 rate expectancy theory (RET); Wagner's 1981 sometimes opponent process (SOP) model) actually try to account for it as opposed to merely assuming its role.

With A, B, and other single letters denoting specific cues that do not initially control behavior, second-order conditioning (B-US pairings followed by A-B pairings yield responding to A that is indicative of an A-US association) and sensory preconditioning (A-B pairings followed by B-US pairings yield responding to A that is indicative of an A-US association) are examples of A-US acquired behavior without pairings of A and US. This appears to contradict the view that contiguity is necessary for acquired behavior. However, the principle of contiguity has been sufficiently successful in so many other situations that, rather than discard it, it has been reframed to focus on contiguity between activation of neural representations rather than contiguity between the actual occurrences of physical events.

Contingency

Stimulus control of behavior is encouraged when a cue and outcome occur together (i.e., are contiguous) and when subject spends time in the training context during which neither stimulus occurs, although the benefit of co-nonoccurrence is far weaker than the benefit of co-occurrence. In contrast, behavioral control is weakened when one of the two stimuli occurs in the absence of the other. Presentations of the cue- or outcome-alone degrade responding to the cue when they occur before the cue-outcome pairings (i.e., CS- and US-pre-exposure treatments) or are interspersed among the cue-outcome pairings (i.e., partial reinforcement and degraded contingency treatments). Cue-alone presentations after the pairings (i.e., extinction treatment) also attenuate subsequent responding to the cue; however, this decrement is often followed by spontaneous or induced recovery of stimulus control. Most contemporary models account for these contingency phenomena, but few address recovery from extinction.

Stimulus Salience (Attention)

Appearance of conditioned responding occurs in fewer pairings and behavioral control is stronger at asymptote when the cue and/or outcome are more salient (increased intensity is one means of enhancing salience). Most models assume that salience is positively correlated with associability (i.e., ease of entering into an association) or attention. However, models based exclusively on

information processing independent of stimulus characteristics (e.g., Gallistel and Gibbon's 2000 RET) are challenged by stimulus salience effects.

Stimulus Interaction

When there are more than two stimuli (e.g., another stimulus, C, in addition to target cue A and outcome B in a given situation, where C is another cue or outcome), such that each stimulus is associated to at least one other stimulus, the presence of C can influence the A-B association or at least its expression. Control of excitatory behavior by the target cue (A) can be either impaired (e.g., cue competition such as overshadowing and blocking, conditioned inhibition, and stimulus interference) or enhanced (e.g., cue potentiation, augmentation, second-order conditioning, and sensory preconditioning) as a result of these interactions. Detrimental interactions are by far the more common (or at least they have received far greater attention). The conditions that favor detrimental as opposed to facilitative interactions are still unclear, but larger numbers of trials appear to more strongly favor detrimental interactions. When cues C and A share a common relationship to outcome B (e.g., AC-B), the altered behavior based on the A-B association is called a cue interaction. Outcome interaction is said to occur if A shares a common relationship with C and B (e.g., A-CB). If the interacting stimuli are presented simultaneously (or at least on the same trials) and the interaction is detrimental to excitatory responding, the interaction is called competition and may depend on the establishment of a within-compound association between the competing stimuli. When the interacting stimuli are presented apart (i.e., on different trials, e.g., A-B followed by C-B), detrimental interactions are called interference and facilitative interactions are rarely observed. Majority of research and theoretical models have attended to cue competition to the exclusion of other forms of stimulus interaction. When interference has been studied, the focus has been on outcome interference far more often than cue interference. When the interacting stimulus, C, is independently revalued (i.e., reinforced or extinguished) following its presence during A-B pairings, the observed change in behavior control by the A-B association is called retrospective revaluation. Retrospective revaluation is a reliable phenomenon, but one that is clearly weaker than changes in behavior obtained by direct manipulations of A or B (i.e., pairings or presentations alone).

Whether stimulus interaction (i.e., modified responding to A) produced by the presence of C is due to a direct change in the A-B association or a change in responding to A that is mediated by C at the time of testing is unclear. For example, a decrease in responding to A based on an A-B association may be due to either a weakened A-B

association or a decrease in the behavioral expression of the A–B association, which is often viewed as a retrieval failure. Both likely occur as a function of parameters. Stimulus competition (competition between stimuli presented together) and stimulus interference (interference between stimuli presented apart) are both subject to recovery through (1) extinction of the competing or interfering stimulus, (2) reminder treatments that preclude new learning, and (3) spontaneous recovery as a result of delayed testing. Such recovery of behavior has been viewed as arising from retrieval of latent memories by some researchers and as new learning by others. Accounts of cue competition as a result of impaired learning at the time of training usually assume that learning depends on a discrepancy between the expected outcome based on all cues present on that learning trial and the outcome that actually occurs. Learning in such models (e.g., Rescorla and Wagner) is said to work to reduce total error in outcome expectation. If learning an A–B association did not occur when subjects were exposed to AC–B pairings (i.e., overshadowing of A by C), this would indicate that contiguity alone was not sufficient for learning to occur in that A and B were paired. Contemporary accounts of cue competition as a product of impaired retrieval assume that learning concerning each separate cue depends on a discrepancy between the expected outcome based on that individual cue and the outcome that actually occurs (i.e., local error reduction). Retrieval-failure accounts of cue competition do not contradict the view that contiguity alone is sufficient because learning is assumed to have occurred but not be expressed. Most contemporary models account for competition, but not interference (however, Bouton accounts for interference, but not competition).

Factors that Differentiate Contemporary Models of Associative Learning

Stimulus Definition

As associative learning is the formation of links between the mental representations of stimuli (or responses), it is essential that the meaning of stimulus is well understood. An early and still prevalent view treats a stimulus as would the layman; for example, a tree is a single elemental stimulus. This position is taken by Rescorla and Wagner's 1972 model and many other more contemporary models. In contrast, some models deconstruct gross stimuli such as a tree into a large number of stimulus elements, thereby replacing the representation of the tree with individual branches, twigs, leaves, or even leaf fragments (i.e., microelements). Such models often concern themselves with the consequences of associations between the elements, which can provide powerful explanatory mechanisms. At the other end of the spectrum of what constitutes a stimulus is Pearce's 1987 configural model,

which treats the entire momentary perceptual field (except for the US) as a single integrated stimulus (i.e., a configured stimulus). Here, rather than perceiving the tree, subjects are assumed to perceive the entire forest including hearing the birds sing. As such, stimuli are not apt to ever exactly repeat from training trial to training trial, or training trial to test trial. Much explanatory power is based, with considerable success, upon generalization between stimuli on different trials being a function of differences in stimulus similarity.

Emphasis on Acquisition or Performance

The chain of events required for associative control of behavior obviously must include acquisition (sometimes called encoding or learning), retention (sometimes called storage), retrieval, and behavioral expression. Acquired behavior would be disrupted by a failure of any of these links in the chain. Thus, any serious model of learned behavior should speak to each of these steps. Surprisingly, most associative models focus their attention on either the acquisition phase or the retrieval/expression phase, paying little heed to the other phases. That is, to explain differences in stimulus control of behavior between treatment groups, most models emphasize differences in acquisition to the exclusion of differential retrieval of stored memories. Other models emphasize differential information processing after acquisition as a function of treatment, rather than differential acquisition. Few models seriously concern themselves with information processing during both acquisition and testing (i.e., retrieval and expression). Notably, neuroscientists have largely focused on acquisition, generally ignoring information processing that occurs during test.

Most acquisition-focused models follow the Rescorla-Wagner model by assuming that acquisition is driven by one or another form of total error reduction. The assumption here is that, on a given trial, learning about each cue present is proportional to the difference between the maximum associative strength that the outcome delivered on that trial can support and the expectation of the outcome based on all cues present on that trial. Models subscribing to this principle use it to account for stimulus interactions (e.g., conditioned inhibition and cue competition). Some models that subscribe to total error reduction assume that this principle results in cue competition by decreasing processing of US representations, whereas other models assume that the total error reduction principle leads to cue competition by decreasing processing of CS representations. Models that focus on processing at the time of performance typically reject the total error reduction principle and use a local error-correction rule to prevent unlimited acquisition across many CS–US pairings (an approach popularized by Bush and Mosteller). Here, acquisition for each specific cue is assumed to be proportional to

the difference between the maximum associative strength that the outcome delivered on that trial can support and the immediate expectation of the outcome based on that specific cue. Such models account for stimulus interactions through processes that occur at the time of testing.

Trial-Wise or Real-Time Processing

Whether they emphasize information processing during acquisition or during performance, most models of learning are trial-wise models. That is, on a given trial on which stimuli are presented, these models assume that organisms first compute responding, then perceive the outcome of the trial, and finally engage the learning mechanism based on what transpired on that trial. Trial-wise models are to be contrasted with real-time models, which assume that organisms are encoding new information instant by instant throughout the treatment sessions, that is, during both the so-called trials and the intertrial intervals. In reality, there is no doubt that organisms are real-time processors of information, but real-time models are often mathematically intractable. Hence, in practice, researchers simulate them by assuming that treatment sessions have a trial-like structure, composed of very small temporal windows throughout the so-called trials and the intertrial intervals. Moreover, trial-wise models sometimes address learning about context that can occur during intertrial intervals by treating each intertrial interval as many trials on which the target cue and outcome are absent (the specifics of parsing intertrial intervals can be critical). When this is done, trial-wise models can closely resemble real-time models that are simulated by parsing the entire treatment session into many trials of short duration. Hence, in simulation, trial-wise models sometimes do not differ greatly from real-time models, despite the fundamental difference in the underlying assumptions.

Learning during test trials must be considered because early parts of a first test trial can influence behavior in later parts of the same test trial. This is fully expected in real-time models (but not trial-wise models) and is commonly observed in some preparations. For example, tests of fear conditioning often consist of a so-called flooding measure, which involves exposing the subject to a potentially fear-inducing cue (or context) for a single long period of time. Initially, the cue disrupts the behavior that was ongoing prior to presentation of the test cue, but as exposure to the cue continues, the ongoing behavior recovers. Clearly, the recovery of ongoing behavior reflects information processing that occurred earlier on that test trial. Alternatively stated, unlike experimenters, subjects do not differentiate between training and test trials; they are real-time information processors.

Context

Context usually refers to the total physical environment exclusive of nominal CSs and USs. The contexts of both treatment (i.e., acquisition, extinction, etc.) and testing can play several important roles in determining associative responding to a target cue. Both types of context can act as another punctate cue that competes with or facilitates responding to the target cue. Most models attempt to account for this by treating the training context as an additional long-duration cue that is present during both CS presentations and intertrial intervals. This approach is conceptually sound, but in simulations it does not perform well because the predicted behavior varies greatly based on the assumed duration of each context-only trial. Less readily addressed at the conceptual level by most contemporary models is the role that the context (or a punctate stimulus) can play as a discriminative stimulus (aka an occasion setter), in which the context at test can serve to direct the organism to respond using information from only one of two contradictory learning experiences regarding the same cue to which the organism has been exposed. For example, after X -US trials in context #1 and X -noUS trials in context #2, responding to X is observed in context #1 but not context #2. Only a few models of associative learning even begin to account for such occasion setting. Bouton does account for such phenomena, but does not provide a general model of learning.

Contemporary Models of Associative Learning

Here, we very briefly describe a few of the more popular contemporary models of associative learning. One might ask why these models are featured as opposed to the many other possible models that could be cobbled together by merging various principles borrowed from the models described below. The aspect that makes the present models popular is that they feature a limited number of principles which makes them tractable; that is, they tend to make unambiguous testable predictions in contrast to more complex models that are often hybrids of these simpler models. Rank-order predictions between groups by the more complex hybrid models tend to be highly dependent on parameters. In contrast, although the predictions of the simpler models are also parameter dependent, rank-order predictions between groups are less dependent on specific parameters.

Acquisition-Focused Models

As previously mentioned, the various contemporary models tend to place most of their explanatory weight on either acquisition or retrieval/expression. There are no

associative models that focus on storage (the time between acquisition and testing) because storage is widely assumed to be reliable, although, in practice, behavior suggests some limited decay of associations with increasing retention intervals.

The Rescorla and Wagner 1972 model assumes that simple stimuli (i.e., neither deconstruction into many elements nor configuration of the entire perceptual field) are processed. This model has long been the most widely cited model of learning. Each stimulus (CSs and USs) is assumed to have an inherent associability (potential to enter into association) that does not change as a function of experience. Acquisition on any given trial is a direct function of how surprising the US is based on all stimuli present (i.e., learning is driven by reduction of the total error in expectation of the US (aka the delta rule), a principle challenged by recent data reported from the laboratories of Rescorla and Miller). Associative strength between a CS and an US varies from positive values, which result in excitatory conditioned responding, to negative values (i.e., a negative expectation of the US), which result in behavior indicative of conditioned inhibition. This contrasts with most other acquisition-focused models which assume that there are two variables of association between a given CS and US, one for conditioned excitation and the other for conditioned inhibition. In order for a CS to change its associative value with respect to a US, the CS (but not the US) need be present. This feature, which is shared with many other acquisition-focused models, prevents the model from explaining retrospective revaluation. Van Hamme and Wasserman proposed a revision of the Rescorla–Wagner model which allows changes in associative value of a CS even when the CS is absent, provided an associate of the CS is present, thereby accounting for retrospective revaluation. Miller, Barnet, and Grahame provide a review of many of the Rescorla–Wagner model's successes and failings.

The Pearce and Hall 1980 model accounts for many phenomena, including cue competition and some contingency phenomena such as CS- and US-pre-exposure effects, through variation in processing of the CS representation, in contrast to Rescorla and Wagner's emphases on variation in US processing. Specifically, Pearce and Hall posit that a cue's associability (potential to enter into an association, as distinct from the cue's potential to elicit responding) changes as a function of experience. Associability to the CS is presumed to increase when the US is surprising and decrease when the US is expected based on all cues present on that trial. Hence, total error drives CS processing, not US processing. Permitting associability to vary contrasts with Rescorla and Wagner's approach which assumes that associability of a given CS or US is constant.

Mackintosh's 1975 model completely rejects total error correction as the key process underlying learning.

Rather, error correction is assumed to occur independently for each individual cue; thus, US processing varies for each cue present depending on the associative status of that cue. To explain phenomena such as cue competition that Rescorla and Wagner address through total error reduction, Mackintosh depends upon variation in CS processing. CSs that best predict their consequences increase in associability, whereas CSs trained in the presence of another cue that is a better predictor of the outcome USs decrease in associability.

The Pearce 1987 configural model, like the Rescorla–Wagner model, assumes that acquisition is driven by variation in the effectiveness of US processing, which is implemented by total error reduction. However, the emphasis of the model is on how stimuli are represented. As previously stated, on a given trial learning presumably occurs with respect to the entire perceptual field (excluding the US) as a single configured stimulus. US anticipation and, hence, conditioned responding is a function of the excitatory status of the configured stimulus presented on that trial plus generalization from other stimuli that are similar to the test stimulus. The model has clear rules for estimating similarity between two (configured) stimuli.

Wagner's 1981 SOP model assumes that all stimuli are composed of many elements, each mentally represented by a node. All nodes are always in one of three states of activation. The first memory state is one of inactivity (I), the second is one of high activity (A1), and the third is one of low activity (A2). Initially, all elements are in state I. Upon presentation of a stimulus, each node of that stimulus that is in state I has a probabilistic chance of being activated into state A1. Unconditioned responding reflects nodes that are in A1. Nodes in state A1 decay rapidly into state A2, and nodes in state A2 decay slowly back into state I. Nodes in state A2 cannot be activated directly into state A1. If a stimulus which is an associate of an absent stimulus is presented, there is a probabilistic chance of any node of the absent stimulus currently in state I being activated into state A2. Conditioned responding reflects nodes in A2. Excitatory learning occurs when two nodes are simultaneously in state A1. Inhibitory learning occurs when nodes of a CS are in A1 and nodes of the US are in A2. Extinction is due to conditioned inhibition; presentation of the CS without the US causes CS nodes to initially go into A1 and US nodes to be activated into A2. Changes in activation and concomitant learning are ongoing, making this a real-time model in contrast to the previously sketched models. SOP does not account for retrospective revaluation, but an extension of it by Dickinson and Burke does. In 2003, Wagner updated his model to accommodate the configuring of stimulus elements (i.e., learning about the compound of several elements independent of the associative status of the individual elements).

There are a large number of additional acquisition-focused models – hybrid models. Most of them combine various features from those already sketched. Among the most notable of these are those of Harris, LePelley, and McLaren and Mackintosh.

Expression-Focused Models

[Stout and Miller's 2007](#) sometimes competing retrieval (SOCR) model assumes that learning *per se* is driven by spatiotemporal contiguity alone, and limited for each stimulus by local error reduction. Unlike the prior models, conditioned responding is not simply a reflection of the association between the test stimulus and the US, plus generalization from other CSs that are similar to the test stimulus. Rather, conditioned responding is assumed to be not only directly related to these sources of US anticipation, but also inversely related to the expectation of the US based on other cues that were present during training; these companion cues can be contextual or punctate. Thus, conditioned responding reflects not the absolute strength of the test CS-US association, but the extent to which the CS predicts an increase (or decrease) in the likelihood of the US over that of the background of training. Notably, unlike the preceding models, the model assumes that there are no negative associations or positively valued CS-no US associations. Instead, behavior indicative of conditioned inhibition arises from the interaction of excitatory associations, specifically, the background being a strong predictor of the US relative to the target CS. This model readily accounts for retrospective revaluation as well as stimulus competition and contingency effects. However, like the previously mentioned trial-wise models, it fails to account for changes in behavior as a function of the interval between two events, such as the interval between CS onset and US onset, or between cue onset and termination (i.e., interval timing effects).

[Gallistel and Gibbon's 2000](#) RET model assumes that subjects encode rates of reinforcement in the presence of each CS and separately in the presence of the training context alone. Conditioned responding emerges when the reinforcement rate in the presence of the CS becomes significantly higher (as assessed by a statistical test) than in the conditioning context alone. This model is similar to Stout and Miller's SOCR model except that subjects are presumed to encode temporal intervals between events and rates of reinforcement rather than CS-US associations; hence, this model can account for interval timing effects. In addition, the model is a real-time model and assumes that animals retain memories of all prior events (not just summary statistics like associative strength) as well as the intervals between them. However, the model erroneously predicts no effect of stimulus salience.

Behavioral Benchmarks Waiting To Be Met

Overemphasis on Acquisition

The study of stimulus-controlled acquired behavior is called learning. However, learning is an intervening variable; all that is observed is a change in behavior. Stimulus-controlled changes in behavior require perception, encoding (i.e., acquisition and learning), maintained storage, retrieval, and mapping of retrieved information into behavior. However, researchers working with animal models of associative learning have largely focused on acquisition to the exclusion of the other steps in the chain of information processing. Gallistel and Gibbon as well as Stout and Miller stand out in emphasizing information processing that occurs at the time of performance. Researchers studying cognition with humans appear more attuned to the importance of retrieval processes (e.g., Bjork; Roediger; and Tulving). There is a need for more uniform attention to each phase of information processing.

What Is a Stimulus

Despite discussion for almost 100 years, there is still controversy concerning how best to describe a stimulus. The layman's view of a stimulus is still the dominant approach. In contrast, some models that assume a simple stimulus is deconstructed into a large number of perceptual elements that get processed independently, whereas other models assume that the entire perceptual field at any given instant (except the US) is treated as a single configured stimulus. If testing later occurs with some fraction of that configured stimulus present, then responding is a function of the similarity of the configured test stimulus to the configured training stimulus. A consensus concerning how to conceptualize a stimulus has not yet been reached.

Summary Statistics

With the exception of Gallistel and Gibbon, contemporary models of learning assume that organisms store a singular association between A and B that is revised with each subsequent relevant event. These stored summary statistics seemingly serve to provide animals with what is sometimes called procedural knowledge, but the approach fails to address animals' memory of specific past events (i.e., episodic knowledge). Much human data and some data from nonhumans document the existence of episodic memories that encode for a specific episode what happened, when it happened, and where it happened. There is a need for associative models to account for

episodic memories, perhaps taking an approach similar to exemplar models of category learning.

Stimulus Interference

Although the occurrence of stimulus interference (competition between stimuli presented on different trials) is widely documented, no contemporary model of learning adequately accounts for all the major interference phenomena. Commonly observed forms of interference include (1) extinction following acquisition (i.e., A–B followed by A-alone), (2) counter-conditioning (i.e., A–B followed by A–C degrading behavior reflecting an A–B reflecting A–B). Bouton, and Miller and Escobar have proposed accounts of stimulus interference, but these accounts by themselves fail to address many non-interference learning phenomena. There is a need for comprehensive models of learning that account for stimulus interference.

Summary

Associative models of learning serve to organize phenomena observed in acquired behavior and they generate contrasting predictions that direct research. In the former role, models also guide neuroscientists who are seeking underlying mechanisms for not a single behavioral phenomenon, but a family of phenomena. The models tell us what constitutes a family of phenomena. That is the good news. The bad news is that all contemporary models of learning are gross simplifications. However, we have to start somewhere and these models are the best currently available.

See also: Active Avoidance and Escape Learning; Amnesia; Cognition: Learning and Memory: Pavlovian; Cognition: Learning and Memory: Spatial; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Eyelid Classical Conditioning; Fear Conditioning; Fear, Anxiety, and Defensive Behaviors in

Animals; Human Fear and Anxiety; Learning and Memory: Computational Models; Memory Consolidation; Neural Basis of Classical Conditioning; Neurogenesis and Memory; Protein Synthesis and Memory; Synapse Formation and Memory.

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Animal Models of Sexual Function

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Glossary

Erectile function – Penile erection is characterized physiologically by a hemodynamic event that results from increased penile blood flow, corpus cavernosal relaxation, venoconstriction, and ultimately increased intracavernosal pressure. In rodent, erection can be facilitated pharmacologically using brain-acting agents, or secondary to sexual stimulation (presence of female, tactile stimulation).

Female consummatory behavior (receptive e.g., lordosis) – A reflex stimulated by pressure on the hind flanks of estrous females when mounted by a male. The female arches her back, which raises the hips to make intromission (penetration) easier. She also moves her tail to one side. Lordosis is displayed by many quadruped mammals, but does not occur in humans.

Female sexual motivation behavior (proceptive) – Appetitive behaviors are displayed by the female rodents to solicit the male to engage in sexual intercourse. In rats, these manifest as approaching the male, crouching, and then darting away to encourage the male to follow (termed ‘hops’ and ‘darts’). The female may also ear wiggle, a very fast flutter of the ears, which is thought to release pheromones to attract the male.

Male consummatory behaviors – A series of complex coordinated motor behaviors and spinal reflexes that underlie mounts, intromissions, and ejaculation.

Male preconsummatory behaviors – Male rodent copulation begins with appetitive behavior and the male approaching the female, followed by anogenital investigations, and this allows the male to understand the female’s estrous state and the degree of female receptivity to his approaches, and if appropriate, then copulation can occur. This then leads to precopulatory behaviors such as pursuit.

or so, there has been an increasing number of studies investigating pharmacological manipulation of sexual function with a view to developing drugs for sexual dysfunction. Early studies were largely focused on male disorders, primarily erectile dysfunction (ED), but also on premature and delayed ejaculation. The successful launch of phosphodiesterase type 5 (PDE5) inhibitors, such as sildenafil, tadalafil, and vardenafil, has demonstrated that animal models can be used to predict human sexual function, at least for ED. More recently, there has been increased attention paid to female sexual disorders, such as deficits in desire and arousal. Studies in women with desire and/or arousal disorders have shown that subjective effects seem to be far more important in modulating sexual responses than in men, which has posed additional challenges to modeling female sexual function in laboratory animals.

The rat is by far the most common laboratory species used for sexual studies, although mice, hamsters, quail, and primates are also used. Rat sexual activity appears somewhat different from that of human, because the specific motor patterns are different, but the overall function of these activities has human correlates (Figure 1). Ethological studies in rats determined that females often mate with several males at the same time in the wild. Appetitive behaviors are displayed by the female to solicit the male to engage in sexual intercourse. These manifest as approaching the male, crouching, and then darting away to encourage the male to follow (termed ‘hops’ and ‘darts’). The female may also ear wiggle, a very fast flutter of the ears, which is thought to release pheromones from behind the ears to attract the male. Mating occurs in bouts with mounting by the male and brief intromission (penetration; ~1 s) before the male and female separate. Each male generally intromits 5–10 times before achieving ejaculation. A female may receive intromissions from multiple males before ejaculation. Given the opportunity, the female paces her contact with the male(s) such that intromission occurs at her preferred interval (typically 60–200 s), which triggers a neuroendocrine reflex that makes pregnancy more likely. Microdialysis studies have demonstrated greater increases in extracellular dopamine levels in the nucleus accumbens when mating occurs at the female’s preferred pacing interval (PPI), suggesting that this is most rewarding for the female. Research has not yet demonstrated for certain whether or not female rats experience orgasm. Male rats prefer to intromit at a faster rate (<60 s), which means that the female must ‘escape’ from the male in order to pace interactions to her PPI.

The field of sexual research in animals is approximately a century old. Despite this, the volume of studies in this area is far lower than most other areas exploring physiological/behavioral functions. Early studies were largely aimed at developing a framework to understand animal, particularly rat, sexual activities, and to start determining the roles of the endocrine and neurotransmitter systems and behavioral manipulations on sexual performance. In the last 20 years

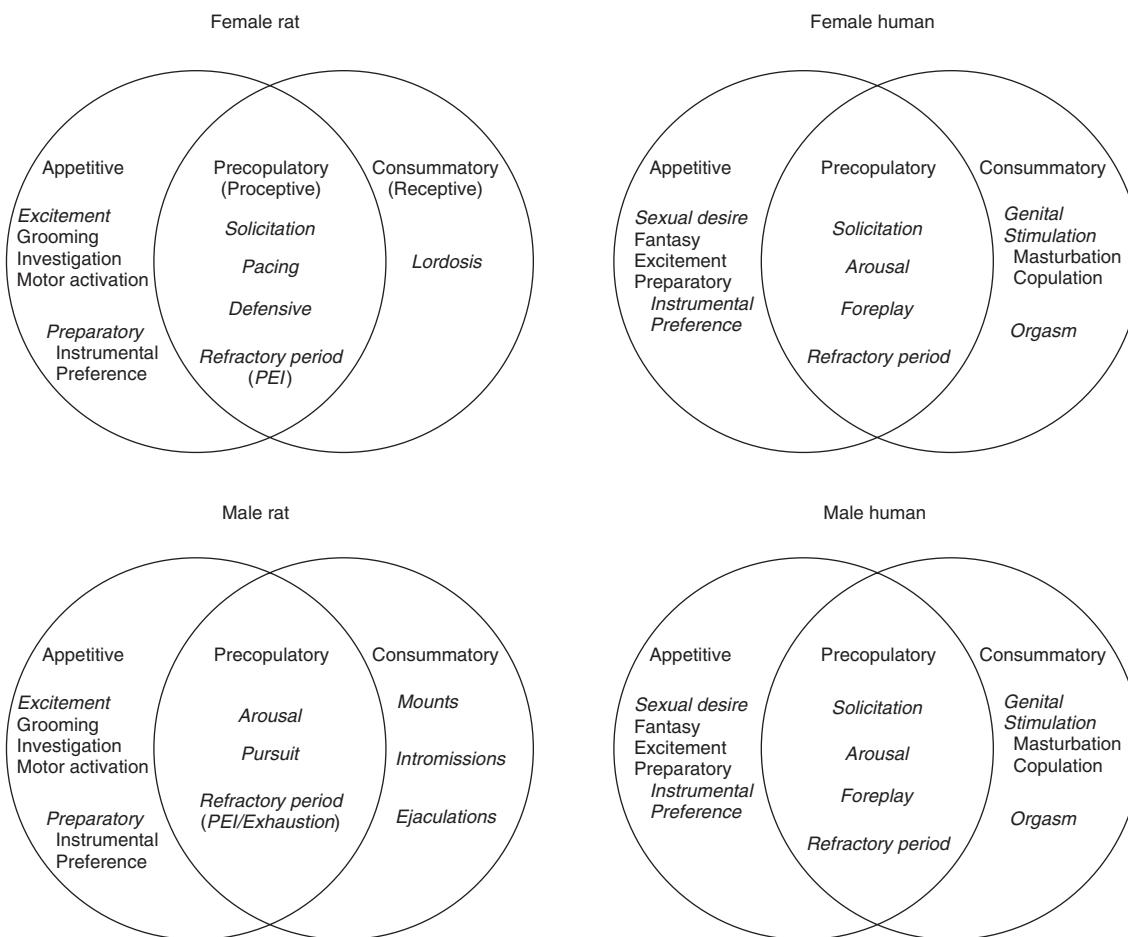


Figure 1 Comparison of sexual behaviors displayed by rats and humans. Redrawn from Pfau JG (1999) Revisiting the concept of sexual motivation. *Annual Review of Sex Research* 10: 120–157.

Female Sexual Behavioral Models

When considering animal models of sexual function, the first thing to take into account is the tight coupling of the estrous cycle with sexual activity. In human females, sexual interest is relatively uniform across the estrus cycle, with a slight rise around the ovulatory period. In contrast, females of most laboratory species will display interest and engage in sexual intercourse only during the estrous stage of the cycle and with very low levels of interest at other times. This presents a practical problem in running animal models of sexual function, especially in species that mate seasonally, such as the dog. To overcome this limitation, the field has concentrated on the use of ovariectomized (OVX) females which are then administered estrogen (for rats, typically 1–100 µg kg⁻¹, 48–52 h before testing) and sometimes progesterone also (250–2000 µg kg⁻¹, 3–8 h before testing) to bring them into an estrous-like state. An additional, important practical advantage is that the OVX prevents pregnancy occurring after copulation, although this could also be

achieved in intact females through the use of vasectomized males. The similarity of this state, physiologically and behaviorally to that of estrous in naturally cycling animals, is fairly well established, although exhaustive studies have not been carried out. For drug studies aimed at assessing pro- or antisexual effects, the hormonal prime can be manipulated: a low level of sexual activity for prosexual studies, and maximal sexual activity to examine drugs that reduce sexual activity.

The only drug approved for use in women to improve sexual function is a testosterone patch. Low levels of testosterone (200–300 pg ml⁻¹ of serum) are required for female sexual activity, although this is a threshold effect and a dose dependency is not observed. Hence, the drug is only approved for use in surgically postmenopausal women with ovary removal, which is known to lower testosterone levels. The lack of widely applicable and clinically approved prosexual agents for women makes it difficult to know which drugs to use as positive controls for preclinical prosexual pharmacological studies. There are some nonhormonal agents have been shown to be

effective in rats and for which there is limited positive data in women with sexual disorders: bremelanotide (melanocortin receptor agonist), apomorphine (nonselective dopamine receptor agonist), buproprion (dopamine agonist, nicotinic acetylcholine receptor antagonist, and noradrenaline reuptake inhibitor), and some serotonergic drugs, such as mCPP (1-(3-chlorophenyl) piperazine).

When testing compounds for pro- or antisexual effects, the hormonal prime used in female rats should be carefully considered. The majority of published studies use fairly large estrogen and progesterone priming doses such that robust sexual activity is observed. A limited number of pharmacological studies have demonstrated an increase in sexual activity above that induced by the high hormonal primes alone, but how to define ‘maximal’ sexual activity is not clear. Despite this uncertainty, it is likely that the window for observing an increase in sexual activity may be limited. One simple solution is that such studies would better run using a suboptimal prime: sufficient to induce some sexual behavior, but of a low-enough level to facilitate potentiation. For antisexual pharmacological studies, an optimal prime is ideal to maximize the effect window for the test compound.

Consummatory Models

By far, the most widely used consummatory sexual model in the laboratory is the lordosis assay. Lordosis is a reflex stimulated by pressure on the hind flanks of estrous females when mounted by a male. The dorsiflexion raises the female’s hips to make intromission easier and also moves her tail to one side. The lordosis test is usually carried out in a small test arena, often a standard husbandry cage bottom. The female, usually OVX with hormonal priming, is introduced into the cage which contains a sexually vigorous male. The female’s response to mount attempts by the male is recorded, typically in a binary (yes/no) manner. The male is allowed a prespecified number of mount attempts (e.g., 10), within the test period (e.g., 5–10 min). The lordosis quotient is calculated as the percentage of mounts resulting in lordosis divided by the total number of mounts/mount attempts. Pro- or antisexual drugs can be tested by administering to the female before testing, although it is important to use an appropriate hormonal prime. The female may be moved between cages of males in order to avoid ejaculation by a male, which would alter the behavior of both partners. The lordosis quotient is a relatively crude measure and the data are often not normally distributed, which make statistical analysis more difficult. Thus, some groups have started using a rating scale for the female’s response, scoring zero for rejection of the male through to three for a willing, full lordosis. This score is then summed over the number of mount attempts. The score has the advantage of giving greater granularity to the female’s response

and the mean group data are normally distributed which facilitates statistical analysis.

The lordosis model is relatively quick to run, simple to execute, and requires no specialist equipment. However, the results are driven largely by the male, as the female has no means to escape from him if she wants to reject mounting and pace her interactions. Additionally, intromissions occur at the male’s preferred rate, which is much faster than that of the female’s PPI. Finally, although lordosis is displayed by many quadruped mammals, it does not occur in humans and so is not a directly translatable endpoint.

Sexual Motivation Tests

Tests designed to examine the motivation of the female for interactions with a male are particularly important for understanding what drives sexual desire, especially as sexual desire disorder affects a significant proportion of women suffering from female sexual dysfunction (FSD). The lordosis test described above largely gives a measure of consummatory sexual behavior (the actual copulation) and can tell us relatively little about the motivation (desire) the female has to approach and engage a male. Sexual motivation tests are designed to prevent full physical contact of the female with the male, so that mounting/intromissions do not confound the measurement of sexual motivation. Early studies of sexual motivation used modified operant methodology which had been successfully applied to drug-abuse motivational studies. Female rats were required to press levers within the test apparatus to obtain access to a male. Although successful, such studies suffered from relatively low response rates, thus making quantitative analyses difficult. Additionally, relatively complex training schedules were required and they have subsequently fallen out of favor. Another early model used classical runway tests, which had been adapted from studies of goal-directed behavior in other fields. In these studies, the speed at which the female runs down the runway to gain access to a male in the goal box at the far end is taken as a measure of motivation for the stimulus animal, in comparison with the speeds where castrated males, other females, or no stimulus (no rat) are used to separate out sexual motivation. In a variation on this test, the runway was fitted with an electrified grid and the current applied could be varied. The theory being that motivation could be inferred from the pain the rat was willing to endure to cross the grid. For example, estradiol benzoate treatment of OVX females increased grid crossings compared with untreated OVX rats.

An alternative experimental approach is to give a female rat the choice between investigating two different stimulus animals and infer that her preference for one over the other is a measure of her sexual desire for that

stimulus animal. This model is run in a large round or oval arena with two small satellite chambers attached on either side of the main arena. The stimulus animals are placed in the satellite chambers, most commonly a sexually active male in one and either a female or a castrated male in the other. The satellite chambers are linked to the main arena through a perforated grid wall, such that the test female in the main arena can see, hear, smell, and have limited tactile contact with the stimulus animal, but cannot fully interact with it. The behavior of the test female is recorded either by direct observation or by an overhead camera. The preference score is the time spent investigating the grid behind which the sexually active male is located, divided by the total time spent investigating either stimulus animal. A score of 0.5 indicates no preference and would be observed for OVX females that have not been hormonally primed. Estrogen and progesterone priming increases the preference score up to a maximum of about 0.75, indicating a preference ('greater desire') for the active male. Likewise, prosexual compounds, such as apomorphine, increase the preference score to a similar degree as hormonal priming.

Combined Motivation and Consummatory Tests

Paced Mating

This test is designed in such a way that the female can escape from the male and thus control the rate of copulation. The apparatus is usually a test arena split into two sections by a divider that has one or more holes in it (**Figure 2(a)**). The test females used are smaller than the males and the holes are small enough such that only the females can pass through them. Alternatively, the males are trained prior to the main test to remain on one side of the chamber by aversive conditioning (e.g., a tap on the nose with a pencil) each time they cross the barrier. Another version of this apparatus tethers the males to one side of

the chamber through a jacket harness. Finally, a bilevel chamber has also been used as a pacing chamber (**Figure 2(b)**). This apparatus is a relatively narrow chamber with a lower and upper platform linked by a ridged ramp. The theory is that the female is more agile than the male and so can outpace him by running up and down between the levels. Both the bilevel chamber and the use of tethered males can have the disadvantage of limiting the movement of the animals and thus, disrupting copulatory behavior.

All pacing chamber studies rely on observers carefully recording the behaviors and interactions of the male and female as well as the time interval between these behaviors. The most common measure used is the exit return latency: the time a female takes to reenter the male's side/area of the chamber after exiting. Additional measures include subcategorizing exit return latency into mount and intromission return latency, recording the number of mounts, intromission, and ejaculations the female receives and also measuring the number of times the female moves between the two chambers. A decrease in the exit return latency and an increase in the amount of time the female spends in the male's part of the chamber are generally taken to indicate a prosexual effect.

Partner-Preference Models

In order to determine whether a female finds different types of sexual interaction rewarding, partner- and/or place-preference models have been used. The place-preference model is widely used to investigate the rewarding effects of pharmacological agents and drugs, such as cocaine or morphine, which will produce a strong preference. The test relies on the observation that when given the choice between two environments, an animal will spend more time in the environment associated with rewarding experiences versus less rewarding/aversive experiences. In the partner-preference test, the female is placed into a test chamber which is usually divided into

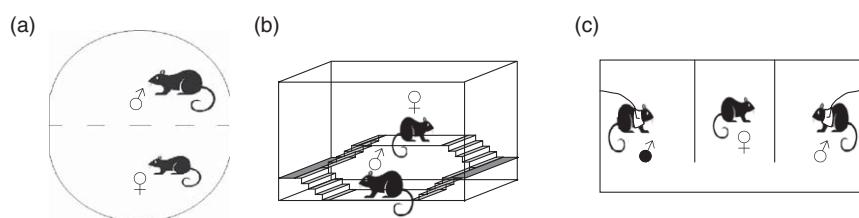


Figure 2 Models of sexual interaction. (a) A paced mating (also known as 'escape chamber') apparatus. The male and female are placed on opposite sides of a dividing wall in the test arena. The dividing wall has four holes, which are large enough to allow the female to pass through, but too small for the larger male rat. The female can control interactions with the male by escaping to the other half of the apparatus. (b) A bilevel chamber is a narrow testing arena with two platforms, one above and the other, linked by ramps. The more agile female can control copulation rates with the male by out-maneuvering him in the apparatus. (c) A partner preference arena has three sections linked by doorways. The test female is placed in the middle and can choose to spend time interacting with either of the stimulus animals tethered in the other chambers through a jacket harness. In this example, there is a sexually vigorous male stimulus (open ♂) and a social stimulus: a castrated male rat (filled ♂).

two or three compartments linked by a doorway or tunnel (**Figure 2(c)**). She is free to move between these chambers and there is a stimulus animal in each compartment. The stimulus animals may be sexually vigorous males, castrated males, or females all with or without drug treatment. The time spent with each animal in a single or repeated test is recorded and expressed as a percentage preference. There are arguments that the presence of the stimulus animals can affect the behavior of the female and thus not give a true representation of her preference. Hence, a place-preference paradigm can be used instead. Here, the female is exposed to a stimulus animal in one compartment of the apparatus with no access to the other compartment. Subsequently, she is exposed to the other compartment with a different stimulus. There may be more than one pairing of the compartment with the stimulus to strengthen the association with that compartment. Finally, the test is run with the female having free access to both sides of the apparatus in the absence of stimulus animals. She will spend more time in the previously rewarding side of the apparatus. An important point in running this study is that the two compartments of the apparatus are contextually different to aid the development of a place-reward relationship. A distinctive context can be provided by distinct wall patterning, smells, and textures such as different flooring types or bedding.

Male Sexual Behavioral Models

Copulatory Behavior/Ejaculation

Rodents are the most commonly used animals in male copulatory behavioral studies, although higher species, such as primates, have been described. There are no pathophysiological rodent models of human sexual dysfunctions, such as ED or premature ejaculation, so studies are performed on 'normal' animals. Male rodent copulation begins with appetitive behavior and the male approaching the female, followed by anogenital investigations, and this allows the male to understand the female's estrous state and the degree of female receptivity to his approaches, and if appropriate then copulation can occur. This then leads to precopulatory behaviors such as pursuit and then, a series of complex coordinated motor behaviors and spinal reflexes that underlie consummatory behaviors such as mounts, intromissions, and ultimately, ejaculation.

Sexually active male adult rats are used in these behavioral assays. Prior to behavioral testing, all rats are habituated to the testing environment, often a perspex observation arena (50–60 cm diameter), for 5 min prior to each trial. Generally, behavioral tests occur between 4 and 8 h after the onset of the dark phase of the light-dark cycle. Following habituation, a receptive female

(usually OVX with hormonal priming) is introduced in the observation arena and copulatory behavior is generally assessed for 30 min. Baseline ejaculatory latencies (ejaculatory latency time, ELT) can be assessed (time taken for rat to ejaculate, from first intromission, following addition of a receptive female into the arena). ELT, similar to humans, varies considerably but is generally fairly reproducible for each animal from study to study. It is possible to reduce the variability in ELT by focusing on a subset of animals, which could be described as rapidly ejaculating animals (ELT < 300 s) proceeding to the main study. There has been some debate as to whether this could be a model of premature ejaculation. Alternatively, rats that show prolonged ELT (>1200 s) can be used to investigate the effects of prosexual pharmacological agents, and these sluggish rats may be considered as a model of delayed ejaculation.

Within 3–4 min after placing the male in the arena, a receptive female is introduced into the arena and the following behavioral parameters noted:

1. *Mount latency (ML)*. The time between addition of a receptive female into the arena and the first mount – a biomarker of sexual drive/desire/libido. A mount is when the male rat mounts the female from the rear and grasps her flanks with his front feet.
2. *Mount frequency (MF)*. The number of mounts to ejaculation (there is no vaginal penetration during a mount – a biomarker of sexual drive/desire/libido).
3. *Intromission latency (IL)*. The time between addition of a receptive female into the arena and the first intromission – a biomarker of sexual drive/desire/libido. An intromission is when the male achieves vaginal penetration during a mount.
4. *Intromission frequency (IF)*. The number of intromissions to ejaculation – a biomarker for erectile function.
5. *ELT*. Time taken from first intromission (vaginal penetration) to ejaculation. Following a mount or intromission, the male usually dismounts from the female, and at the end of a series of intromissions, the male ejaculates. Ejaculation is readily identified by a prolonged intromission, often with energetic thrusting and a slower withdrawing 'crucifix' posture postejaculation.
6. *Copulatory efficiency (CE)*. Ejaculatory latency/the number of intromissions to ejaculation, that is, the number of seconds between intromissions – a biomarker of sexual drive/desire/libido.
7. *Intromission ratio (IR)*. The number of mounts with intromissions divided by the total number of mounts with and without intromission – a biomarker of erectile function/dysfunction.

There are no approved therapies for the treatment of rapid or premature ejaculation. Currently, the only available treatments for premature ejaculation are the off-label

use of selective serotonin transporter inhibitors (SSRI's) and tricyclic antidepressant drugs, and/or behavioral therapy. Behavioral counselling does provide short-term symptomatic relief for some patients; however, long-term relapse rates are high. The clinical efficacy of on-demand SSRI's is frequently debated and a more robust delay in ejaculatory latency is observed after daily dosing. SSRI's, such as paroxetine and sertraline, have been shown to increase ejaculatory latency in rodents on acute administration and greater increases in ejaculatory latency are observed after chronic dosing. However, the doses used are proportionately significantly higher than those approved in humans.

Penile Erection

Assessing penile erection in conscious animals is far more complex than behavioral copulatory studies and to date erection has been routinely assessed by quantification of intracavernosal pressure in terminally anesthetized animals following electrical stimulation of the nerves that innervate the penis, that is, pudendal, cavernosal, or dorsal penile nerves and by direct electrical stimulation of brain centers that control erection, for example, medial preoptic or paraventricular nuclei of the hypothalamus. In conscious animals, two techniques have been employed to assess erections, and these possess an advantage over anesthetized models in that ascending and descending neuronal pathways are left intact. Additionally, the animal can be studied in its home cage, which helps to avoid any secondary behavioral influences such as anxiety. The two main quantification techniques are:

1. *Observational quantification of the number of penile erections.* Seeing rodent erections directly is difficult, but generally erection is followed by distinct genital grooming which is easy to observe and count.
2. *Radiotelemetric recording of intracavernosal or intraspengiosal pressure (ICP or ISP) during penile erection.* Penile erection is a hemodynamic effect and the hardness is produced by large increases in cavernosal/spongiosum blood pressure within the constricted cavernosal bodies (**Figure 3**).

Once the method to quantify penile erection has been determined there are a number of experimental paradigms that can be used to initiate a sexual response. These can include the use of pharmacological agents and behavioral paradigms where the male rats are kept in isolation in the presence of a female partner whom he cannot access, that is, noncontact erection, and if ICP/ISP radiotelemetry is being used during copulation itself with a receptive female animal. During 'pharmacological agent-induced erections,' the test compound is administered to male rats in the absence of a female and the animal is monitored for erectile events over a period of time depending on the pharmacokinetic properties of the agent. Active agents in this test are mostly centrally acting (spinal and/or supraspinal), such as serotonin 5HT2C agonists (e.g., m-CPP), dopamine agonists, such as apomorphine, or melanocortin agonists, such as bremelanotide or MB243 ((2S)-N-[(1R)-2-[4-cyclohexyl-4-[[[(1,1-dimethylethyl)amino]carbonyl]-1-piperidinyl]-1-[(4-fluorophenyl)methyl]-2-oxoethyl]-4-methyl-2-piperazinecarboxamide). Care must be taken when performing these type of studies as handling rats or the presence of

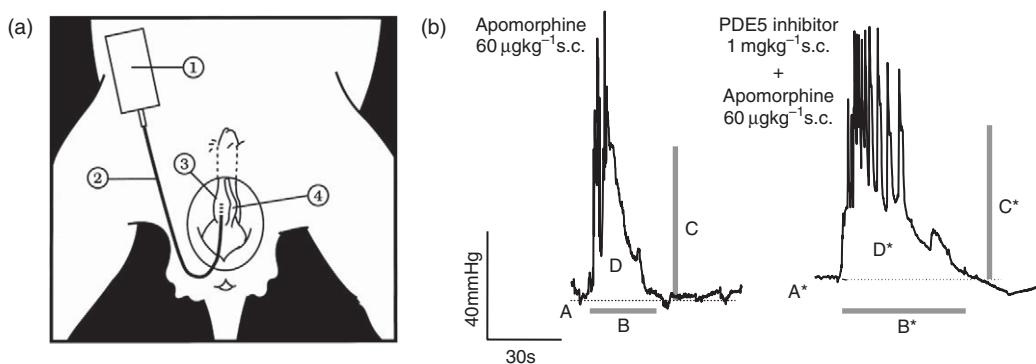


Figure 3 Radiotelemetric recording of rodent penile erection (a) implantation of radiotelemetry device and cannulation to cavernosal/spongiosal body. From Bernabé J, Rampil O, Sachs BD, and Giuliano F (1999) Intracavernous pressure during erection in rats: An integrative approach based on telemetric recording. *American Journal of Physiology* 276: R441–R449. Ventral view of rat pelvic area. Device (1) is subcutaneously located. Open-tip catheter (2) is inserted in corpus cavernosum (3). Dashed line in circled area is intracavernosal route of catheter. Corpus spongiosum (4) is laterally shifted to correctly expose corpus cavernosum during surgery. (b) Intracavernosal pressure (ICP) traces illustrating apomorphine-induced penile erection, in the presence and absence of a PDE5 inhibitor. Typical measurements include (A) basal ICP, (B) duration of erectile response (C) increase in ICP (a measure of erection hardness), and (D) area under ICP time response curve. In this study, apomorphine induced erectile responses, and the duration of the erectile response and the area under ICP curve were increased in the presence of a PDE5 inhibitor (B* and D*), while the amplitude and baseline ICPs were largely unaffected (A* and C*). There were no erectile responses following treatment with a PDE5 inhibition alone.

female odors can induce spontaneous erections. The disadvantage of this type of model is that the erection is directly induced by drug treatment. While this has a positive end result (erection), it is not linked to sexual motivation, which would certainly be preferred for treatment of human erectile disorders.

The ‘noncontact erection model’ uses a female stimulus animal to increase sexual arousal resulting in ‘natural’ erections. A male rat is placed in the presence of an estrous female rat in a cage or observation chamber divided into two halves. Direct physical contact is prevented by the partition wall. The Perspex or plastic/metal mesh dividing wall still allows the passage of auditory, visual, and olfactory cues and it is these that elicit a genital response from the male. The number of erections or changes in ICP/ISP can then be quantified over a set period of time.

A third method to induce erection in conscious animals is through tactile stimulation, and these erections are often referred to as ‘*ex copula* penile erections.’ The rat is restrained, lying on its back, and a tactile reflexive mechanism is triggered by making physical contact with the skin surrounding the penis. This response is believed to be under the influence of forebrain regions of the brain, and centrally acting agents, such as MB243, have been shown to enhance the number of excopula erections.

In the last 10 years, the treatment of impotence or ED has been revolutionized by the development and approval of selective PDE5 inhibitors, such as sildenafil, tadalafil, and vardenafil, and these agents have become the gold standard in ED therapy. Interestingly, PDE5 inhibitors have very little effect in normal conscious rats, and preclinically the effects can only be observed using anesthetized preparations, where ICP/ISP are assessed following nerve stimulation. This suggests that these agents are facilitators of erectogenic mechanisms, as clearly illustrated above, when a PDE5 inhibitor facilitated the proerectile effects of an inducer of erection, yet had no direct effect by itself (**Figure 3(b)**). Novel, centrally acting agents are currently being developed as future therapies for ED; and the conscious models described here may prove highly valuable in the

assessment of inducer mechanisms that initiate proerectile effects. Moreover, conscious animals are useful for the simultaneous determination of therapeutic windows between efficacious doses and centrally mediated side effects, for example, nausea/vomiting, hemodynamic effects, or sedation/somnolence or ataxia.

See also: Hormonal Contributions to Arousal and Motivation; Hormones and Female Sexual Behavior; Impulsive–Compulsive Sexual Behavior; Male Sexual Behavior; Mating Behavior; Pleasure; Sex Hormones, Mood, and Cognition; Sexual Motivation; Stress and Arousal/Sleep.

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Animal Tests for Anxiety

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Glossary

Antidepressants – Drugs traditionally used for the clinical management of major depressive disorder and, increasingly, the anxiety disorders. These include first-generation compounds (monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs)) and second-generation compounds (serotonin-selective reuptake inhibitors (SSRIs), noradrenaline-selective reuptake inhibitors (NARIs), noradrenaline and serotonin reuptake inhibitors (NSRIs), reversible MAOIs, atypical antidepressants).

Anxiolytics – Drugs traditionally used for the clinical management of anxiety disorders, including the benzodiazepines, beta-blockers, and serotonin (5-HT)_{1A} receptor partial agonists (e.g., buspirone). The related term ‘anxiogenic’ refers to treatments that induce and/or increase anxiety.

Benzodiazepines – A group of drugs originally developed in the late 1950s and which work by facilitating the function of GABA_A receptors in brain. Benzodiazepines (such as Librium® and Valium®) have a limited therapeutic spectrum (primary clinical application in the treatment of generalized anxiety disorder) and a variety of acute and chronic side-effects.

Buspirone – A serotonin (5-HT)_{1A} receptor partial agonist, buspirone is the only truly novel anxiolytic drug to have received regulatory approval in the last 45 years and is believed to work by reducing 5-HT activity in the forebrain. Although equi-effective with benzodiazepines in clinical trials, buspirone is relatively ineffective in benzodiazepine-experienced patients and, like SSRIs, suffers a therapeutic delay.

Cognitive behavior therapy – Commonly known as CBT, this is a form of psychological therapy widely used in major depression and the anxiety disorders. It is based on the combined use of conditioning techniques to alter behavior and psychotherapeutic techniques to alter negative thinking. In clinical trials, CBT and drugs appear equi-effective although CBT has a better relapse profile. Combination treatments are increasingly being advocated in clinical practice.

DSM – The accepted abbreviation for the *Diagnostic and Statistical Manual of Mental Disorders*, published by the American Psychiatric Association, and now in its fourth and text-revised edition (i.e., DSM-IV-TR (2000)). Alongside ICD-10 (*International Classification of Diseases*, published by the World Health Organization

(WHO), DSM is the major diagnostic/classificatory source in modern psychiatry.

Endophenotype – Coined by Gottesman and Shields some 35 years ago, the term endophenotype refers to an internal, intermediate phenotype (e.g., neuroanatomical, neurophysiological, biochemical, perceptual, cognitive, motivational, and/or affective) that is not obvious to the unaided eye but which fills the gap in the causal chain between genes and distal diseases.

Panicolytics – Drugs used specifically for the treatment of panic disorder as distinct from other subtypes of clinical anxiety. These agents include the first- and second-generation antidepressants, a pharmacotherapeutic profile fundamental to the DSM-IIIR classification of panic as a clinical condition distinct from other anxiety disorders. The related term ‘panicogenic’ refers to treatments that induce/increase panic.

Over the past three quarters of a century, numerous animal tests have been developed to enable and facilitate preclinical research on anxiety and its treatment. These procedures typically involve the exposure of laboratory rodents to stimuli (threats) that provoke physiological and/or behavioral responses (defenses) similar, if not identical, to those seen in anxious or frightened humans. This strong cross-species correspondence in the overt expression of anxiety/fear not only provides high face validity for the animal tests but is also consistent with evidence that the neural circuits for detecting and responding to danger have been highly conserved in evolution. Although widely referred to animal ‘models’ of anxiety, it is important to note that this terminology is simply a convenient (although, as we shall see, misleading) shorthand. Quite apart from problems surrounding the implied unitary nature of anxiety, these tests are by definition limited to physiology and/or behavior and do not access the subjective experience of anxiety. The reader is therefore asked to bear in mind that, as commonly used, animal models of anxiety are really no such thing; rather, they are tests for anxiety-like physiological and/or behavioral responses in animals. Models are something altogether different. Despite this caveat, preclinical tests have been invaluable both in advancing our understanding of the basic neurobiology of anxiety and in laying the foundations for the treatment of its disorders.

Anxiety and its Disorders

Anxiety can be both normal and abnormal. Normal anxiety is adaptive insofar that it helps organisms to cope with a wide variety of threats using a range of stimulus- and context-specific defenses. Pathological or maladaptive forms of anxiety are defined as a marked, persistent, and excessive/unreasonable fear that is experienced to a degree that significantly impairs quality of life. Such disorders of anxiety are thought to reflect the exaggeration and/or inappropriate activation of normally adaptive defenses. It should therefore come as no surprise that the major diagnostic tools in psychiatry (e.g., DSM-IV-TR) recognize multiple disorders of anxiety including generalized anxiety disorder (GAD), panic disorder (PD), agoraphobia, posttraumatic stress disorder, obsessive-compulsive disorder, specific phobia, and social phobia.

Until quite recently, many of these illnesses have either been untreatable or treatable only by psychological techniques, for example, behavior therapy and cognitive behavior therapy (CBT). The historical exceptions to this rule have been GAD and PD, the former having been successfully treated since the late 1950s by benzodiazepines (e.g., Librium® and Valium®) and the latter since the 1980s by first- and then second-generation antidepressants (e.g., MAOIs, TCAs, and SSRIs). Indeed, this pharmacotherapeutic distinction formed a (if not the) core element of the case for the nosological separation of GAD and PD in earlier versions of DSM. More recently, however, things have begun to change. Stemming from clinical dissatisfaction with the acute (e.g., sedation and cognitive impairment) and chronic (e.g., tolerance and dependence) side-effects of benzodiazepines, the search for safe and effective alternate medications has led to identification of the broad-spectrum efficacy of SSRIs – not only in major depression and PD but also in other anxiety disorders such as GAD and social phobia. Indeed, with the exception of short-term crisis management (for which benzodiazepines are still recommended), these developments have culminated in the widespread acceptance of SSRIs as the first line treatment for virtually all anxiety disorders. As will become apparent, these advances present both a considerable challenge and a major opportunity to those engaged in preclinical research.

Animal Tests for Anxiety: Applications and Validation

Tests involving physiological and/or behavioral observations in animals have long been employed in the search for novel therapeutic agents (screening) and as a means to unravel the complex etiology and neural mechanisms of fear and anxiety (simulations). These applications are by no means mutually exclusive in that the same test may be

used for several purposes while advances in our basic understanding of the causes and mechanisms of anxiety disorders should, at least in principle, inform refinement of the basic screening tests. Over 30 animal tests for anxiety, mostly behavioral, have been developed since the invention of the classical open field and conditioned emotional response tests in the 1930s and 1940s. Before examining some of the procedures in some detail, it is pertinent to ask why they are generically referred to as tests for anxiety.

In contrast to tests that bear little or no resemblance to the condition in question ('predictive only' tests such as antagonism of drug-induced seizures), most behavioral tests for anxiety possess a reasonable degree of face validity. In other words, as already noted, the fear/anxiety responses displayed by rodents and other nonhuman mammals appear analogous with human reactions to similar circumstances. While analogy is by no means indicative of homology (i.e., common origin; construct validity), these tests share another common property – namely, sensitivity to established anxiolytics and, in particular, to benzodiazepines such as Librium® and Valium®. Historically, this criterion (known as predictive or pharmacological validity) has been the 'gold standard' in establishing the credentials of a given procedure as a test relevant to anxiety as opposed to depression or schizophrenia. However, there are several disadvantages in using this criterion or at least this criterion alone. First, there is the risk of simply rediscovering the wheel ('groundhog day'), that is, identifying me-same compounds as opposed to those with a truly novel mode of action. Second, there is very real possibility that benzodiazepine validation produces models not of anxiety-like responses but of benzodiazepine (or, more correctly, GABA_A receptor-related) psychopharmacology. For example, the test may just happen to induce/elicit a benzodiazepine-sensitive subtype of anxiety-like behavior or, worse still, some unrelated facet of behavior that just happens to be sensitive to drugs with this mechanism of action. Third, as traditionally employed, predictive validity is curiously retrospective in that it involves detection of compounds with already known clinical indications. Such pitfalls were recognized long after the introduction of most currently available tests, a chronology that undoubtedly goes some way toward explaining the relative insensitivity of these tests to more recently developed anxiolytics such as buspirone.

Animal Tests for Anxiety: Classification and Overview

The conventional grouping of animal tests for anxiety is into two broad categories involving either unconditioned or conditioned defense responses. For reference purposes,

Table 1 Defensive behaviors commonly displayed by mammals, including laboratory rodents

<i>Behavior</i>	<i>Brief description and eliciting stimuli/contexts</i>
Freeze	Sudden and complete cessation of all movement except respiration (e.g., pain)
Startle	A reflex twitch or jump (e.g., sudden noise or tactile stimulation)
Bury	Covering of noxious object with substrate (e.g., shock prod)
Flee	Running away from source of danger (e.g., approach by predator or aggressive conspecific)
Threat and attack	Rearing to face threat, vocalizing (sonic) jumping, and biting (e.g., cornered by predator or aggressive conspecific)
Tonic immobility	Death feigning: inhibition of all reactivity including pain (e.g., captured by predator or persistent conspecific attack)
Ultrasonic vocalization (USV)	Vocalizations in ultrasonic range for humans (18–25 kHz), for example, pain (adults), separation from mother (pups)
Thigmotaxis	Staying close to vertical surfaces (wall-hugging), for example, potentially dangerous (unfamiliar) environments
Risk assessment	Information-gathering (stretched attend postures), for example, potentially dangerous (unfamiliar) environments

Table 1 summarizes the most common defensive behaviors in mammals and the contexts in which they can most readily be observed, while **Tables 2 and 3** summarize many of the more widely used animal tests for anxiety. Irrespective of classification, all tests require careful attention to alternative explanations of treatment effects on the main dependent measures (i.e., the fear/anxiety responses). Control data (from within the same test or independent tests) are invariably necessary to rule out indirect drug effects mediated via changes in locomotor activity, motor coordination, pain sensitivity, hunger, thirst, learning and memory, and body temperature. Some of these controls (e.g., locomotion) are test-general while others (e.g., pain sensitivity and body temperature) are very much more test-specific.

Tests of Unconditioned Behavior

A wide variety of unconditioned behaviors (and a smaller number of unconditioned physiological responses) have been used as tests for anxiety (**Table 2**). These may be

grouped into six broad categories which, conveniently, divide equally into those involving response emission (aggression, antipredator behavior, and somatic stress responses) and those involving response suppression (consummatory, exploratory, and social behaviors). An anxiolytic-like response in the former is generally indicated by a reduction in the dependent measure/s and, in the latter, by a disinhibition of the dependent measure/s.

Aggressive Behavior

The earliest documented behavioral effect of the benzodiazepines was their ability to calm aggressive zoo animals. Unsurprisingly, therefore, inhibition of aggressive behavior has since been used by some to screen for anxiolytic-like activity in laboratory rodents. While there is little doubt that diverse anxiolytics can and do inhibit aggression (particularly its defensive forms), anti-aggressive activity is by no means limited to drugs of this type. Furthermore, paradoxical pro-aggressive effects have quite frequently been reported with benzodiazepines both in animals and humans. For these reasons, a

Table 2 Common animal tests for anxiety based on unconditioned responses

<i>Type of test</i>	<i>Examples</i>	<i>Typical anxiolytic-like response</i>
Aggressive behavior	Isolation-induced fighting	Reduction in offensive elements of the behavioral repertoire
Antipredator behavior	Fear (FDTB) and anxiety (ADTB) defense test batteries	Reductions in subcomponents of the defensive repertoire (defensive threat, biting, risk assessment) but not freezing or flight
Consummatory behavior	Food consumption in novel environments	Increased consumption of preferred food in novel (but not home) cage
Exploratory behavior	Open field test; elevated plus-maze; elevated zero-maze; light/dark exploration; free exploratory task	Increased exploration of potentially dangerous open/brightly lit areas of environment
Social behavior	Rat social interaction test; separation-induced ultrasonic vocalizations (USV)	Increased conspecific social interaction under brightly lit unfamiliar test conditions; reduction of separation USV
Somatic stress responses	Responses elicited by aversive stimulation: hypertension, hyperthermia, elevated plasma corticosterone, and USV	Reductions in all stress-elevated parameters

Table 3 Common animal tests for anxiety based on conditioned responses

Type of test	Examples	Typical anxiolytic-like response
Aversive brain stimulation	Electrical or chemical stimulation of the dorsal periaqueductal gray (dPAG)	Reduction of stimulation-produced defense responses (freezing or escape); elevation of stimulation thresholds for such responses
Conditioned taste aversion	Biologically prepared learning: avoidance of novel flavors as consequence of prior association with illness-inducing chemical (e.g., lithium)	Attenuation of the avoidance or aversion
Defensive burying	Biologically prepared learning: burying of noxious objects (e.g., shock prod)	Reduction in the burying response and disinhibition of approaches to noxious object
Drug discrimination	Two-choice lever task; animals trained to selectively respond to drug cue on one lever, saline on other	For animals trained on anxiolytic – generalization to drug lever; for animals trained on anxiogenic – suppression of responding on drug lever
Pavlovian (defensive) conditioning	Conditioned emotional response (CER); fear-potentiated startle (FPS)	Inhibition of freezing or exaggerated startle responses elicited by presentation of a cue previously paired with aversive footshock
Punishment procedures	Four-plates test; Geller-Seifter conflict; passive avoidance; Vogel conflict	Disinhibition of behavior (locomotion, lever-pressing, exploration, licking) suppressed by electric shock or cue previously paired with shock

reduction in aggressive behavior cannot be used as a definitive marker of anxiolytic activity.

Antipredator Behavior

Over the past 20 years, pioneering research on the anti-predator defenses of wild and laboratory rodents has culminated in the Fear/Defense Test Battery (FDTB) and the Anxiety/Defense Test Battery (ADTB). Each battery comprises a number of subtests designed to elicit in a controlled and predictable fashion specific components of the defensive repertoire. The FDTB elicits defensive responses (e.g., avoid, freeze, flee, defensive threat and attack) to explicit, proximal, imminent, and unambiguous threat stimuli, whereas the ADTB elicits defensive responses (hypervigilance and risk assessment) to potential, distal, and ambiguous threats. Anxiolytics (benzodiazepines and buspirone) consistently inhibit defensive threat and attack as well as risk assessment whereas, mimicking the clinical situation, panicolytics acutely enhance but chronically suppress flight responses. In each case, changes in defensive behavior can be differentiated from more general behavioral effects.

Consummatory Behavior

Alongside anti-aggressive activity, an enhancement of food (and fluid) intake was one of the earliest behavioral effects observed with benzodiazepines. Initially reported in rats and dogs, such effects have since been documented in species as diverse as pigeons and humans. Although hyponeophagia (an inhibition of novelty-suppressed food intake) is still used in some laboratories as a test of

anxiolytic-like activity, the fact that anxiolytics increase the consumption of familiar foods in sated animals tested in their home cages would suggest a direct stimulatory effect on appetite. Indeed, there exists a huge anxiety-independent literature on the effects of benzodiazepines and related GABA_A receptor compounds on appetite; interestingly, 5-HT_{1A} receptor agonists and partial agonists (e.g., buspirone) also potently stimulate food intake. Although some of these concerns can be addressed by contrasting treatment effects on food intake in novel and familiar surroundings, inhibition of the hyponeophagia response as a pure and simple index of anxiolytic-like activity is fraught with interpretative difficulties – a situation made worse by the fact that hyperphagia is also seen in response to antipsychotics and, in particular, atypical antipsychotics such as clozapine and olanzapine.

Exploratory Behavior

Tests of this type originated with the development of the open field test in the 1930s. Now including behaviorally very much more sophisticated procedures such as the elevated plus-maze, its derivatives (such as the elevated zero-maze) and the light/dark exploration paradigm, these tests are based on the natural tendency of rodents to avoid open and/or brightly lit environments. Placed directly into the test apparatus (i.e., forced exposure), the animal can choose to spend its time in relative safety (near the walls of the open field, in the closed arms of the plus-maze, or the dark compartment of the light/dark apparatus). Alternatively, it may explore potentially more dangerous areas of the apparatus (center of the open field, open arms of the plus-maze, light compartment of

the light/dark apparatus). Anxiolytics of many types (except buspirone and antidepressants) reduce avoidance of the danger areas in a behaviorally selective manner. As clear evidence of bidirectional sensitivity, many anxiogenics produce the opposite behavioral signature of enhanced avoidance. The more recent adoption of behavioral (ethological), as well as conventional spatio-temporal, measures has not only enhanced test sensitivity but, ethically, has also facilitated the detection of behavioral selectivity without recourse to separate control tests. Little wonder then that exploration-based paradigms are currently the most widely used (if not necessarily the most popular) of all animal tests of anxiety, for example, some 3000 papers on the plus-maze been published in less than 25 years.

Social Behavior

Although several variants exist, by far the most common version of this procedure is the rat social interaction test developed in the late 1970s. It is based on the observation that levels of active social interaction between pairs of male rats vary as a function of familiarity with the test partner and lighting levels in the test arena. Social interaction is lowest in the high light-unfamiliar test condition and, independent of effects on locomotor activity, anxiolytics disinhibit social behavior under such aversive test conditions. In contrast, anxiogenics inhibit the higher levels of social interaction obtained under less aversive (low light-familiar) test conditions. An alternative test of social interaction revolves around mother–infant interaction, has been used with mice, rats, and guinea pigs, and is based on the observation that young pups emit ultrasonic distress vocalizations (USVs) when separated from their mother/nest/littermates. Somewhat exceptionally among the multiple animal tests for anxiety, these USVs are suppressed not only by all classes of anxiolytic, but also by certain antidepressants. However, as separation-USVs are proximately caused by the drop in body temperature that accompanies removal from the nest and contact with cohabitants, controls for potential treatment effects on core body temperature *per se* are essential.

Somatic Stress Responses

Physical stressors (e.g., restraint or electric shock) and/or psychological stressors (e.g., social defeat) are widely known to elevate blood pressure, heart rate, body temperature, and plasma corticosterone levels. Although such effects are sensitive to anxiolytics of various classes, they are also reduced by other psychotropic drugs and cannot, by themselves, be used as reliable indicators of anxiety. The one probable exception is stress-induced hyperthermia, a test developed in mice and which appears to show good sensitivity both to conventional and novel

anxiolytics. However, since intrinsic hypothermic (as opposed to anxiolytic) effects could account for any reduction in stress-induced hyperthermia, controls for treatment-induced changes in core body temperature are once again obligatory.

Tests of Conditioned Behavior

Given its potency in eliciting defensive reactions, it is unsurprising that electric shock has figured so prominently in traditional procedures used to study learned fear and anxiety in laboratory rodents (**Table 3**). However, the precise reaction observed (e.g., flinch, startle, jump, or run) will depend upon specific features of the test situation, including shock parameters and size of the test apparatus. Indeed, the categorization of animal tests for anxiety into those involving either response suppression or response emission nicely reflects the basic nature of behavioral defense, that is, passive (e.g., freezing) versus active (e.g., flight or startle).

Aversive Brain Stimulation

Electrical or chemical stimulation of dorsal aspects of the periaqueductal gray matter (dPAG) of the midbrain elicits abrupt escape/flight reactions in rats and intense fear reactions in humans, both accompanied by strong autonomic activation. Unsurprisingly, therefore, strong parallels have been drawn between the effects of dPAG stimulation in rats and the symptoms of panic attack in humans. Pharmacologically, dPAG-induced flight is inhibited by panicolytic agents (e.g., SSRIs) and exacerbated by panicogenic agents (e.g., caffeine and yohimbine). However, it is curious that the inhibitory effects of antipanic drugs on flight are seen with acute administration, whereas, clinically, these agents are effective only after chronic treatment. In fact, acute SSRI treatment is frequently reported to intensify the symptoms of panic in patients. Furthermore, some panicogenic drugs actually inhibit flight (e.g., mCPP), while others (e.g., CCK-4) have little/no apparent effect. Nevertheless, as very few animal tests for anxiety purport to specifically address panic disorder, it would be foolish to exclude dPAG stimulation as a potentially useful procedure. Indeed, this test has very successfully been employed in delineating the midbrain neurochemistry and neural circuitry of defense.

Conditioned Taste Aversion

Rats are biologically predisposed (prepared) to more readily make certain associations than others. The earliest example of this biologically adaptive learning

phenomenon is referred to as conditioned taste aversion (CTA) whereby, on the basis of a single pairing, rats learn to avoid a novel taste (conditioned stimulus, CS) that has been followed by feelings of nausea (unconditioned stimulus, UCS). Numerous studies have shown that CTA can be blocked by a wide range of anxiolytics but not by non-anxiolytics. It has consequently been argued that CTA not only represents a more naturalistic version of traditional approach/avoid conflict (see below) but, since taste aversions are also common in humans, may also be a useful tool in translational research. However, there are some important constraints on this test. For example, a very wide range of drugs (paradoxically, including anxiolytics themselves) will support CTA when paired with novel flavors, begging the question as to exactly what is driving the learned response. Furthermore, as already noted, anxiolytics are renowned for their ability to increase fluid as well as food consumption. Therefore, in single-bottle tests, it could simply be the case that a suppression of CTA reflects a general dipsogenic (not anxiolytic) action of the drug. Although this difficulty can be circumvented using a two-bottle preference task (i.e., paired flavor vs. unpaired flavor), the effects of anxiolytics are not so clear-cut in this version of the test. Thus, despite strong claims for face, predictive, and construct validity, it would seem unwise to use CTA alone as a definitive screening test for anxiolytic-like activity.

Defensive Burying

Another rodent behavior that can be considered a biologically prepared form of learning is conditioned defensive burying, that is, the apparently natural tendency of rats, following initial contact with an aversive object, to bury that object using cage bedding or similar materials. Although the formal version of this test has been developed using a shock prod as the aversive object, identical behavior has been reported in response to sources of aversive air blasts, light flashes, and noxious odors. As for CTA, a single learning trial is sufficient for the emergence of the burying response. A large volume of empirical evidence indicates that conditioned defensive burying behavior in rats is rather selectively suppressed by anxiolytic agents, an action that cannot be alternatively explained in terms of motor impairment or analgesia. Understandably, ceiling effects render this test (and several other conditioned behavior tests) relatively unsuited to the study of anxiogenic manipulations, that is, it lacks bidirectional sensitivity. Overall, the defensive burying test is arguably more useful for research on the neural mechanisms of fear/anxiety than as a primary screen for anxiolytic drug action.

Drug Discrimination

Unlike other tests in this review, drug discrimination is not a test for anxiety *per se* but can legitimately be considered a test for the subjective effects of anxiolytic-like drugs. It rests on the fact that rats can be trained to discriminate the subjective (cue) effects of a training drug from those of placebo. This is typically achieved in a two-lever operant chamber where responding on one lever will only produce reward when the animal has been treated with drug while responding on the other lever will yield reward only under placebo. If rats have been trained to discriminate a chlordiazepoxide (i.e., Librium[®]) cue, then it would be expected that they would respond on the drug lever when tested with compounds producing the same subjective cue as the training drug, that is, varying degrees of cue generalization would be observed depending upon how closely the test drug resembled the training drug. In contrast, if rats have been trained to discriminate an anxiogenic cue (e.g., pentylenetetrazol, PTZ), then it would be expected that responding on the drug lever would be suppressed by an anxiolytic test drug, that is, antagonism of the PTZ cue. In both cases, behaviorally nonselective activity can be determined via monitoring treatment effects on responses on the placebo lever. Both sets of effect (i.e., generalization and antagonism) have been widely reported in the literature, but are largely limited to within-class comparisons (e.g., GABA_A receptor-related drugs). Nevertheless, recent research has confirmed the value of drug discrimination in differentiating compounds within a class, for example, drugs with selective actions on particular protein subunits (e.g., $\alpha 1$ vs. $\alpha 2/3/5$) of the GABA_A receptor complex. This is a significant development in view of genetic and pharmacological evidence for the involvement of $\alpha 1$ -subunits in the sedative effects of benzodiazepines and the $\alpha 2/3$ subunits in their anxiolytic actions.

Pavlovian (Defensive) Conditioning

An early version of this type of test was developed in the late 1930s from Pavlov's original research on defensive conditioning in dogs, and is generally known as the conditioned emotional response (CER) test. Sometimes also referred to as conditioned suppression, it is a simple form of associative learning in which rats are presented with an unavoidable electric shock (UCS) that is reliably preceded by a auditory or visual cue (CS). The subsequent presentation of the CS alone during a food-reinforced operant task suppresses lever pressing and this suppression is the CER. Despite continuing popularity with a number of research groups, the test is renowned for inconsistency. Other more recent versions of the test omit the operant component, relying instead on cue- or context-induced defensive freezing. Due to the relatively

unambiguous fear stimulus and response, cue- or context-induced freezing has been used with considerable success over the past two decades in delineating the neural and cellular mechanisms of fear learning and memory in laboratory rats. One particular variant of CER has, however, proven to be very useful both as a screening tool and as a means for exploring the neural basis of emotion. This test is widely known as fear-potentiated startle (FPS) and is based on the potentiation of the noise-induced startle reflex by a CS (light) that has previously been paired with aversive footshock. FPS, but not basal startle, is reliably blocked by conventional anxiolytic drugs and by buspirone but, like so many other tests, is relatively insensitive to antidepressants.

Punishment Procedures

These tests have in common the suppression of ongoing behavior by punishment. Passive avoidance is based on footshock-induced suppression of exploratory behavior but, due to the 24h interval between training and testing, is more useful in learning and memory research than as test for anxiety. The four-plate test involves the suppression of locomotor activity by electric footshock as subjects move from one quadrant of an metal-floored arena to another. Although several classes of anxiolytic are effective in increasing punished crossings, the test is still being validated as a screening test for anxiolytic-like activity. The more commonly employed Vogel conflict test uses water-deprived animals and is based upon the suppression of drinking by electric shock delivered via the spout of the water bottle. The Vogel test responds well to a broad range of anxiolytics and, with some modification of shock parameters and baselines, to anxiogenics. For obvious reasons, it is imperative to control for possible treatment effects on pain sensitivity. However, by far the most behaviorally and technically sophisticated of all punishment procedures is the ‘Geller–Seifter conflict test.’ Unlike the three other tests above, Geller conflict requires extensive training of the experimental animals prior to drug testing. This training involves food-deprived animals learning that lever pressing in the absence of a tone produces food reward (unpunished; CS⁻), whereas lever-pressing in the presence of a tone produces food reward plus electric footshock (punished; CS⁺). Trained to criterion, rats naturally suppress responding during CS⁺ periods. With the now almost inevitable exceptions of buspirone and antidepressants, anxiolytics release punished responding while a wide range of psychostimulants and analgesics are ineffective. However, as with any procedure based on food deprivation, electric shock and extensive training protocols, it is essential to control for possible treatment effects on primary motivation (in this case hunger and pain) and/or learning and memory processes. Furthermore, there is

very little evidence that animals well-trained in this protocol actually display any behavioral or physiological signs of fear/anxiety during CS⁺ presentation. Along with the extensive training involved, concerns such as these are undoubtedly responsible for the current unpopularity of Geller–Seifter conflict both as a screening test and a simulation.

Animal Tests for Anxiety: Relative Merits?

Aggressive behavior, comummatory behavior and certain somatic stress responses have quite limited value as animal tests for anxiety. Although conditioned taste aversion and drug discrimination procedures would also appear to have few merits in this specific context, such tests are not completely irrelevant to the understanding of anxiety and its treatment. For instance, major differences in the interoceptive cues provided by benzodiazepines and 5-HT_{1A} receptor partial agonists help to inform our understanding of patient noncompliance when switched from diazepam to buspirone. Nevertheless, such tests are demonstrably less relevant than others as tests for anxiety. On present analysis, the procedures that fare best in this regard are the unconditioned tests involving antipredator, exploratory and social behavior, and the conditioned tests based on aversive brain stimulation, defensive burying, CER, and punishment.

There is little to choose among the three most preferred categories of unconditioned test. All have equivalent face and predictive validity, and each involves comparable time and effort in data collection/analysis. Nevertheless, it is worth noting that, of all of these tests for anxiety, the elevated plus-maze and social interaction tests have been by far the most thoroughly validated (pharmacologically, physiologically, and behaviorally). Although the four categories of conditioned test are also reasonably equivalent in terms of face and predictive validity, several require extensive training and/or surgical preparation of the animals (e.g., FPS, Geller conflict, defensive burying, and aversive brain stimulation) and, as such, would not normally be considered frontline screening tests. In contrast, CER and Vogel conflict are much more user-friendly.

Traditional comparisons between tests of conditioned and unconditioned fear/anxiety behavior hold that the former permit much greater experimenter control over behavioral baselines and, through within-subject designs, the use of fewer animals. On the downside, and as already noted, they normally require considerable time for the training of subjects, necessitate food or water deprivation and/or use electric shock. Extensive control tests are therefore needed to negate the possibility that treatment effects on the measure of interest are merely secondary to alterations primary motivation (hunger, thirst, and pain) and/or learning and memory processes. By comparison,

Table 4 Some of variables affecting rodent performance in the elevated plus-maze and other tests of exploratory behavior

Species, strain, age, gender, and estrus
Housing conditions and light cycle
Prior handling and injection experience
Prior stress
Prior test experience (same or different test)
Adaptation to test laboratory
Time of testing and lighting level
Presence of experimenter in test room
Maze construction (e.g., opaque/transparent walls, open arm ledges)
Method of scoring (e.g., live, manual/automated, videotape/DVD)
Measures scored: conventional; ethological
Definition and validation of measures

Modified with permission from Rodgers RJ (1997) Animal models of 'anxiety': Where next? *Behavioural Pharmacology* 8: 477–496 and Carobrez AP and Bertoglio LJ (2005) Ethological and temporal analyses of anxiety-like behavior: The elevated plus-maze model 20 years on. *Neuroscience and Biobehavioral Reviews* 29: 1193–1205.

tests involving unconditioned behavior generally have a higher degree of ecological validity, are much less susceptible to confounds arising from interference with learning/memory, hunger/thirst, or nociception, and allow for the truly comprehensive 'behavioral profiling' of experimental interventions (i.e., a multivariate as opposed to univariate approach). On the debit side of the balance sheet, however, these procedures are notoriously more susceptible to variable baselines arising from a greater sensitivity to factors associated with the experimental animals, holding conditions and/or test procedures (**Table 4**).

In short, all tests for anxiety have advantages and disadvantages and, as such, no single procedure is better than any other as a screening tool or a simulation. The current domination of the literature by unconditioned tests (such as the plus-maze) undoubtedly owes more to practical than to theoretical considerations, that is, simplicity, economy, rapidity, and bidirectionality coupled with the absence of extensive training, food/water deprivation, and/or pain. The conclusion that no one test is objectively superior to any other should convince of the need for a broad-based multitest strategy incorporating both conditioned and unconditioned procedures. However, given the often profound influence of prior test experience on behavioral baselines and anxiolytic pharmacology, the advised multitest strategy should ideally not take the form of sequential testing of the same animals in a test battery. This advice is most apposite for those using animal behavior tests in drug discovery programs.

to their therapeutic benefits, these drugs had unwanted effects as well as a limited therapeutic spectrum. This realization in turn stimulated the search for novel molecular targets of relevance to anxiety and its treatment. In recent years, however, many authors (and CEOs of pharmaceutical companies) have commented ruefully upon the dearth of truly novel psychotherapeutic drugs to have emerged since the psychopharmacology revolution of the 1960s. Indeed, buspirone is the only really 'new mechanism' anxiolytic to have been marketed as such since the discovery of the benzodiazepines. Although many factors are at play, serious inadequacies in preclinical screening tests is chief among the suspected culprits for such lack of progress. The argument runs that were these tests actually 'fit for purpose' then many more novel anxiolytics would by now have made it from the laboratory, through clinical trials, and onto the market. This is not to say that preclinical tests have failed to identify potentially useful targets/agents but rather that these compounds have frequently failed to convince in human trials while (at least at the time of writing) none has gained regulatory approval. This profile indicates that, as used, existing animal tests for anxiety are prone to false positives. Unfortunately, as implied by their failure to detect the anxiolytic potential of buspirone and to display sensitivity to first- or second-generation antidepressants, these tests are also prone to false negatives. This particular form of 'bidirectionality' really does appear to be a most serious indictment of current methods.

Herein lies something of a paradox, in that the obvious shortcomings of animal tests as screening tools stand in marked contrast to their successes as simulations in helping to illuminate the basic neurobiology of emotion. Thus, over precisely the same timeframe (past 25 years), there has been truly astonishing progress in our

Animal Tests versus Animal Models

The introduction of benzodiazepines almost 50 years ago revolutionized the treatment of anxiety disorders. However, it gradually became apparent that, in addition

understanding of the neural circuitry of fear and anxiety as well as the diverse gene products (precursors, enzymes, transmitters, modulators, hormones, receptors, transporters, second messengers, etc.) via which such mechanisms function. This difference in pure versus applied progress emphasizes the substantial conceptual and practical differences between ‘animal tests’ on the one hand and ‘animal models’ on the other. Namely, the vast majority of currently available animal tests for anxiety are based upon quite normal behavioral responses to threat and not (at least not usually or indeed knowingly) upon some pathology or abnormality of these highly adaptive defense mechanisms. Our present understanding is of the former and not necessarily the latter, a situation that has arisen quite logically as a consequence of researching the perfectly normal defensive reactions of normal animals to various threats. However, clinical anxiety disorders are characterized as an exaggeration of normal reactions and/or their display in inappropriate circumstances (contexts). Thus, in anxiety patients, there may well be nothing fundamentally awry with the basic response systems (effector mechanisms) but rather with the upstream mechanisms of perception and appraisal, that is, those mechanisms responsible for the detection and evaluation of stimulus input and triggering the appropriate defense response. Referring to the benzodiazepine gold standard in test validation, the late Richard Lister remarked that “pharmacological validity alone does not make a test a model of anxiety” (1991, p.323). Despite extensive repetition over the past quarter century, we have yet to fully appreciate the implications of this insightful remark – tests for normal fear reactions are not models of fear psychopathology.

For many years, psychological research on anxiety and mood disorders has emphasized the importance of cognitive biases, for example, the tendency of anxiety patients to interpret even neutral stimuli as threatening/potentially threatening. A considerable body of literature now suggests that these cognitive biases operate below the level of conscious awareness and have their origins in genetic predisposition, epigenetics, or adverse life experience. In view of major recent advances in our understanding of gene–environment interactions, it will not be too long before the cognitive biases typical of, for example, generalized anxiety disorder are linked to the early adverse experiences of individuals with a specific version (polymorphism) of this or that gene. These fresh insights into etiology, which are already emerging in the literature (e.g., the importance of serotonin transporter (5-HTT) polymorphisms in coping with stress), should help create the necessary mindset change in those currently using animal screening tests. In essence, the problem with our existing animal tests is not so much with the tests themselves as it is with how they are used. If real

progress is to be made, this crucial point must be fully taken on board by those managing drug discovery programs.

Toward Viable Animal Models of Anxiety Disorder

Elsewhere in biomedical research, the term ‘model’ is used with reference to the induction in animals of a condition/state closely resembling a specific human disorder, for example, hypertension, stroke, and epilepsy. What is therefore required in anxiety research is a means (or several means) of ‘creating’ emotional reactivity outside the normal range that is, hyperanxiety profiles. Such profiles, whether due to genetic, environmental, or interactive factors, could then legitimately be referred to as animal models of abnormal anxiety. In terms of genetic models of high anxiety, there are numerous potentially useful rat lines (Maudsley reactive reactive and nonreactive; Roman high and low avoidance; Flinders sensitive and insensitive; high anxiety HAB and low anxiety LAB) and inbred mouse strains (high anxiety BALB/c and DBA/2 vs. low anxiety C57BL/6J). Furthermore, the vast majority of genetically engineered mice that have been phenotyped for anxiety-like behavior have been found to display high anxiety phenotypes relative to wild-type controls (e.g., 5-HT_{1A} and CRF2 receptor null mutants). On the other hand, considerable evidence has accumulated concerning enhanced adult levels of anxiety in animals that have experienced early-life maternal separation/neglect or, alternatively, some form of physical/ psychological trauma in adolescence or early adulthood. Unsurprisingly, perhaps, some genetic strains are already known to be more sensitive (susceptible) than others to such negative life experiences.

In essence, therefore, the model of anxiety disorder is the ‘susceptible’ animal, while the test for anxiety is essentially the means via which the anxiety-relevant response is elicited and quantified. Defined in this manner, models are clearly theory driven and, with their stronger etiological basis, have considerable merit in the context of contemporary translational research. These tools will be all the more convincing (certainly as models of trait as opposed to state anxiety) if they can be shown to display abnormally intense reactions to a range of (but perhaps not all) threatening stimuli/situations. This specific wording is not just insurance against the occasional awkward finding, but rather an awareness that the awkward finding (the exception) itself may provide much-needed clues as to the model’s relevance for a specific human anxiety disorder.

Table 5 Cardinal symptoms/endophenotypes of human anxiety disorders, possible animal analogues/homologs, and relevant animal tests

<i>Human symptom/endophenotype</i>	<i>Animal analog/homolog</i>	<i>Animal test</i>
Avoidance of potentially dangerous environments (generalized anxiety disorder)	Increased avoidance of exposed, well-lit areas	Exploration-based tests (e.g., open field; plus-maze; light/dark exploration)
Anxiety provoked by social situations (social phobia)	Low social interaction with unfamiliar nonaggressive conspecific	Rat social interaction test
Sudden and intense fear, leading to avoidance (panic attack)	Increased flight from predator	Rat or mouse fear defense test battery
Anxiety provoked by specific feared object (specific phobia)	Rapidly acquired avoidance of novel stimulus associated with pain or discomfort	Conditioned taste aversion (CTA)
Re-experiencing traumatic event leading to enhanced arousal and avoidance (posttraumatic stress disorder)	Increased freezing to fear-conditioned cue or context	Conditioned emotional response (CER)
Flashbacks of traumatic events (posttraumatic stress disorder)	Impairment in extinction of fear memory	CER extinction paradigm
Distress caused by separation from object of attachment (separation anxiety)	Ultrasonic vocalizations (USVs)	Maternal separation-induced distress USV in pups
Cognitive bias to weak/ambiguous threat cues	Increased fear conditioning to partial threat cue	Cue variation in CER
Heightened startle response in unfamiliar settings	Basal and potentiated startle	Fear-potentiated startle (FPS)

Modified from with permission Cryan JF and Hulmes A (2005) The ascent of the mouse: Advances in modelling human depression and anxiety. *Nature Reviews: Drug Discovery* 4: 775–790.

Models, Tests, and Endophenotypes

If, as strongly suspected, specific human anxiety disorders reflect dysfunction in different defensive subsystems, it follows that the validity and reliability of our animal tests will depend rather critically upon test selection and, in particular, the precise nature of the associated defensive reaction/s (see **Table 1**). As of now, those working in preclinical research are unanimous in accepting the sheer impracticality (never mind illogicality) of attempting to develop rodent tests that will feature all symptoms (i.e., the entire ‘syndrome’) of a particular human mental disorder. Mirroring pioneering animal research in the area of depression, which has concentrated upon tests of one or two cardinal features of the disorder (e.g., anhedonia, behavioral despair), several authors have more recently argued for adoption of a similar endophenotype-based approach to animal studies on anxiety. As originally conceived by Gottesman and Shields in the 1970s, an endophenotype is an internal phenotype (e.g., neuroanatomical, neurophysiological, biochemical, perceptual, cognitive, motivational, and/or affective) that is itself not obvious to the naked eye but which is manifest in certain response tendencies or biases.

Table 5 (with due deference to John Cryan and Andrew Holmes) exemplifies some cardinal features/endophenotypes of human anxiety disorders and suggests which animal defensive behaviors and tests may be most relevant to these endophenotypes. Combining this much more realistic approach to phenotyping with an understanding that

the model is the (genetically, epigenetically, and/or environmentally) altered animal while the test is a device to elicit and measure behavior comes close to a much-needed and long overdue paradigm shift in the preclinical pharmacology of anxiety. In view of emerging ideas concerning the close relationship between anxiety disorders and major depressive disorder, such an approach to animal modeling may help explain some long-standing conundrums. Not least the question as to why animal tests for normal anxiety, while exquisitely sensitive to benzodiazepines (and a very large number of other pharmacological and genetic manipulations) are so recalcitrant when it comes to 5-HT_{1A} receptor partial agonists and SSRIs.

See also: Active Avoidance and Escape Learning; Amnesia; Animal Models of Learning and Memory; Cognition: Learning and Memory: Pavlovian; Communication of Emotions in Animals; Comorbidity – Depression; Depression; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Effects of Stress on Learning and Memory; Emotion–Cognition Interactions; Emotions; Evolution of Emotions; Fear Conditioning; Fear, Anxiety, and Defensive Behaviors in Animals; Fear: Potentiation and Startle; Genes and Behavior: Animal Models; Human Fear and Anxiety; Maternal Deprivation; Motivation; Mouse Genetic Approaches to Psychiatric Disorders; Neural Bases of Defensive Aggression; Neural Substrates of Conditioned Fear and Anxiety; Neural Substrates of Unconditioned Fear, Defense, and Anxiety; Neuropsychological Aspects

of Anxiety Disorders; Novelty; Perinatal Influences on Behavior and Neuroendocrine Functions; Physical and Emotional Pain; Psychiatric and Substance Use Disorder Comorbidity; Stress and Emotionality; Value of Animal Models for Predicting CNS Therapeutic Action.

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Cognition: Attention and Impulsivity

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Glossary

Attention-deficit hyperactivity disorder (ADHD) – A developmental, behavioral disorder which comprises three main symptoms: inattention, hyperactivity, and impulsiveness. The basis for clinical diagnosis is typically guided by the fourth edition of the *Diagnostic and statistical manual of mental disorders* (DSM-IV) or the *International statistical classification of diseases and related health problems – 10th revision* (ICD-10). Although estimates vary, ADHD is thought to affect 3–5% of children under age seven. ADHD is less prevalent, in adulthood.

Covert attention – Refers to the ability to focus and cognitively process information of a particular perceptual feature and/or a particular spatial location without directing or orienting the sense organs to the source. It can be contrasted with overt attention in which the sense organs are reoriented. Covert and overt attention may be either reflexive or voluntary.

Divided attention – Refers to the ability to process and/or perform multiple perceptual and/or cognitive operations simultaneously.

Selective attention (also referred to as focused attention) – Refers to the ability to focus cognitive processing resources on a subset of available sensory and/or cognitive information, to the exclusion of other, potentially competing information.

Stop-signal reaction time (SSRT) – The principal measure of impulsivity in the stop-signal reaction time task which assesses the ability to cancel or stop a prepotent response already initiated and being executed. SSRT is defined as the latency to successfully stop, or complete the cancellation of, an ongoing response. As there is no clearly observable behavioral endpoint for the response inhibition process, SSRT cannot be measured directly and must be inferred or estimated from SSRT task data. In 1984, Logan and Cohen proposed a quantitative method for computing SSRT based on a horse race model in which it is assumed that constant-duration ‘stop’ and ‘go’ processes compete independently to determine the outcome of a given trial on the SSRT task.

Sustained attention (also referred to as vigilance) – Refers to the ability to maintain or continuously monitor perceptual and/or cognitive processing over a period of time. Vigilance may be subtly distinguished from sustained attention and is traditionally defined more

specifically as a state of readiness to detect and respond to unpredictable, rare events.

Temporal discounting (also referred to as delay discounting) – Refers to phenomena in which the effect of reinforcement, or subjective value of the reinforcer, tends to decline as a function of the delay to reinforcer availability. Typical functions used to model discounting behavior are exponential curves – in which the effect of reinforcement or subjective value declines by a fixed amount per unit time – or hyperbolic curves – in which the value of reinforcement or subjective value declines inversely proportional to delay. In hyperbolic discounting models, under certain parameters, a striking reversal in preference is predicted whereby preference shifts from a larger, delayed reinforcer to a smaller, more immediate reinforcer as the choice draws near.

Animal Models of Attention

Varieties of Attention

In a general sense, the term ‘attention’ refers to an enhancement in focus or limiting selection of sensory and/or cognitive processing, in spite of potential competing distractions, over a period of time. However, there is great diversity in opinion over issues such as attentional capacity (e.g., number of items, spatial size, or resolution), timescale (e.g., milliseconds to hours or longer), global and/or domain-specific attentional processing for different types of information (e.g., attending to a particular sensory input dimension Vs. attention during learning or performance output of a motor skill), and whether attention in these different contexts is implemented by distinct or overlapping psychological and neural mechanisms. Indeed, it has long been hypothesized that attention does not represent a single, unitary entity but is rather a concept which encompasses a variety of psychological processes. While many such hypothetical attentional processes have been developed primarily in conceptual terms, recent research is beginning to illuminate potential subdivisions of attentional processes based on neurobiological correlates and mechanisms. However, as yet, no universally accepted definition or taxonomy of attention has been achieved.

Despite the lack of definitional consensus, certain major categories of attention (e.g., selective, divided or sustained attention) have been identified which may represent somewhat independent processes. Moreover, it is clear that such attentional processes encompass and interact importantly with a number of cognitive and behavioral functions including learning and memory, executive control, decision-making, and consciousness. Disturbances in attentional processes are thought to underlie some of the dysfunctional symptoms associated with many neuropsychiatric disorders such as anxiety, depression, schizophrenia, Alzheimer's disease, and attention-deficit hyperactivity disorder (ADHD). Thus, the development and validation of animal models of attention could serve to enhance the characterization of neurobiological mechanisms of normal and disrupted attentional processing, and to facilitate the testing and development of therapeutic interventions. A variety of behavioral paradigms have been proposed to assess certain aspects of attention in nonhuman animals. These paradigms will be surveyed in the context of the broad categories of selective, divided, and sustained attention, with particular emphasis on the procedures most widely used, and which attempt clear, comparative links to attentional tasks in humans.

Experimental Paradigms

Selective attention

1. *Orienting*. The manner in which certain locations or objects are given priority over others epitomizes selectivity in processing of the visual environment, and is typically thought to engage attentional orienting processes. Such spatial selectivity may recruit a shift of the eyes and/or head to place the region of interest onto the foveal visual field (overt orienting), or may alter internal processing resources in the absence of changes in gaze direction (covert orienting). Whether orienting is overt or covert, its control may be further classified as being directed voluntarily (endogenous orienting) or reflexively by external stimuli (exogenous orienting).

A well-established paradigm for measuring visuospatial attention is the cued-target detection (CTD) task first developed for use in human subjects by Posner and colleagues. The main procedure requires that a subject fixate on a central point and produce a speeded motor response upon detection of a stimulus presented briefly within the periphery of the fixated visual field. An 'informative' cue to the location of the imminent target stimulus is typically presented 100–1000 ms preceding the onset of the stimulus and is presented either within the central visual field (endogenous cues – generally thought to promote endogenous orienting) or in the periphery (exogenous cues – generally thought to promote

exogenous orienting). The primary outcome variables of the CTD task are accuracy in visual localization or discrimination, and reaction time (RT) to respond. Cues that correctly predict the location of the target ('valid' cues) typically increase performance accuracy and decrease RT, whereas cues that predict an opposite location ('invalid' cues) decrease accuracy and increase RT – the difference in performance between valid and invalid trials is known as the 'validity effect'. Neutral cues, which precede the target but provide no information about its imminent location, may also decrease RT by generally preparing the subject for the target ('alerting effect') as compared to control trials in which no cue is presented.

The CTD paradigm has been adapted to examine attentional orienting across various species such as primates, pigeons, and rats. In procedural tests of covert orienting, rhesus macaques appear to consistently demonstrate validity effects in their attentional shifts to peripheral target stimuli in response to both exogenous and endogenous cues. However, compared with human subjects, rhesus monkeys typically show faster RTs, smaller validity effects that decline more rapidly, and are considerably less sensitive to changes in the probability of valid cues (in humans, the ratio of presented valid to invalid cued targets influences the magnitude of the validity effect), which may suggest some differences in the cognitive processes used by the two species within the paradigm. Nonetheless, several brain regions have been related to task performance in the primate that correspond well with data from human functional imaging and from brain-damaged individuals: the frontal eye fields and midbrain superior colliculus have been implicated in both overt visual saccades and covert attentional shifts, the lateral pulvinar of the thalamus has been implicated in attentional engagement at a new locus, and the posterior parietal cortex has been implicated in disengagement of attention.

Furthermore, investigations into the psychopharmacology of covert orienting indicates similar effects of certain neurotransmitter systems on task performance between primates and humans. For instance, lesions of the basal forebrain nuclei in monkeys, causing 20–70% reductions in choline acetyltransferase (ChAT) activity within several cortical areas, slows RTs to invalidly cued targets (with only moderate slowing of RTs to validly cued targets). This pattern of results is similar to that seen when rhesus monkey or human subjects are systemically administered the muscarinic antagonist, scopolamine (exhibiting slowed RTs for both valid and invalid cues while not affecting RTs in no-cue trials), or in patients with Alzheimer's dementia who have reduced cholinergic innervation of the cerebral cortices. These results may reflect impaired disengagement of attention from an invalidly cued location. Conversely, systemic administration of the cholinergic agonist, nicotine, in monkeys or

humans tends to speed RTs following invalid cues as compared to valid cues, resulting in a reduced validity effect. Manipulations of acetylcholine (ACh) do not greatly affect the alerting effect. The opposite pattern of results for validity and alerting effects appears to hold for norepinephrine (NE) or dopamine (DA). Low, nonsedative doses of clonidine or guanfacine, alpha-2 receptor agonists which act to reduce NE release, significantly reduce the alerting effect by selectively increasing RTs on double, neutral-cue trials relative to no-cue trials in both primates and humans. Antagonists of alpha-2 adrenoceptors, such as yohimbine or idazoxan, block these reductions in the alerting effect caused by clonidine. The D2 receptor antagonist, droperidol, also reduces the alerting effect in primates but appears to do so by selectively increasing RTs on no cue trials, with little to no effect on double, neutral cue trials. Taken together, converging results from both humans and primates strongly suggest a dissociation between CTD performance based on neurotransmitter function: the cholinergic system influences selective attentional orienting in space, but not alerting, while catecholamines alter alerting but not orienting.

Attempts to model orienting attention in the rat include: a two-choice lever task in which rats (located centrally within a central food well at the start of each trial) press the appropriate lever in response to brief visual targets located above them, preceded by an informative peripheral visual or auditory cue; and a task in nine-hole boxes in which rats withdraw their nose from a central hole and nose poke into an adjacent hole in response to a brief visual target, preceded by a informative peripheral visual cue. There are differences in performance between the rodent and human paradigms: in rats, cues appear to exert effects on target detection for a longer period than in primates or humans, and cues do not change their effect on target detection as a function of the probability of cue validity as observed in human subjects. Furthermore, rats are afoveate and their eye movements are neither monitored nor controlled, which complicate interpretations based on covert attention. However, studies using both rodent CTD task versions have demonstrated validity and alerting effects on response accuracy and RT, where the lever-based task in particular has included several parametric controls such as target salience, probability of target location, inclusion of neutral cue and no cue trials, and varying the probability of cue validity. Such controls suggest that task performance cannot simply be explained by nonattentive mechanisms such as stimulus additivity or response preparation, and thus reinforce explanations based on shifts of selective attention. Moreover, bilateral infusions of a selective cholinergic immunotoxin, 192 IgG-saporin, into the substantia innominata of rats, which causes substantial loss of cholinergic input to the cortex, results in decreased accuracy and RTs to an

invalidly cued target (but not on valid or no cue trials, e.g., not an alerting effect) compared with controls. Pharmacological manipulations within the rat CTD task are relatively few and are difficult to reconcile with data from humans: systemic injections of nicotine or haloperidol produce no significant changes in accuracy or latency, while scopolamine and clonidine reduce both response accuracy and increase RT.

2. Stimulus features and associative learning In addition to paradigms in which cues are presented prior to stimuli in order to attract or orient attention, there are paradigms in which attention is summoned or captured by the properties of stimuli themselves (e.g., brightness, shape, etc.) and their relation to outcome contingencies. Two prominent examples of such paradigms are latent inhibition and intradimensional/extradimensional (ID/ED) set-shifting.

Latent inhibition (LI) refers to the observation that nonreinforced presentations of a particular stimulus renders that stimulus less effective, as compared with a novel one, to subsequent associative conditioning. A typical LI procedure in animals includes three phases: a first phase in which an animal learns, or spontaneously performs, behaviors with a relatively high rate of occurrence (e.g., exploratory locomotion or lever pressing on a VI schedule for food or water) – it is during this first phase in which the to-be-conditioned stimulus (CS, e.g., tone or light) can be repeatedly pre-exposed; a second phase in which a Pavlovian conditioning procedure is employed in which the CS is paired with an unconditioned stimulus (US, e.g., shock); and a third phase in which the animal is re-exposed to the conditions of the first phase and, whilst the animal is behaving, the CS is presented. The extent of CS-US conditioning is assessed by the impact of CS presentation on ongoing behavior in the third phase. The typical LI effect is that animals pre-exposed to the CS tend to show reduced conditioning as compared to those not pre-exposed. Disruptions in LI are thus observed as exhibiting an enhancement in conditioning effects, which is convenient toward ruling out several types of nonspecific deficits in performance.

LI has been observed across a wide variety of behavioral paradigms and in many different species. One major proposed interpretation of LI is that it results from a stimulus-specific decline of attention as a function of repeated irrelevant CS pre-exposure, which reduces the subsequent associability of that CS. Based on such an interpretation, LI has been proposed as a model for attentional dysfunction in schizophrenia, that is, disruptions in LI represent a deficit in the ability to selectively screen out irrelevant stimuli (cf., to the pre-exposed CS). Patients with schizophrenia show disruptions in LI, which tend to be ameliorated by treatment with D2 dopamine receptor antagonists (e.g., neuroleptics). Correspondingly, dopamine receptor agonists that produce psychotic-like effects in humans produce

disruptions in LI in animals, which can be counteracted by administration of neuroleptics (which, in rats, also produce an increase in LI performance) at clinically relevant doses. It should be noted, however, that there is considerable theoretical debate over the attentional interpretation of LI, and it has proven difficult to distinguish this from other associative or learning explanations. Further research is needed to attribute LI more definitively with processes of selective attention.

The ID/ED set-shifting task is based on the premise that control over selective attention is required to optimize performance when different stimulus dimensions are specifically reinforced. In a typical ID/ED set-shifting task, a subject is presented with a series of compound stimuli comprised of several dimensions and is trained to discriminate between attributes which vary along one stimulus dimension (e.g., color or shape). It is believed that an attentional set is formed, rather than simple responding to alterations in different attribute reward contingencies, which can be illuminated by the subject's performance when confronted with discriminations involving novel compound stimuli. If the subject learns more readily to discriminate attributes within a previously reinforced dimension (ID shift) when that dimension remains reinforced as compared to discriminating attributes along a new dimension when a different stimulus dimension is reinforced (ED shift), it is concluded that the subject is selectively attending to the relevant stimulus dimension.

Variants of the ID/ED set-shifting task have been devised for primates, pigeons and rodents. In terms of translation, the ED shift is a main component of the Wisconsin Card Sorting Test, which has proven sensitive (ED shift disruptions) for patients with frontal lobe dysfunction as well as patients with psychiatric conditions such as autism, obsessive-compulsive disorder and schizophrenia. Correspondingly, it has been demonstrated that attentional set shifts require the dorsolateral prefrontal cortex of monkeys and the medial frontal cortex in rats. ED shifting appears to be differentially sensitive to selective lesions of cholinergic and dopaminergic projections to the prefrontal cortex in the marmoset. In rats, noradrenergic (NA), but not cholinergic, deafferentation produces attentional set-shifting impairments while leaving the ability to ignore irrelevant stimuli intact, suggesting that the attentional deficit results from an overly focused attentional state that retards learning when a new stimulus dimension becomes reinforced.

3. Prepulse inhibition. A widely-used model of preattentive or automatic processing is prepulse inhibition (PPI) of the acoustic startle response. The acoustic startle response is a widespread, reflexive muscular contraction in reaction to a sudden, loud auditory stimulus. PPI refers to the phenomenon in which a weaker (nonstartling) prestimulus (prepulse), presented 30–200 ms prior to a

startling stimulus, reduces the magnitude of the startle response. The prepulse is usually acoustic, but visual or tactile stimuli are also effective. Decreases in prepulse inhibition are typically ascribed to abnormalities in sensorimotor gating, interpreted as an inability to filter out unnecessary information. PPI may thus represent an automatic mechanism of selective attention which regulates reflexive responsiveness and may be most closely akin to concepts of alerting attention or motor readiness.

PPI has been observed in numerous species ranging from mice to humans. Reductions in PPI have been noted in patients suffering from psychiatric disorders such as schizophrenia or Alzheimer's disease, and in people subject to brain injury or surgical manipulation. Due to its ease of implementation, precision in the control of task parameters, and robustness and reliability of the response, PPI is a useful paradigm for probing the neurobiology and genetics of gating deficits in these disorders.

There is an extensive literature examining the neural and pharmacological bases of PPI. One obvious mediating factor is sensory threshold (animals that cannot detect the prepulse will not exhibit PPI). Beyond sensory threshold, both the acoustic startle reflex and PPI appear to be mediated by inputs to brainstem nuclei: particularly the ventral cochlear nucleus and nucleus reticularis pontis caudalis. The fact that PPI remains intact after acute trans-collicular decerebration in the rat suggests that the expression of acoustic PPI does not require the forebrain. However, PPI has also been observed to be modulated by various brain structures such as the hippocampus, ventral striatum, and medial prefrontal cortex (mPFC) across many different species. Moreover, infusions of D1/D2 dopamine receptor agonists such as apomorphine tend to disrupt PPI, and these disruptions can be remediated by a number of antipsychotic agents to an extent significantly correlated with their clinical potency.

Divided attention

It is generally assumed that organisms are limited both in their ability to monitor a number of simultaneous sensory inputs, as well as in their ability to perform concurrent responses (e.g., dual-task performance). Thus, the concept of divided attention suggests that rapid switches in attention between input or output channels are necessary to effectively deal with multiple, simultaneous demands. In humans, neuro-imaging studies of divided attention of bimodal sensory information have implicated recruitment of the middle-dorsolateral prefrontal cortex. These activations are distinct from those required for selective attention toward only one of the presented sensory inputs, which is achieved primarily via modulation of the sensory cortices. There have been several paradigms devised to measure divided attention in experimental animals. In one task, Robbins and colleagues devised a method analogous to a paradigm developed for human subjects where

rats were required to monitor and discriminate between two simultaneous, laterally presented, visual stimuli and respond specifically to the stimulus which terminates first. By manipulating the difference in the termination times, a minimum switching time could be inferred. Systemic administration of d-amphetamine produced a dose-dependent reduction in choice accuracy, which was blocked by depletion of dopamine in the nucleus accumbens. In another task, rats were trained to respond on one of two levers under a random ratio schedule of food reinforcement – the correct lever was redetermined before each response and was signaled by a light. Systemic d-amphetamine increased the proportion of responses made on the correct lever at low and intermediate doses, but reduced the proportion at a higher 3.2 mg kg^{-1} dose. A third protocol demanded that rats make a temporal discrimination of stimuli presented either alone or together. Rats with lesions of the frontal cortex (FC) or nucleus basalis magnocellularis (NBM) were able to time each stimulus when it was presented alone, but not when they were presented together. FC or NBM lesioned rats appeared to only time the intruding stimulus whilst ignoring the other, suggestive of an impairment in divided attention. Rats with lesions of the fimbria-fornix (FF) or medial septum (MS) performed the dual stimulus task normally, but exhibited a general failure to remember the duration of a stimulus, suggesting a failure of working memory. In the cross-modal divided attention task, rats are initially trained in consecutive operant auditory and visual conditional discrimination tasks. This is followed by trials comprising a sequence of stimuli from auditory and visual modalities as well as from different stimulus properties (flashing/pulsing or constantly turned on). Although response accuracy remained unchanged in the bimodal condition, response latencies were generally longer in comparison to unimodal trials suggestive of an increased switching cost. Both, the muscarinic receptor antagonist scopolamine and the benzodiazepine receptor agonist chlordiazepoxide (CDP), increased the response latencies, with scopolamine eliciting greater latency increases in the bimodal condition.

Sustained attention

In a general sense, sustained attention refers to the ability or capacity to maintain attention over some specified period of time and is a fundamental component of cognitive capacity in humans and other animals. Since the work of Mackworth in the late 1950s, the highly related concept of vigilance has been operationally defined, based on human task performance, as a state of readiness to detect and respond to unpredictable and rare events under conditions of relatively low arousal. Vigilance is thus quite difficult to sustain for prolonged intervals and typically results in decreased accuracy of signal detection and

increased latencies to respond over time. Performance on sustained attention or vigilance tasks appear to vary systematically in response to relevant parametric manipulations, which are believed to influence attentional capacity or load, including signal intensity (brightness or duration), trial rate, signal predictability in type or location, and demands on working memory. Two general classes of paradigms have been proposed as models of sustained attention: continuous performance tasks and tests of vigilance.

1. *Continuous performance.* Rosvold and colleagues first devised a task for human subjects in which decisions were continuously required in the detection of a target item. For instance, individuals were asked to press a key whenever they saw a target stimulus X. Many variations of the task have been developed, such as including one or more distractors (e.g., nontarget stimuli) or conditional rules (e.g., respond only to X if preceded by the stimulus A). A further variant was developed in which subjects had to continually monitor five distinct locations in order to detect a target stimulus. Carli and colleagues later developed the five-choice serial reaction time task (5-CSRTT) as a rodent analog of such human continuous performance tests of sustained attention.

On the 5-CSRTT, a large number (100 or more) of discrete consecutive trials are presented. Each trial is initiated when the subjects nose poke into a rear panel magazine tray. Subjects are then required to turn and wait during a short (e.g., 5 s) inter-trial interval (ITI) while scanning a horizontal array of five apertures on the front panel for the presence of a brief light stimulus. There are four possible response outcomes for each trial. Correct responses are recorded when animals nose poke in the aperture having the same spatial location as the brief visual stimulus, and earn food reinforcement delivered to the rear panel magazine tray. The next trial begins immediately upon food collection. Incorrect responses are recorded when subjects nose poke into a different spatial location as the brief light stimulus and are followed by a 5 s timeout period. Omissions are recorded if the animal does not nose poke into any of the front panel apertures within a short (e.g., 5 s) limited hold period after onset of the brief visual stimulus, and are followed by a 5 s timeout period. Premature responses are recorded if subjects respond during the ITI, prior to the presentation of the brief visual stimulus, and are also followed by a 5 s timeout period. For incorrect, omitted, and premature trials, the next trial begins when subjects nose poke into a rear panel magazine tray after the timeout period has elapsed.

A valuable feature of the 5-CSRTT is that it provides several relatively independent measures of attentional performance, including accuracy of target detection (defined as the ratio of correct to the total number of correct and incorrect responses), response speed as

well as response inhibition (a putative measure of impulsivity – see below). Furthermore, the task parameters can be manipulated in order to further evaluate and specify the characteristics of attentional performance within the 5-CSRTT. The stimulus duration or luminance can be altered to vary task difficulty (or attentional load) and to test for changes in sensory threshold. The ITI or the event rate can be altered or made variable such that animals can no longer rely on internal timing to control orientation and thus have to maintain readiness on a continuous basis. Distracting stimuli, such as bursts of white noise, can be used to concurrently assess selective attention. Motivational factors may be captured by examining food collection latencies; however, it should be noted that one common problem with continuous performance tasks in experimental animals is that, owing to the large number of trials, ingestion of rewards may lead to satiety over the course of a session.

In the rat, lesion and pharmacological studies have implicated a variety of brain regions and neurotransmitter systems on attentional performance in the 5-CSRTT. For example, rats given large excitotoxic lesions of the mPFC exhibit substantial reductions in response accuracy and increases in response latency compared with sham-lesioned controls. More discrete excitotoxic lesions show that this effect on discrimination accuracy, particularly following variable ITI and noise burst challenges, may be restricted to damage of the anterodorsal mPFC. Similar decrements on response accuracy and lengthening of response latencies are observed following lesions of the dorsomedial striatum and are consistent with the observed connectivity between anterodorsal mPFC and dorsomedial striatum. The importance of this functional circuitry is further strengthened by the observation that asymmetrical, disconnection preparations (in which a lesion to the mPFC in one hemisphere is combined with a lesion to the medial striatum in the other hemisphere), produce effects similar to that of the bilateral lesions. These results are also consistent with the hypothesis that several components of 5-CSRTT performance are mediated by somewhat distinct corticostriatal circuits.

Ascending cholinergic (ACh) and monoaminergic (NA, DA, and 5-HT) systems appear to contribute to both distinct and overlapping aspects of attentional performance on the 5-CSRTT. Excitotoxic lesions of the NBM in rats, or lesions produced by the selective cholinergic immunotoxin 192 IgG-saporin, generally produce impairments in discrimination accuracy, particularly during the increased attentional demand imposed by shortened stimulus durations or the concurrent presentation of auditory distractors. Similarly, decreases in response accuracy and increases in response latency are observed following intra-NBM doses of the GABA agonist, muscimol, at doses sufficient to produce reductions in cholinergic activity. Infusions of 192 IgG-saporin

directly into the ventromedial PFC impair 5-CSRTT performance accuracy, specifically under high event rates. And intra-mPFC infusions of the muscarinic receptor antagonist, scopolamine, induce significant deficits in response accuracy. These results are compatible with the hypothesis that cholinergic afferents in the mPFC serve to optimize attentional resources, particularly under conditions of increased attentional demand or load. Destruction of the ascending noradrenergic projections to the FC by infusions of 6-hydroxydopamine (6-OHDA) into the dorsal noradrenergic ascending bundle also impair attentional accuracy, but only following variable ITI challenge or in the presence of white noise distraction.

Based on these and similar findings, the ACh and NA systems, although functionally distinct, appear to contribute to overlapping operational processes relevant to visual attention, presumably in a manner serving to maintain discriminative selectivity in the face of interference. Less is known of the role of the prefrontal DA systems in the 5-CSRTT, but combined depletion of NA and DA from the mPFC has been observed to result in attentional impairments, specifically during a variable short ITI contingency. Manipulations of 5-HT systems have generally been observed to affect impulsivity more than attentional processes and are described below.

2. Vigilance. Early attempts to model vigilance in experimental animals typically employed a discrete trial procedure in which a signal (e.g., light) had to be detected above a background stimulus and a unitary response performed. These early models relied heavily on signal detection theory, which helped provide a quantitative basis for dissociating discriminative sensitivity for the signal from other factors such as response bias. However, such procedures are often subject to interpretative and computational issues regarding signal detection and response bias and, subsequently, two-response discrete trial tasks (e.g., one response for reporting signal detection, and a separate response for reporting no signal detected) were developed to help obviate these difficulties. In one version of a two-response task, rats were required to respond to the presentation of brief visual signals by pressing one lever and a second lever in the absence of a signal in order to receive reward. The face validity of the task was assessed through manipulation of signal intensity, background noise (flashing chamber houselight), event rate, and signal asynchrony, parameters which are known to alter performance in human vigilance tasks. Predictable performance decrements were observed as the signal length was decreased (fewer correct detections), as background noise increased (decreased discrimination between signal and nonsignal events as well as a vigilance decrement over blocks of trials observed for shortest signals) and as the event rate increased (vigilance decrement over blocks of trials). However, no reliable effects were observed by increasing signal asynchrony.

Within this two-lever task, basal forebrain depletions of ACh via 192 IgG saporin lesions of the NBM in rats led to a persistent decrease in the ability to detect signals (while leaving the ability to correctly reject nonsignals unaffected). By contrast, 6-OHDA lesions of the dorsal noradrenergic ascending bundle produced no consistent effects. Administration of the benzodiazepine receptor (BZR) agonist CDP resulted in a decrement in discriminability between signal and nonsignal events, which was exclusively due to an increase in the number of misses. The BZR partial inverse agonist RU 33965 (0.1, 0.5 mg kg⁻¹) impaired the vigilance performance in a dose-dependent manner, whereas BZR ligands with weak or selective inverse agonist properties (e.g., ZK 93426; beta-CCtB) did not affect the vigilance performance. Amphetamine administration also impaired the vigilance performance, but these impairments may have been due to effects unrelated to attentional mechanisms, such as increased perseveration and/or 'lever switching.'

Although two-response procedures provide the most sophisticated assessment of vigilance to date, they have been criticized on the basis that alerting or arousal mechanisms cannot be completely accounted for, and because there is often a lack of interaction of treatment effects across trial blocks, as is typically observed in humans.

Animal Models of Impulsivity

Varieties of Impulsivity

There are currently a variety of definitions and uses of the term 'impulsivity' within cognitive and behavioral neuroscience, none of which are universally accepted. This definitional multiplicity is generally believed to result from heterogeneity in the timescale, context, population, or category of behavior within which impulsivity is ascribed. In other words, impulsivity is likely not a unitary construct but rather a concept incorporating a range of phenomena spanning several dimensions – from immediate, ongoing actions to more enduring personality traits; from adaptive or functional behaviors to those considered as maladaptive or dysfunctional; and from 'normal' acts of impulsiveness to symptoms of certain psychiatric or pathological conditions. Within such dimensions, definitions of impulsivity also commonly encompass several, seemingly distinct, classes of behaviors – such as actions elicited with less consideration of future consequences; acts reflective of a deficit in the ability to withhold, inhibit, or stop; or decisions reflective of increased risk-taking. Moreover, both within and between such classes of impulsive behaviors, individual actions likely recruit distinct or overlapping sets of psychological processes – such as attention, motor control, switching, timing, arousal, or sensitivity to reward or punishment – processes which themselves may

be multifaceted and have distinct or overlapping neurobiological implementations.

Despite the difficulties in achieving a precise definition of impulsivity, in the clinical setting, measures of impulsivity continue to play a prominent role both as correlates and diagnostic symptoms of a variety of psychopathological conditions including ADHD, conduct disorder, borderline personality disorder, and addiction. Given its various definitions, it is not surprising that there are a diverse methods by which impulsivity can also be measured. Many clinical approaches assess impulsivity by introspection and use of self-report questionnaires. Scale design and principal-component analyses within and between questionnaire test items suggest that these scales are assessing multiple, independent dimensions. While useful as correlative tools, self-report scales are primarily subjective and are designed to measure longer lasting personality characteristics – the majority have little focus on the dynamics of ongoing impulsive behaviors or the influence of relevant situational variables. To this aim, a number of paradigms have been developed to more objectively quantify behaviors which are thought to reflect impulsivity. The extent to which such behavioral indices are related to the rating scale assessments of impulsivity is the subject of ongoing research. However, behavioral paradigms provide useful clinical correlates in their own right and have the added advantages of being amenable to the tools of neurobiological analysis and enabling the development of analogous paradigms for testing in humans and nonhuman animals. The next section will summarize some of the expanding research efforts aimed at investigating impulsivity in animals.

Experimental Paradigms

Behavioral paradigms designed to measure impulsivity in animals have traditionally been divided into two broad categories: paradigms that assess some form of behavioral inhibition of a motor response (i.e., impulsive action), which are typically inclusive of actions that are premature, mistimed, or difficult to suppress or control; and those that assess altered decision-making processes (i.e., impulsive choice), which are typically inclusive of actions that are initiated without accounting for, or in spite of, other possible options or outcomes. The most common tasks designed to measure behavioral inhibition in animals are the go/no-go task, the stop-signal reaction time (SSRT) task, the 5-CSRTT as well as other delayed response tasks (e.g., differential reinforcement of low rate of responding (DRL) task).

Impulsive action

1. *Go/No-go tasks.* In a typical go/no-go paradigm, a subject is reinforced to make a particular response (e.g.,

approaching/touching a stimulus or pressing a lever) when signaled by a sensory cue (the 'go' signal – typically visual, auditory, or olfactory). This reinforced response is often made prepotent by the presentation of a number of such 'go' trials. However, on a subset of 'no go' trials, in order to receive reinforcement the subject must inhibit this prepotent response when a distinct, 'no-go' signal is presented prior to, or concurrently with, the 'go' signal. Higher impulsivity in the go/no-go task is typically defined by a reduced ability to withhold responding on 'no-go' trials relative to accuracy of performance on 'go' trials.

Brain-imaging studies investigating the neural basis of human go/no-go task performance have generally observed bilateral activations within the inferior frontal cortex (IFC), anterior cingulate cortex (ACC), supplementary motor area, dorsolateral PFC, and inferior parietal cortex. In rats, the situation is somewhat less clear. While early results indicated significantly impaired go/no-go performance on an odor discrimination task following aspiration lesions of the orbitofrontal cortex (OFC), later studies inducing bilateral OFC lesions with the excitotoxin, N-methyl-D-aspartate (NMDA) found no impairments in acquisition or performance of go/no-go responding. Similarly, no consistent effects have been observed following NMDA lesions of the prelimbic and infralimbic areas of the mPFC. However, ibotenic lesions of the ACC in the rat disrupt the acquisition of a go/no-go task requiring behavioral temporal sequencing, but do not affect more traditional go/no-go responding when selection processes were based on a tone/light conditional rule that does not require temporal patterning. Of the ascending neurotransmitter systems, 5-HT has been most heavily implicated in go/no-go performance in both humans and rats. In rats, 5-hydroxytryptamine (5-HT) depletion following intra-cerebroventricular (ICV) infusions of 5,7-DHT (5,7-dihydroxytryptamine) profoundly and selectively disrupts the acquisition of go/no-go task performance. Similar results are observed following para-chloroamphetamine administration, which also produces a profound and selective loss of 5-HT in the brain.

2. *SSRT tasks.* The SSRT paradigm follows a similar logic to the go/no-go task with the exception that, in the subset of 'no-go' trials, the 'no-go' signal (or 'stop' signal) is presented some time after the presentation of the 'go' signal. Therefore, while the go/no-go task is thought to measure inhibition of a motor response before the response has been initiated (e.g., action restraint or withholding), the SSRT task is thought to measure inhibition of a motor response which has already been initiated (i.e., action cancellation or stopping). The primary outcome variable of the SSRT task is SSRT, which is an estimate of the latency of a subject to successfully cancel or stop the prepotent 'go' response. As the endpoint of withholding a response cannot be observed directly, SSRT is typically

estimated within the theoretical framework of the 'race' model, a model which assumes that 'go' and 'stop' processes proceed independently from one another. Higher impulsivity in the SSRT task is defined by an increase in SSRT.

Brain imaging studies in humans have implicated many of the same brain regions as the go/no-go task. However, further work has suggested that IFC, and particularly the right IFC, is critical to inhibition on the SSRT task, while other activated structures are better correlated with other task-related processes such as maintaining the go response or error monitoring. Patients with lesion damage to the FC display a strong correlation between lesion size within the right IFC and increased SSRT. There is no such correlation with lesion size and SSRT for other neighboring regions within the right hemisphere or anywhere in the left hemisphere. At the moment it is unclear whether there is a region homologous to the IFC in the rat. Neurotoxic lesions of several subregions of the PFC have been reported to have no effect on SSRT measures – to date, only lesions of the OFC have produced increases in SSRT. Unlike the go/no-go task performance, SSRT does not appear to be reliably altered by manipulations of 5-HT in humans or rats. Neither acute tryptophan depletion (which reduces brain 5-HT levels) nor acute administration of the 5-HT reuptake inhibitor, citalopram, has any significant effect on SSRT in humans. Moreover, global (ICV) 5,7-DHT lesions have no effect on any SSRT task parameters in rats and 5-HT transporter knockout mice show no impairments in SSRT. Recent work has demonstrated that acute administration of the NE reuptake inhibitor, atomoxetine, can improve SSRT in both healthy human subjects as well as in normal rats, suggestive of a potential role of catecholamines in action cancellation forms of behavioral inhibition.

3. *Delayed response tasks.* In both the go/no-go and SSRT paradigms, a separate and explicit signal is used to indicate a subset of trials requiring behavioral inhibition. The absence of an operant response on such trials is typically reinforced. By contrast, on the 5-CSRTT and other forms of delayed response tasks, the absence or delay of responding on any given trial results in a loss and/or delay of reinforcement (e.g., all trials are similar to prepotent 'go' trials). In other words, in typical delayed response tasks, there are no trials with an explicit signal to inhibit responding, nor any immediate feedback that a trial has been successfully inhibited.

As described above, the 5-CSRTT consists of a number of discrete consecutive trials in which subjects are required to wait (e.g., restrain or withhold responding) during a fixed or variable inter-trial interval (ITI) while scanning a horizontal array of five apertures, and to nose poke in the spatial location of a brief visual stimulus in order to earn reinforcement. Nose pokes during the ITI,

prior to the presentation of the visual stimulus, are classified as premature responses. Higher impulsivity is defined as increase in the number of premature responses.

Of many brain regions examined, neurotoxic lesions of the postgenual ACC (Cg1) and infralimbic cortex (IL), as well as local NMDA receptor antagonism in the IL, stand out with respect to the magnitude of the increased impulsivity observed on the 5-CSRTT in the rat. While central depletion of ACh and NE produces effects mainly on 5-CSRTT performance accuracy (described above), a long-lasting increase in impulsive responding (along with a transient increase in accuracy) is observed on the 5-CSRTT following global (ICV) 5-HT depletion produced by infusions of 5,7-DHT into the dorsal raphe nucleus (which mainly innervates the neocortex and striatum). Psychostimulants, which exert marked increases in DA neurotransmission, generally increase impulsivity on the 5-CSRTT task and, in the case of acute amphetamine administration, can be reversed by selective DA depletion in the nucleus accumbens. Overall, these results are consistent with studies using delayed response tasks in humans which heavily implicate both fronto-striatal circuitry and the ascending serotonergic and dopaminergic neurotransmitter systems in the regulation of impulsive behavior.

On a DRL schedule of reinforcement, the subject is required to space operant responses by a specified time interval to obtain reinforcement. Impulsivity is generally attributed to a greater number of responses made before the interval has elapsed and/or lower levels of efficiency (reward per response ratio). On a fixed consecutive number (FCN) schedule, the subject is required to perform a minimum number of responses on one operandum before a response on a second operandum will deliver reinforcement. A reduction in the number of consecutive responses made on the first operandum before responding on the second is typically interpreted as increased impulsivity. On a variant of this task – the paced FCN – the two levers are withdrawn from the chamber each time the rat responds. The paced FCN schedule helps to control for the potential confounding effects of alterations in general levels of activity. A key methodological distinction between the 5-CSRTT and standard DRL and FCN schedules is that in the 5-CSRTT the end of the waiting period (i.e., the time after which an operant response will be reinforced) is explicitly signaled.

While less is known regarding the neural substrates of behavior on DRL and FCN schedules, the effects of psychopharmacological agents shows remarkable overlap with the 5-CSRTT. For example, destruction of the ascending serotonergic systems using 5,7-DHT impairs DRL performance, similar to the effects of selective lesions to the median raphe nucleus. Experiments examining specific destruction of 5-HT neurons in the dorsal raphe suggested that, while processes of temporal

discrimination remained intact, behavioral ‘switching’ mechanisms may be dysregulated following 5-HT loss. Moreover, akin to the 5-CSRTT, administration of psychostimulants such as amphetamine cause increases in impulsivity in both the DRL and FCN procedures.

Impulsive choice

Another category of procedures used to assess impulsivity relates to decision-making or impulsive choice. Impulsive choice is typically measured in the delay discounting paradigm where impulsivity is defined by a greater tendency to value or choose smaller, more immediate reinforcers over larger, more delayed reinforcers – even where it is economically advantageous to value or choose the latter. This tendency to devalue delayed reinforcers appears highly consistent across species, exemplified by characteristic hyperbolic discounting curves for reinforcer as a function of increasing delay. While this obviously implies that delay discounting has adaptive value in natural environments, it is also clear that when the tendency to show preference for immediate reinforcers over more lucrative delayed reinforcers is exaggerated, it may lead to maladaptive behavior. Another variant of impulsive choice procedure includes probability or risk discounting (i.e., a tendency to choose larger, but less probable rewards over smaller more certain rewards despite smaller economic value); however, less work has been performed using this paradigm in experimental animals.

As an example, in one recent rodent version of the delay discounting procedure, rats were first trained to initiate trials (upon activation of a light cue) at regularly spaced intervals to gain access to one of two levers, and to press the extended lever to receive a food pellet reward. Subsequently, rats initiate a series of free-choice trials in which both levers are extended. Rats choose between pressing one lever that always results in the immediate delivery of a single food pellet and pressing another lever that always results in four pellets, but only after a delay that is increased progressively across blocks of trials in each session. Impulsivity, or impulsive choice, in this paradigm is commonly attributed to subjects who show a greater choice of the lever giving the smaller, more immediate reward at some or all of the increased delays on the lever giving the larger reward.

Higher levels of impulsive choice have been documented in individual suffering from disorders such as addiction and ADHD. Further, human brain imaging studies of subjects performing impulsive choice tasks involving primary (e.g., juice) or secondary (e.g., money) rewards have shown that lateral prefrontal and intraparietal cortical regions are activated independently of the delay – which are proposed to participate in ‘rationally’ evaluating the relative value of immediate and delayed rewards, while limbic regions including the ventral striatum and medial OFC are preferentially activated by

relatively immediate rewards – which are proposed to promote choice of imminent rewards without consideration of delayed alternatives. In rats, the implication of prefrontal cortical regions is less clear. Neither mPFC lesions (including both prelimbic and infralimbic regions) nor perigenual ACC lesions appear to influence impulsive choice behavior. While some studies have observed increases in impulsive choice following neurotoxic lesions of the OFC, others have observed decreases in impulsive choice. It should be noted, however, that large mPFC lesions (including prelimbic, infralimbic, Cg1, and Cg2) as well as more selective ACC lesions do appear to affect choice between smaller rewards obtained with low-effort (no ramp) and larger, high-effort alternatives (steep ramp to climb) by reducing preference for the high-effort option. Thus, the ACC may be involved in promoting the selection of effortful, rather than delayed, alternatives. Subcortical brain regions have been more strongly implicated in delay discounting in the rat. Excitotoxic lesions of the nucleus accumbens, hippocampus, and basolateral amygdala all result in increased impulsive choice, while lesions of the subthalamic nucleus tend to decrease impulsive choice.

Forebrain 5-HT depletion has generally been observed to elicit higher levels of impulsivity across a variety of impulsive choice paradigms in the rat, typically by steepening the discounting function. In humans, lowering 5-HT levels via dietary tryptophan depletion, which decreases levels of 5-HT metabolites in cerebrospinal fluid (an indirect indicator of brain 5-HT levels) has not been observed to increase impulsive choice. Psychostimulants such as amphetamine or methylphenidate, which potentiate DA neurotransmission and are widely used in the treatment of ADHD, produce mixed effects on impulsive choice behavior in the rat. Some studies have found that they promote choice of delayed reinforcers while others have observed the opposite effect. One factor that may explain some of these discrepant effects is the presence of cues or signals present during the delay to the large reward alternative, however, these discrepancies require further clarification. While little work has investigated the role of NE in delay discounting, it has recently been observed that acute administration of the NE reuptake inhibitor, atomoxetine (a nonstimulant medication also used in the treatment of ADHD) can decrease impulsive choice in the rat.

Summary

A number of paradigms and procedures have been developed to assess attention and impulsivity in experimental animals. Many of these tasks are automated and highly sophisticated and are readily scalable to analogous paradigms in humans, including selected patient groups. As such, they provide tangible models of core cognitive and behavioral processes relevant to neuropsychiatric disorders, which are amenable to investigative neurobiological research. The continued development of tasks to measure attention and impulsivity in animals is inevitable as research continues to refine the taxonomy of attention and impulse control in humans.

See also: Analysis of Learning in Invertebrates; Animal Models of Learning and Memory; Cognition: Learning and Memory: Pavlovian; Cognitive Decline in Laboratory Animals: Models, Measures, and Validity.

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Cognition: Learning and Memory: Pavlovian

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Glossary

Conditioned response (CR) – A response elicited by a conditioned stimulus.

Conditioned stimulus (CS) – In Pavlovian conditioning, a conditioned stimulus (CS; e.g., a tone) comes to elicit a conditioned response (e.g., salivation) by being paired with an unconditioned stimulus (US; e.g., food). The term ‘conditional’ is sometimes used in place of ‘conditioned.’

CS-US interval – In Pavlovian conditioning, the time between the onset of the CS and the onset of the US.

Extinction – In Pavlovian conditioning, the presentation of the CS without the US that it was previously paired with.

Intertrial interval (ITI) – In Pavlovian conditioning, the time between trials, where a trial is one pairing of the CS with the US.

Unconditioned response (UR) – A natural response elicited by a stimulus in the absence of conditioning.

Unconditioned stimulus (US) – In Pavlovian conditioning, a US (e.g., food) elicits an unconditioned response (e.g., salivation) regardless of the experience or learning history of the subject. Pairings of a neutral stimulus (e.g., a tone) with US (e.g., food) may convert the neutral stimulus into a CS that elicits a conditioned response (e.g., salivation). The term ‘unconditional’ is sometimes used in place of ‘unconditioned.’

can come to do so, given the appropriate conditions. In particular, a previously neutral stimulus such as a sound can come to elicit a CR such as salivation if it has been established as a valid and reliable signal for a US such as food.

It should be noted that some authors use the terms ‘conditional’ and ‘unconditional’ in place of ‘conditioned’ and ‘unconditioned,’ respectively, when referring to the stimuli used in Pavlovian conditioning procedures. One pairing of a CS with a US is called a ‘trial.’ An experimental session generally consists of multiple trials. The time between trials is called the ‘intertrial interval’ (ITI). The time between the onset of the CS and the onset of the US is called the ‘CS-US interval.’

Four possible temporal arrangements of a CS and US are represented by delay, trace, simultaneous, and backward conditioning procedures. In delay conditioning, the CS is presented first and then, while the CS is still present, the US is delivered. The essential features of delay conditioning are that the CS precedes the US and that the two stimuli partially overlap in time. In trace conditioning, the CS also precedes the US, but they do not overlap each other. There is a period of time between the offset of the CS and the onset of the US where neither stimulus is present. This period is called the ‘trace interval.’ Pavlov called this procedure trace conditioning because it was assumed that the aftereffect of the CS (i.e., the CS trace), rather than this CS directly, was associated with the US. Both delay and trace conditioning procedures reliably produce conditioned responding and are the most commonly used temporal arrangements of CS and US. In simultaneous conditioning, the CS and US perfectly overlap. The two stimuli commence as well as terminate at the same time. Simultaneous conditioning is not considered to be as effective as delayed or trace conditioning procedures. In backward conditioning, the US is presented before the CS. Pavlov originally reported that backward conditioning was entirely ineffective, although more recent research has found that learning can be generated with backward conditioning in certain preparations.

Introduction and Basic Terminology

In Pavlovian conditioning (also known as classical or respondent conditioning), a conditioned stimulus (CS) comes to elicit a conditioned response (CR) by being paired with an unconditioned stimulus (US). For example, Ivan Pavlov found that a sound (the ticking of a metronome) that was repeatedly presented several seconds before the delivery of food was capable of eliciting salivation in dogs. In this case, the metronome was the CS, the food was the US, and salivation was the CR.

The US is a stimulus that elicits an unconditioned response (UR) regardless of the learning history of the subject. For example, salivation in response to food being placed in the mouth is an unlearned, innate response. In contrast, sounds do not normally elicit salivation, but they

Pavlovian versus Operant Conditioning

The distinguishing feature between Pavlovian and operant (instrumental) procedures lies in the nature of the relationship between the presentation of stimuli and the subject’s behavior. In Pavlovian conditioning, the CS and

US are presented independently of the behavior of the subject. For example, the sounding of the metronome was followed by food regardless of what the dog was doing. The salivation CR was elicited by the CS, but did not affect the presentation of the CS or US. In contrast, in operant conditioning, the presentation (or removal) of the reinforcer depends on the subject's behavior. For example, a food reinforcer might be presented if a rat presses a lever. The food is not presented otherwise and therefore its presentation is dependent on the lever-press response of the rat. Although a simplification, the following heuristic may be useful in highlighting this essential difference between the two paradigms: in Pavlovian conditioning, a stimulus (e.g., a sound) produces a response (e.g., salivation); in operant conditioning, a response (e.g., a lever-press) produces a stimulus (e.g., food).

Major Procedures Used Today

Eyeblink Conditioning

In the eyeblink conditioning preparation, the US is a puff of air delivered to the eye or a brief electric shock applied to an area near the eye. The UR elicited by this US is an eyeblink. A stimulus, such as a tone or a light, can be converted into a CS by being repeatedly paired with the air puff or shock. Such a CS will come to elicit an eyeblink CR. A special feature of the eyeblink conditioning preparation is the shortness of duration of the optimal CS-US interval. Research has shown that conditioned responding is best generated by intervals of 200–400 ms, with less conditioned responding occurring when shorter or longer CS-US intervals are used. Several hundred trials may be necessary to firmly establish the CR.

Eyeblink conditioning has most often been studied in rabbits, although humans have also served as subjects. In addition to eyelids, rabbits have a nictitating membrane, which is located below the eyelid and serves a very similar function to the eyelid (it has been called a 'third eyelid'). Closure of the nictitating membrane has often been the CR directly measured in studies with rabbits. Thus, the CR in studies with rabbits is often referred to as the 'nictitating membrane response.'

Fear Conditioning

Rats are commonly used as subjects in the conditioned fear preparation. A stimulus, such as a tone or light, is presented and paired with a brief electric shock that is applied to the metal grid floor of the chamber. This footshock US elicits a UR that may consist of an initial jump or startle reaction, occasionally accompanied by a vocalization, followed by a longer period of immobility. A CS that has been paired with footshock elicits a CR that has been referred to as 'freezing.' This freezing response is

characterized by the absence of all movement except that required for breathing. The CS in conditioned fear experiments typically lasts from several seconds to several minutes in duration. The CR can be acquired after relatively few trials. In the simplest version of the conditioned fear procedure, the freezing CR is measured directly by observing and scoring video recordings or through the use of movement-analyzing video software.

Conditioned suppression is a frequently used procedure that involves the indirect measurement of conditioned fear. This technique has also been referred to as the 'conditioned emotional response,' or CER, procedure. Subjects are first trained to make an operant response such as lever-pressing for food. Once a stable baseline of operant performance (e.g., a regular pattern of lever pressing) is established, classical conditioning is conducted where a CS such as a tone is paired with the footshock US. After several CS-US pairings, the CS comes to suppress performance of the operant behavior.

Autoshaping/Sign-Tracking

One of the most commonly used procedures today in the study of Pavlovian conditioning is autoshaping, which is also known as 'sign-tracking.' The autoshaping response typically involves approaching and contacting a CS that has been paired with an appetitive US. In the standard autoshaping procedure with pigeons, the CS is the brief (e.g., 8 s) illumination of a keylight which terminates with delivery of a food US. After a relatively small number of CS-US pairings (usually <100), the CS comes to elicit a pecking CR. Pigeons peck the keylight CS at high rates even though the food US is delivered regardless of their behavior. Remarkably, pigeons continue to peck the keylight CS, although at a reduced rate, even when it is arranged so that pecking leads to the omission of the food US on that trial. This modification of the standard autoshaping procedure is called the 'negative automaintenance' or 'omission' procedure. Autoshaping is also frequently studied with rats as subjects. In the standard rat version of the procedure, insertion of a retractable lever (typically for 10–15 s) is the CS. Upon retraction of the lever, a food pellet US is delivered. The CR soon elicited by the CS is vigorous touching, biting, gnawing, and licking behavior directed at the lever CS. Similarly to pigeons, rats will persist in contacting the CS, although at a reduced rate, even when doing so cancels delivery of the food US.

It has been argued that the term autoshaping is a misnomer since it implies that that operant behaviors or procedures (such as shaping) are involved. The original investigators that coined this term described the procedure as a more efficient and standardized alternative to operant shaping as a method for training pigeons to acquire the keypeck response. However, a procedure is

a Pavlovian one if the CS and US are presented independently of the subject's behavior. Furthermore, results from negative automaintenance studies (described above) suggest that the autoshaping response is a Pavlovian CR rather than an operant behavior since the autoshaping response appears and persists even when it has never been reinforced. The term sign-tracking has been suggested as an alternative to autoshaping since it is an accurate description of the phenomenon and it does not have the operant connotations. Despite these advantages, autoshaping has remained the more commonly used term.

The identities of the CS and US determine the form of the autoshaping CR and whether or not autoshaping occurs at all. Auditory stimuli or diffuse visual stimuli do not generate the robust autoshaping behavior that is produced by keylight CSs in pigeons or by retractable lever CSs in rats. While food has been the most commonly used US in autoshaping studies, CSs paired with other appetitive stimuli have also been shown to elicit approach and, sometimes, contact responses. For example, a keylight CS paired with a water US elicits a drinking- or slurping-like CR that is directed at the key-light in pigeons. In rats, a lever CS paired with reinforcing intracranial stimulation (ICS) elicits approach and contact behavior directed toward the lever. Recently, there have been reports of approach elicited by CSs paired with drugs of abuse, such as cocaine or alcohol in rats, although such autoshaping behavior appears to be less robust than that elicited by CSs paired with food.

Orienting responses, such as looking at a CS that has been paired with food, are similar to autoshaping responses in that both include the observation and tracking of a stimulus that is predictive of a US. However, in autoshaping, the subject moves toward and, usually, physically contacts the CS (sometimes biting or grabbing it), whereas mere orientation does not involve the forward locomotion and tactile interaction with the CS that characterize autoshaping.

Magazine Entry/Goal-Tracking

'Magazine entry' (sometimes called head entry) is a frequently used procedure to study Pavlovian conditioning in rats. A briefly presented (e.g., 30 s) tone or light CS is paired response-independently with a food pellet US. After several such pairings, the rat begins to anticipate delivery of the food pellet during the CS and puts its head into the receptacle (sometimes referred to as a 'magazine') or aperture where the food pellet is soon presented. The number of times that the rat checks for the food pellet during the CS can be measured by counting the number of times that a photobeam projected across the food aperture is broken. This behavior has been called 'goal-tracking' to contrast it with sign-tracking (see above). In goal-tracking, the approach CR elicited by the CS is

directed toward the site of US delivery. In sign-tracking, the approach CR elicited by the CS is directed at the CS itself (the sign). Characteristics of the conditioning procedure (e.g., whether the CS is auditory or visual) determine whether goal-tracking or sign-tracking occurs.

Conditioned Taste Aversion

In conditioned taste aversion learning, a novel taste CS, such as saccharin-flavored water, is paired with an aversive US, such as the injection of an emetic drug. In a commonly used version of the procedure, thirsty rats are allowed to drink a saccharin solution for several minutes prior to injecting them with lithium chloride. With as little as one saccharin CS–lithium US pairing, rats learn to avoid drinking the CS solution in the future. A special feature of conditioned taste aversion learning is that the CS–US association can be learned with very long CS–US intervals (e.g., up to 24 h). In addition to using lithium chloride as the US, conditioned taste aversions have been demonstrated in rats when the US was an injection of a drug of abuse, such as cocaine or morphine.

Extinction and Related Phenomena

If, after having established a CS–US association, the CS is no longer paired with the US, the CS will gradually cease to elicit the CR. For example, Pavlov noted that when a metronome that had previously been paired with food and that had elicited salivation was presented alone for several trials, it took progressively longer for salivation to be elicited and the amount of salivation elicited grew progressively smaller. The procedure where CS–US pairings are discontinued and the CS is presented alone is called 'extinction.' The weakening of the CR resulting from such a procedure has also been referred to as extinction.

Pavlov performed much research on extinction and discovered many of its properties. He noted that a CR that had been thoroughly extinguished would reappear if the CS were presented again after a period of time during which it was not presented. For example, a visual CS that had come to elicit salivation by being paired with a food US was repeatedly presented by itself (extinction) until it no longer elicited salivation. A 2-h period was allowed to pass during which time no stimuli were presented. When the visual CS was then presented, the dog began salivating again. Pavlov called this phenomenon 'spontaneous recovery.' Pavlov also observed that presentation of some extraneous stimulus, such as a loud sound, could cause a CS that was previously subjected to extinction, and that had ceased to elicit the CR, to begin to elicit the CR again. He called this phenomenon 'disinhibition.'

Much recent research has investigated other situations where behavior reappears after it had been eliminated through extinction. In reinstatement, presentation of the US alone leads to the reappearance of the CR when the CS is subsequently presented by itself. For example, a tone previously paired with shock might be subjected to extinction during multiple sessions so that it no longer elicits a freezing CR. If the shock is then presented by itself a number of times, the tone will once again elicit the freezing CR if the tone is subsequently presented. In rapid reacquisition studies, the CS that had been subjected to extinction is again paired with the US. Thus, this is similar to the reinstatement procedure except that the US is not presented alone, but is paired with the CS. The typical result is that the CR appears much sooner than during initial conditioning and much sooner as compared to a control group receiving CS-US pairings for the first time.

In context renewal, pairings of the CS and US occur during the first phase in one context and then, in a second phase that is conducted in a different context, the CS is subjected to extinction until it no longer elicits the CR. When the CS is presented by itself in the original context it again elicits the CR. For example, rats might experience pairings of a tone with footshock in an experimental chamber that has black-and-white striped walls, a low level of illumination, and a eucalyptus-scented ambient odor. By being paired with shock, the tone CS comes to elicit a freezing CR. Then, extinction is conducted and the tone is presented by itself over the course of several sessions conducted in a different experimental chamber that has solid white walls, a high level of illumination, and a vinegar-scented ambient odor. Even if the tone no longer elicited any conditioned freezing by the end of the extinction treatment conducted in the different context, it will again produce a robust freezing CR if it is presented to the subjects in the original training chamber. This is the context renewal effect.

The five phenomena just described involving a reappearance of the CR after extinction – spontaneous recovery, disinhibition, reinstatement, rapid reacquisition, and context renewal – all indicate that extinction does not lead to the loss of, or unlearning of, the CS-US association. Rather, such findings suggest, in accordance with Pavlov's ideas as well as more recent accounts, that extinction involves a type of inhibitory learning that acts to suppress expression of the CR while leaving the original CS-US association intact. Much current research has been concerned with investigating the mechanisms involved in extinction learning.

Inhibitory Pavlovian Processes

A CS that has been paired with a US and comes to elicit a CR because of this association is said to have excitatory

properties and is called an 'excitor.' A stimulus that signals the absence of a US and comes to control a behavioral tendency opposite that of an excitor is called a 'conditioned inhibitor.' For example, a tone that signals that food will not be presented and thereby comes to suppress salivation is a conditioned inhibitor.

There are a number of ways to create inhibitory stimuli. In the most commonly used conditioned inhibition procedure, originally described by Pavlov, one stimulus (denoted A) is first established as an excitor by being paired with the US. Trials where stimulus A is paired with the US are called A+ trials. On other trials that are intermixed with A+ trials, another stimulus (called B) is presented simultaneously with stimulus A and the US is omitted. On these AB- trials, stimulus B signals the absence of the US. The CR eventually stops being elicited on these AB- trials, while it remains intact on A+ trials. This method of creating inhibition has been called the 'A+/AB- conditioned inhibition procedure.'

Another method of establishing an inhibitory stimulus is through the use of differential conditioning, or what Pavlov referred to as the 'method of contrast.' Two types of trials are presented in an intermixed fashion: A+ trials, where one stimulus (e.g., a tone) is paired with the US; and B- trials, where another stimulus (e.g., a light) is presented alone. Stimulus A becomes an excitatory CS that elicits the CR and stimulus B becomes an inhibitor capable of suppressing the CR.

Pavlov called the instances of inhibitory learning described above 'internal inhibition' to contrast it from external inhibition. In external inhibition, the suppression of the CR is not due to inhibitory associative learning, but rather is due to the disruptive or distracting effect of some extraneous stimulus on CR performance. For example, an unexpected loud noise coincidentally occurring at about the same time that a CS for food is presented might temporarily suppress the salivation response, but this suppression is not due to the type of learning that is involved in conditioned inhibition.

Two major methods used for measuring conditioned inhibition are the summation test and the retardation-of-acquisition test. In the summation test, the putative inhibitor is presented simultaneously with an excitor for the first time (not the same excitor that was used to create the inhibitor). Evidence for conditioned inhibition is provided if the presence of the putative inhibitor leads to a reduction in the amount of conditioned responding normally elicited by the excitor when it is presented alone. In retardation of acquisition, the putative inhibitor is paired with the US and the rate of acquisition of the CR is measured. Evidence of conditioned inhibition is provided if the rate of excitatory conditioning of the putative inhibitor is slower than that of a novel or neutral stimulus that was not previously established as a signal for the absence of the US.

Control Procedures Used in Pavlovian Conditioning

In studies of Pavlovian conditioning, control conditions are often included to determine whether a CS-US association has been formed and to rule out potential alternative explanations of changes in behavior. A number of procedures have been used for this purpose.

CS-Only Control

In the simplest procedure, the experimental group receives pairings of the CS and US, while the control group receives only presentations of the CS. This control procedure is often used in conditioned taste aversion and conditioned fear studies. For example, an experimental group may receive access to saccharin (the CS) that is followed by an injection of lithium chloride (the US), while a control group receives access to saccharin that is followed by an injection of saline (or other inert substance). Changes in saccharin drinking over successive trials are then measured and a conditioned taste aversion is assumed to have been produced if saccharin drinking in the experimental group is reduced relative to that observed in the CS-only control group.

A CS-only group is not an ideal control procedure, although it may be the only one that is practical or feasible in some situations. The problem is that nonassociative effects of the US are not controlled for because a CS-only group does not experience the US. This is a critical shortcoming in situations where simple exposure to the US might affect the subject's reaction to the CS in a way that makes it appear as though a CS-US association has been learned when, in fact, such an association has not been learned. For example, an intense electric shock might cause subjects to freeze in response to any subsequently presented unfamiliar stimulus. If an experimental group receiving tone-shock pairings quickly happened to freeze in response to the tone (or any other stimulus), while a CS-only control group did not freeze in response to the tone, it might be erroneously concluded that subjects in the experimental group have extremely rapidly learned a tone-shock association. To address such potential pseudoconditioning effects, control procedures that involve presentation of both the CS and the US are necessary.

Explicitly Unpaired Control

In the explicitly unpaired procedure, both the CS and US are presented, but never together. The US is presented during the ITI. For example, in a rat autoshaping experiment, the experimental group might receive trials

consisting of a 15-s lever insertion CS that is followed immediately by a food pellet US, with ITIs lasting a mean of 90 s. An explicitly unpaired control group might receive the same number of presentations of both the lever and the food pellet, but with a food pellet presented at a varying time points during each ITI that is no more than 20 s before or after presentation of the lever CS.

A concern with the explicitly unpaired control procedure is that it may produce inhibitory conditioning. That is, a stimulus that is never paired with the US is a signal for the absence of the US. If such a stimulus acquires inhibitory properties, it may come to produce a level of CR performance that is below the true baseline, control level that would be elicited by a neutral CS. Therefore, comparing the response elicited by a CS that has been paired with the US with that elicited by a stimulus subjected to explicitly unpaired treatment may lead to overestimates of how much learning was produced by CS-US pairings.

Truly Random Control

In the truly random control procedure developed by Robert Rescorla, the CS and the US are both presented as frequently as in the experimental condition, but they are presented independently with respect to each other. To use the rat autoshaping example described above, a truly random control group might receive presentations of the 15-s lever CS with a mean ITI of 90 s. Deliveries of the food pellet US would be programmed to occur at varying intervals that also have a mean of 90 s. However, the schedules controlling the varying intervals at which each stimulus is presented would operate independently. This would mean that the lever and the food pellet would occasionally be presented together by chance, in contrast to the explicitly unpaired procedure where they would never be presented together.

Because in the truly random control procedure the likelihood of the US occurring during the CS is the same as the likelihood of the US occurring during the absence of the CS, there is no contingency between the CS and US. Therefore, the CS should become a true control stimulus, without either excitatory or inhibitory properties.

Contiguity, Contingency, and Predictive Value

Temporal Contiguity

Pavlov believed that the most essential factor for the learning of a classically conditioned response was the temporal contiguity of the CS and US. This was based on his observation that a CR readily developed when the CS preceded and overlapped with the US, but not when

this temporal relation was absent. More recent work has shown that temporal contiguity between the CS and US plays an important role, but that such contiguity is not sufficient for associative learning and that the effects of CS-US temporal relations vary depending on the specific conditioning preparation that is used.

Contingency

Research by Rescorla demonstrated that contingency between the CS and US is necessary for the learning of Pavlovian associations. Contingency refers to the degree to which presentation of the US depends on the prior presentation of the CS. If the US is more likely to occur when the CS has been presented than when the CS has not been presented, then there is a positive contingency between the CS and US. This arrangement produces excitatory conditioning as evidenced by the CS's elicitation of CRs. If the US occurs following presentation of the CS just as frequently as it occurs when the CS has not been presented, then there is no contingency between the CS and US. This zero contingency does not produce learning of an association between the CS and US. If the US occurs less frequently when the CS is presented than when the CS is not presented, then there is a negative contingency between the CS and US. This type of contingency may lead to inhibitory conditioning.

Predictive Value

In experiments by Leon Kamin, a noise stimulus was paired with a shock US in a first phase. Subjects learned this noise-US association and the noise came to elicit a conditioned fear response. Then, in a second phase, the noise was presented simultaneously with a light and this noise + light compound stimulus was paired with the shock US. Even after many pairings of the noise + light compound with shock, the light did not elicit any conditioned fear when it was presented by itself despite the facts that (1) the light was always paired with the shock US, and (2) the light elicited a strong conditioned fear response in a control group that did not experience the phase 1 training where the noise alone was paired with shock.

This phenomenon is known as 'blocking' because the conditioning history of the noise prevented, or blocked, learning about the light. Blocking has been explained in terms of CS information value or predictiveness. When the light was presented simultaneously with the noise in phase 2 of the experiment described above, it did not provide any new information relevant to the occurrence of the shock US. This is because the shock was already fully predicted by the noise.

Blocking and related findings indicate that an essential condition for the formation of CS-US associations is that the CS must provide nonredundant information relevant to the occurrence of the US. If the outcome that follows a stimulus is already fully predicted and expected, then no learning about that stimulus results because it provides no valuable predictive information. If the outcome that follows a stimulus was not yet fully predicted, then learning about that stimulus occurs. That is, associative learning is a function of the discrepancy between the outcome that the CS predicts and the outcome that actually occurs (i.e., the US). The idea that this discrepancy, or prediction error, drives learning is the basis of error-correction theories of Pavlovian conditioning, such as the influential Rescorla-Wagner model.

See also: Animal Models of Learning and Memory; Blocking, Neural Basis of; Cardiovascular Conditioning: Neural Substrates; Cerebellum: Associative Learning; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Eyelid Classical Conditioning; Fear Conditioning; Fear, Anxiety, and Defensive Behaviors in Animals; History of Behavioral Neuroscience; Learning and Memory: Computational Models; Neural Basis of Classical Conditioning; Neural Substrates of Conditioned Fear and Anxiety.

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Cognition: Learning and Memory: Spatial

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Glossary

Allocentric – An other-centered spatial frame of reference, which is independent of the organism's current viewpoint or location.

AMPAR – The α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA). AMPARs are ionotropic ligand-gated receptors for glutamate that mediate fast synaptic transmission in the central nervous system and are essential for the maintenance and expression of long-term potentiation (LTP). AMPARs are composed of four subunits, previously known as GluR-A to GluR-D or alternatively as GluR1 to GluR4, but now known as GluA1-4. In this article, we use the nomenclature of the original cited studies (i.e., GluR-A).

Egocentric – A self-centered spatial frame of reference, which is dependent on the organism's current viewpoint or location.

GluR-A – A subunit of the AMPAR, which has been selectively knocked out in GluR-A^{-/-} mice. These mice are deficient in certain aspects of LTP.

Long-term potentiation (LTP) – An experimental model of the type of synaptic plasticity that may provide the neural substrate for learning and memory. In practice, LTP refers to a long-lasting increase in synaptic efficacy that follows stimulation of a chemical synapse.

Spatial reference memory – A form of hippocampal-dependent, long-term memory which involves the formation of associations between particular spatial locations and specific outcomes, such as associating one arm of a maze with food reward or associating the location of a fixed platform with escape from an aversive environment. GluR-A^{-/-} mice are not impaired in spatial reference memory tasks.

Spatial working memory – A form of hippocampal-dependent, short-term memory, which requires the ability to distinguish between the relative familiarity of previously encountered spatial locations. GluR-A^{-/-} mice are impaired in spatial working memory tasks.

Introduction

The ability to learn and remember spatial locations, and to associate them with other stimuli, is essential for adaptive behavior. Birds need to remember nesting sites or where they have cached food; rodents need to learn about

their surroundings to locate provisions and avoid predators; humans need to navigate complex environments, such as cities. A network of brain regions is involved in spatial learning and memory, including the hippocampus, dorsal striatum, and the entorhinal, retrosplenial, prefrontal, and parietal cortices. Different types of spatial representation may recruit different components of this network. For example, some spatial tasks can be solved purely on the basis of egocentric (self-centered) information (e.g., vestibular, proprioceptive, or sensory cues) that will change every time the animal moves. In contrast, other spatial tasks require encoding of the relationship between salient features of the environment to create an allocentric (other-centered) representation that is independent of the animal's current location. Such a representation has been termed a 'cognitive map.' This article concentrates on animal studies of allocentric spatial learning and describes some of the dominant models that have been used to investigate spatial learning and memory, with particular focus on studies of the hippocampus and related brain structures in rodents.

The Hippocampus and Allocentric Spatial Learning

Evidence from a variety of sources suggests that the human hippocampus plays an important role in spatial memory. Human patients with hippocampal pathology, whether as a result of surgery, ischemic brain damage, or as a consequence of clinical conditions such as Alzheimer's disease, have problems with spatial orientation and get lost even in surroundings with which they have considerable experience. Moreover, normal, healthy individuals exhibit activation of brain areas in the hippocampal formation during functional neuroimaging, when performing navigational tasks in virtual reality environments. Interestingly, in structural magnetic resonance imaging (MRI) studies, London taxi drivers with many years experience of navigating around a busy city were found to possess larger hippocampi than age-matched controls. Spatial information is likely to provide an important contextual cue for retrieving other memories, and thus it has been widely suggested to be a key component of human episodic memory.

In rodents, hippocampal lesions impair allocentric but not egocentric spatial memory. There has been considerable debate as to what constitutes an allocentric spatial cue or

what makes a behavioral task spatial in nature (it certainly should not be defined on the basis of sensitivity to hippocampal lesions). It is generally considered that spatial cues are complex, multimodal representations of the environment, comprising information from different sensory modalities, and diffuse in nature. O'Keefe and Nadel proposed two distinct systems to guide spatial learning and memory. The first of these, the taxon system, utilizes egocentric cues and specific behavioral responses to specific landmarks or stimuli (e.g., always turn right, always approach stimulus X, always avoid stimulus Y, etc.) to allow for route-based navigation. The second system, the locale system, underlies allocentric spatial encoding and the formation of a cognitive map of the environment. The locale system provides the mechanism for solving the conflict that arises when it is not always the correct thing to do to approach stimulus X or avoid stimulus Y. In such situations, by using current position and/or heading direction, the correct response can be selected for efficient navigation to occur. O'Keefe and Nadel proposed that the locale system is hippocampal dependent with place cells as its basic functional units (see below).

Electrophysiological Correlates of Spatial Processing

Place Cells in the Hippocampus

Although scientists had studied rats in mazes since the early twentieth century, it was not until the 1970s that spatial processing was linked to a specific neural substrate. The breakthrough came when O'Keefe and colleagues found cells in the hippocampi of behaving rats that selectively increased their firing rate only when the rat occupied a well-defined region of the environment, the place field, and rarely fired outside the place field. Logically, these cells were named place cells. (NB: It is important to note that the firing rate of hippocampal neurons has also been shown to correlate with nonspatial stimuli in other experimental situations.)

In open-field arenas, place cells are nondirectional in that they will fire irrespective of the direction from which the animal enters the place field (see **Figure 1(a)**). This suggests that place cells are not dependent on specific sensory input such as the local view of the animal. Subsequent studies found that place fields can be altered in predictable ways by manipulating the spatial environment. For example, rotating salient cues around the testing arena causes the place fields to rotate accordingly. Further, when several cues are present, removing one or two cues typically does not abolish the place field. These results argue that place cells depend on the configuration of distal (extramaze) cues rather than specific local (intra-maze) cues, and as such place cells exist within an allocentric frame of reference.

However, on linear tracks or mazes, many place cells only fire when the animal enters the place field from a particular direction, demonstrating that the egocentric local view can influence place-cell activity. Moreover, place cells do not require visual input at all as they develop normally in rats that are blind from birth and can remain stable in sighted rats when tested in the dark. Thus, in addition to extramaze visual cues, it is likely that rodents can use local olfactory, auditory, and somatosensory cues as well as vestibular, proprioceptive, and other motor signals to help maintain a representation of their current location within the environment.

Movement is particularly important for place cells as they tend only to fire when the animal moves through the place field and not when it is immobile within the place field. More specifically, the firing rates of place cells are modulated by spatial location only in the presence of theta activity, the regular 5–12 Hz oscillation in hippocampal electroencephalogram (EEG) that occurs during voluntary behaviors such as walking, running, and swimming. Within a given place field, a place cell will fire in bursts at different phases of theta. When entering the place field, the place cell fires at the late phase of theta and as the animal traverses the place field, the firing bursts occur at progressively earlier stages in the theta cycle, so-called phase precession.

Although there is no obvious topographical organization of place cells, recent investigations have shown that the spatial resolution of place cells differs systematically along the longitudinal (septo-temporal) axis of the hippocampus. Place field size progressively increases (i.e., spatial resolution decreases) from the septal (dorsal) to the temporal (ventral) pole of the rat hippocampus, suggesting that the environment is mapped simultaneously at many different spatial resolutions.

Of course, knowing your current location (information potentially provided by hippocampal place cells) is necessary, but not sufficient, for successful navigation. The cognitive map proposed by O'Keefe and Nadel requires not only information about individual places but also information about the direction and distance between places. Evidence for cells that can provide this directional and distance information came from the discovery of head direction (HD) cells in the dorsal presubiculum (and anterior thalamus) and grid cells in the entorhinal cortex, respectively.

Head Direction Cells in the Presubiculum

HD cells, first reported by Ranck and colleagues, signal a single preferred head direction, irrespective of body orientation or current position; whether the animal is moving or stationary (see **Figure 1(c)**). HD cells appear to be controlled by distal sensory input and, like place cells, realign to the rotation of salient environmental cues.

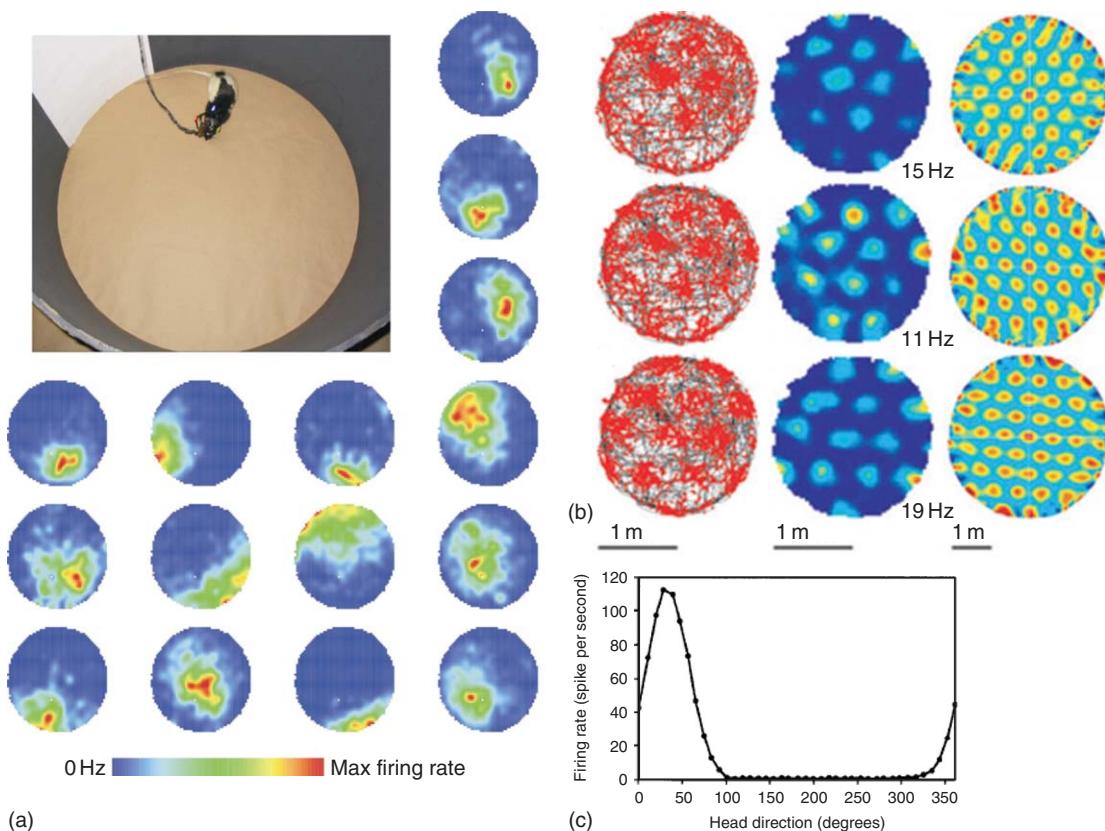


Figure 1 Place cells, grid cells, and head direction cells. (a) Place cells in the hippocampus. Place fields recorded from the hippocampus of a freely moving rat. The picture shows a rat implanted with 16 tetrodes in the hippocampus foraging for food pellets in a circular open-field (75 cm diameter). The 15 density plots represent the firing rate of 15 pyramidal cells at different locations within the open-field. The cells fired only when the rat was at a specific location in the open-field. (b) Grid cells in the entorhinal cortex. Firing fields of three simultaneously recorded cells from layer II of the dorsal medial entorhinal cortex during a 30 min session in a large circular enclosure. Left column, trajectory of the rat (black) with superimposed spike locations (red). Middle column, color-coded rate map with the peak rate indicated. Red is maximum, dark blue is zero. Right column, spatial autocorrelation for each rate map. The color scale is from blue ($r = -1$) through green ($r = 0$) to red ($r = 1$). (c) Head direction cell in the postsubiculum. The plot shows firing rate against head direction for one cell in the postsubiculum. The firing rate is above a background rate of 0 spikes per second for a $\sim 90^\circ$ range of head directions centered on 27° . (a) Unpublished data from Kevin Allen, used with permission. (b) Adapted with permission from Hafting T, Fyhn M, Molden S, Moser M-B, and Moser EI (2005) Microstructure of a spatial map in entorhinal cortex. *Nature* 436: 801–806. (c) Adapted with permission from Müller RU, Ranck JB, Jr, and Taube JS (1996) Head direction cells: Properties and functional significance. *Current Opinion in Neurobiology* 6: 196–206.

Importantly, even when the environmental cues controlling HD cells are disrupted, the angular distance between any two HD cells remains constant. This constancy provides a fixed frame of directional reference for the animal.

Grid Cells in the Entorhinal Cortex

It is likely that HD cells exert their influence on hippocampal place cells via grid cells in the medial entorhinal cortex (MEC), which were first reported by the Moser group in 2005. Unlike hippocampal place cells, which fire in only one part of a given environment, grid cells fire at several regularly spaced locations, with marked inhibition of firing outside of these locations. Thus, a map of peak firing rates resembles a hexagonal lattice (see Figure 1(b)). The distance between grid-cell firing locations depends on

the depth of the recording site on the dorsal–ventral axis of the MEC. Cells recorded from the dorsal MEC have a short distance between firing locations and thus code distance on a fine scale. The distance between firing locations progressively increases from dorsal to ventral MEC, and this change in scale maps onto the increase in place field size along the dorsal–ventral axis of the hippocampus. Thus, grid cells could provide the distance metric by which space is coded.

In summary, detailed electrophysiological investigations in the last 40 years have laid the foundations for subsequent neurobiology theories of spatial learning and memory. However, electrophysiological experiments are correlational by nature – they can tell us that a cell responds under certain circumstances but they cannot tell us that this response is necessary for the

accompanying behavior. Thus, to demonstrate that a particular anatomical structure or neurobiological feature is necessary for a particular behavior, researchers use interference methods. Chief among these techniques are selective brain lesions, pharmacological interventions, and genetic manipulations. Critically, these techniques must be combined with the appropriate behavioral paradigms.

The Morris Watermaze Task

Perhaps the most influential behavioral paradigm demonstrating hippocampal-dependent spatial learning and memory is the watermaze task, devised by Morris in the early 1980s. In this task, rodents have to locate a hidden escape platform submerged just beneath the surface of the water in a large cylindrical tank (e.g., ~2 m diameter for rats). The water is made cloudy by adding milk or non-toxic paint to ensure that the animal cannot see the submerged platform. Therefore, the animal must use the extramaze cues around the room to form an allocentric representation of the environment, including the platform location.

In the standard reference memory version of the task, the animal is trained to the same fixed platform location over several days. Platform locations should be counterbalanced between experimental groups to counteract unconditioned preferences for particular areas of the pool (e.g., half of the animals in the control and experimental groups might be trained to a platform in the

northwest quadrant, while the remaining animals are trained to a southeast quadrant location). Although the platform remains in the same position throughout training, crucially, the starting position changes on each trial to prevent the use of egocentric strategies (e.g., body turn). During acquisition, subjects are given a time limit to find the platform (e.g., 120 s) and those that fail can be guided or placed on the platform by the experimenter. The subject is then allowed to remain on the platform for a short period (e.g., 30 s). Learning is assessed by measuring the amount of time or, preferably, the swimming path length taken to find the platform over successive trials. Importantly, spatial memory of the platform location is then also assessed specifically using a probe trial (transfer test) in which the platform is removed from the pool and the subject allowed to swim freely for a set time (e.g., 60 s). Subjects that remember the platform location spend more time searching in the appropriate part of the pool. Accurate measurement of time, distance, and swim speed can be made using a ceiling-mounted camera and specialized tracking software. Control tasks can be used to ensure that impaired performance is not due to sensorimotor or motivational deficits. For example, rats can be made to swim to a visible platform or a highly salient cue can be used as a beacon to indicate the platform location.

Hippocampal lesions made before training dramatically impair both the acquisition stage of the watermaze task and performance in the transfer test (Figure 2). Hippocampal lesions made after training also impair retrieval; with a flat gradient of retrograde amnesia observed in rats lesioned up to 3 months after the initial

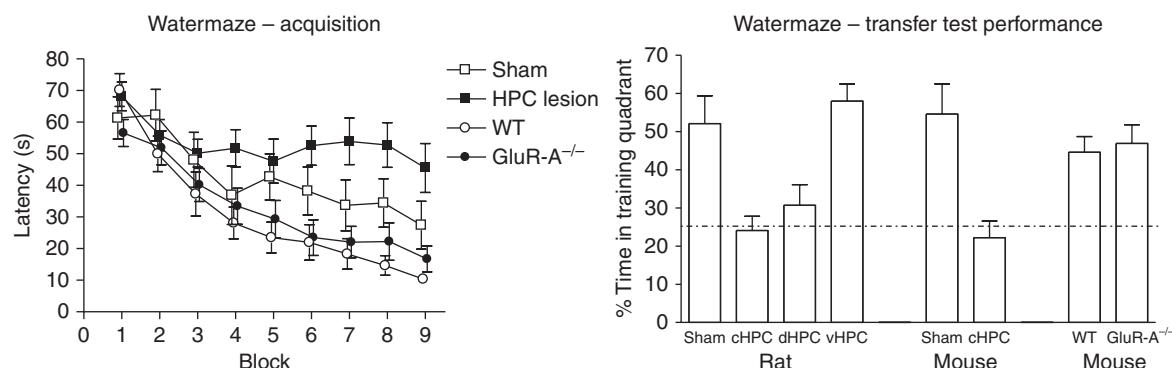


Figure 2 Watermaze. *Watermaze acquisition:* Complete and dorsal hippocampal lesions but not GluR-A deletion impair transfer test performance following training on the standard, fixed location, hidden escape platform, (spatial reference memory) version of the Morris watermaze task. *Transfer test performance:* Mean percent time in the training quadrant (\pm SEM) during a probe trial in which the platform is removed from the pool and the rats allowed to swim freely. Left: Performance of sham, complete (cHPC), dorsal (dHPC), and ventral (vHPC) lesioned rats. Center: Performance of sham and complete (cHPC) hippocampal lesioned mice. Right: Performance of wild-type and GluR-A-knockout mice. Broken line equates to chance performance (25%). *Transfer test performance:* (Left) Reproduced with permission from Bannerman DM, Yee BK, Good MA, et al. (1999). Double dissociation of function within the hippocampus: A comparison of dorsal, ventral, and complete hippocampal cytotoxic lesions. *Behavioral Neuroscience* 113: 1170–1188. (Center) Reproduced with permission from Deacon RM, Bannerman DM, Kirby BP, Croucher A, and Rawlins JN (2002) Effects of cytotoxic hippocampal lesions in mice on a cognitive test battery. *Behavioural Brain Research* 133: 57–68. (Right) Reproduced with permission from Reisel D, Bannerman DM, Schmitt WB, et al. (2002) Spatial memory dissociations in mice lacking GluR1. *Nature Neuroscience* 5: 868–873.

learning. Hippocampal-lesioned animals are not impaired when a visible platform is used, arguing against a sensorimotor or motivational explanation of the deficit.

More recent work has shown that tissue at the septal end of the hippocampus (dorsal hippocampus in rodents) is more important for watermaze spatial learning than tissue at the temporal end (ventral hippocampus; **Figure 2**, right panel). Moser and colleagues found that the severity of the watermaze impairment was proportional to the degree of dorsal hippocampal damage and that relatively small volumes of dorsal tissue (25–30%) could support water maze acquisition and retrieval. This finding is consistent with the higher spatial resolution exhibited by dorsal hippocampal place cells and dorsal MEC grid cells. However, ventral hippocampal lesions made after training can impair subsequent retrieval and, under certain training regimes, the ventral hippocampus does appear able to support spatial learning in the standard reference memory task. Thus, it is important not to exclude the ventral subregion from a role in spatial processing altogether.

Studies of lesioned animals can inform as to whether a particular brain region is necessary for a particular task but they offer little insight into the physiological or molecular mechanisms that underlie normal memory function. Importantly, experiments using pharmacological manipulations or transgenic mouse studies, in which specific neurobiological mechanisms can be targeted, have revealed dissociations between different types of spatial memory that were not evident on the basis of lesion studies.

Hippocampal Long-Term Potentiation and the Spatial Learning Hypothesis

Donald Hebb suggested that memories are stored as changes in the strength of the synaptic connections between neurons. The subsequent discovery that high-frequency stimulation of an input pathway (e.g., the perforant pathway from the entorhinal cortex to the dentate gyrus) can produce long-lasting changes in synaptic efficacy has led to long-term potentiation (LTP) becoming the dominant experimental model of the cellular mechanisms of learning. It has been widely suggested that LTP-like events in the hippocampus might underlie allocentric, hippocampus-dependent spatial learning abilities. The induction of LTP is dependent on the activation of N-methyl-D-aspartate receptors (NMDARs). Its subsequent maintenance and expression are thought to involve changes in α -amino-3-hydroxy-5-methyl-4-isoxazolopropionate receptor (AMPAR) number and function in the postsynaptic neuron. Thus, the 'hippocampal LTP/spatial learning hypothesis' can be tested by interfering with these receptors and assessing the consequences on spatial learning.

Experiments by Morris and colleagues confirmed that blocking NMDARs, by infusing the NMDAR antagonist 2-amino-5-phosphopentanoate (AP5) into the cerebral ventricles, impaired reference memory acquisition in the watermaze at concentrations that also blocked LTP *in vivo*. However, subsequent experiments showed that rats treated with AP5 were in fact capable of forming an association between a spatial location and the platform, and of navigating to that location, if they had received watermaze pretraining prior to testing with the drug.

Genetic Manipulations of NMDA and AMPA Receptors

The hippocampal LTP/spatial learning hypothesis has also been tested in numerous studies of genetically modified mice in which key proteins required for either the induction or expression of LTP are deleted, altered, or overexpressed. For example, mice lacking the GluR-A (also known as GluR1 or GluA1) subunit of the AMPA receptor (GluR-A^{-/-} mice) are particularly deficient in the early component of LTP in the Schaffer collateral pathway (CA3 → CA1) yet they show normal acquisition and retention in the standard reference memory watermaze task. However, they are profoundly impaired on tests of spatial working memory. The concept of distinct working memory and reference memory systems was first proposed by Honig and developed by Olton and colleagues, primarily on the basis of studies using the radial arm maze (see below). It is important to note that the term working memory has been used by different researchers to reflect different processes. For example, researchers working with primates use working memory to refer to a short-term, online system supported by frontal lobe structures. In the present context, however, we use the term to refer to a flexible short-term memory system in which conditional, trial-specific information is used to select between response options that are variably correct or incorrect, and which is dependent on the hippocampal formation.

Simultaneous Dissociation of Spatial Reference and Working Memory

Spatial reference and working memory can be assessed simultaneously in the same animals using a radial maze. The radial maze consists of a number of arms (commonly 6, 8, or 12) radiating out from a central area like spokes on a wheel. The aim of the task for the animal is to collect hidden food rewards located at the ends of the arms by using the distal extramaze cues around the laboratory. Because the food rewards are not replaced, the animal has to adopt a win-shift strategy and remember which arms it has already visited. This provides a test of spatial working memory. By baiting only certain arms but always

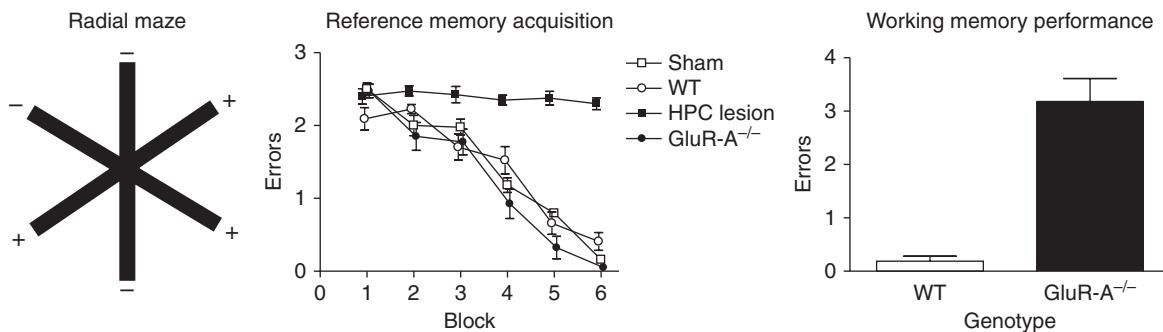


Figure 3 Radial arm maze. Hippocampal lesions but not GluR-A deletion impair acquisition of the spatial reference memory component of the radial maze task. *Left:* Mice were trained to discriminate between three baited arms (+) and three nonbaited arms (−) of a six-arm radial maze. Doors prevented mice re-entering arms they had already visited on that particular trial (i.e., the doors prevented working memory errors) during this stage of the experiment. *Middle:* Mean number of reference memory errors per trial (\pm SEM) during six blocks of training (four trials per block). Sham lesioned (white squares), wild-type (WT: white circles), and GluR-A-knockout mice (GluR-A^{-/-}: black circles) all acquired the task at an equivalent rate. Hippocampal lesioned mice (HPC: black squares) were completely unable to learn which arms were baited and which arms were not baited. *Right:* GluR-A deletion impairs spatial working memory performance on the six arm radial maze task. Mice were still rewarded in the same three arms of the maze and not rewarded in the three nonbaited arms, but now they were allowed to re-enter arms as often as they liked, and rewards were not replaced within a trial. Mean number of working memory errors per trial (\pm SEM) for wild-type (white bar) and GluR-A-knockout mice (GluR-A^{-/-}: black bar). Reproduced with permission from Schmitt WB, Deacon RM, Seuberg PH, Rawlins JN, and Bannerman DM (2003) A within-subjects, within-task demonstration of intact spatial reference memory and impaired spatial working memory in glutamate receptor-A deficient mice. *Journal of Neuroscience* 23: 3953–3959.

baiting the same arms, spatial reference memory can be assessed simultaneously (Figure 3, left panel).

For example, mice can be first trained to discriminate between three baited and three never-baited arms on a six-arm maze. Once an arm has been chosen, a door prevents access to that arm for all subsequent choices during the rest of that trial. This prevents the animals from making working memory errors during the reference memory acquisition phase. This is important because working memory errors made during initial training can interfere with reference memory acquisition. Reference memory errors (maximum of 3) are scored when an animal enters a never-baited arm. This measure provides a pure test of spatial reference memory acquisition and rats or mice with hippocampal lesions are impaired on this stage of the task. GluR-A^{-/-} mice successfully acquire the 3/6 spatial reference memory task, making progressively fewer errors as training proceeds, and they are perfectly able to associate a particular arm with reward (Figure 3, middle panel).

Once at a high level of performance, the working memory component of the task is now introduced. The same three rewarded arms are baited as before but now the mice are allowed free access to all arms on each choice and thus can re-enter arms that have previously been visited on that trial. As the rewards are not replaced within a trial, efficient performance relies on a win-shift strategy. Under these circumstances, GluR-A^{-/-} mice continue to make few reference memory errors (they rarely visit the never-baited arms) but they make significantly more spatial working memory errors than their

wild-type counterparts (Figure 3, right panel). In other words, GluR-A^{-/-} mice learn which arms are never baited but cannot remember which arms they have recently visited. This radial maze procedure is important because it clearly dissociates working memory and reference memory spatial processes within the same animals and the same trials, with the same sensorimotor and motivational demands, and using the same spatial cues to guide performance.

T-Maze Alternation

Spatial working memory can also be assessed on a simple T-maze using a discrete trial, rewarded-alternation procedure. In this task, each trial has two runs. In the sample run, one of the goal arms is blocked off, forcing the animal to enter a particular goal arm (e.g., the left arm), whereupon it receives a reward. Prior to the subsequent choice run, the block is removed and the animal is then given a free choice of either arm. If the animal re-enters the previously visited arm, it receives no reward; if it enters the alternate arm (e.g., the right arm), it does receive a reward (see Figure 4, top panel). Thus, accurate performance depends on remembering which arm was visited most recently during the sample run in order to guide appropriate responding in the choice run, with the correct spatial response varying from one trial to the next. The amount of time between the sample and choice runs can be varied to increase/decrease the working memory demands. Rewarded alternation cannot be solved by a fixed association between reward and location because

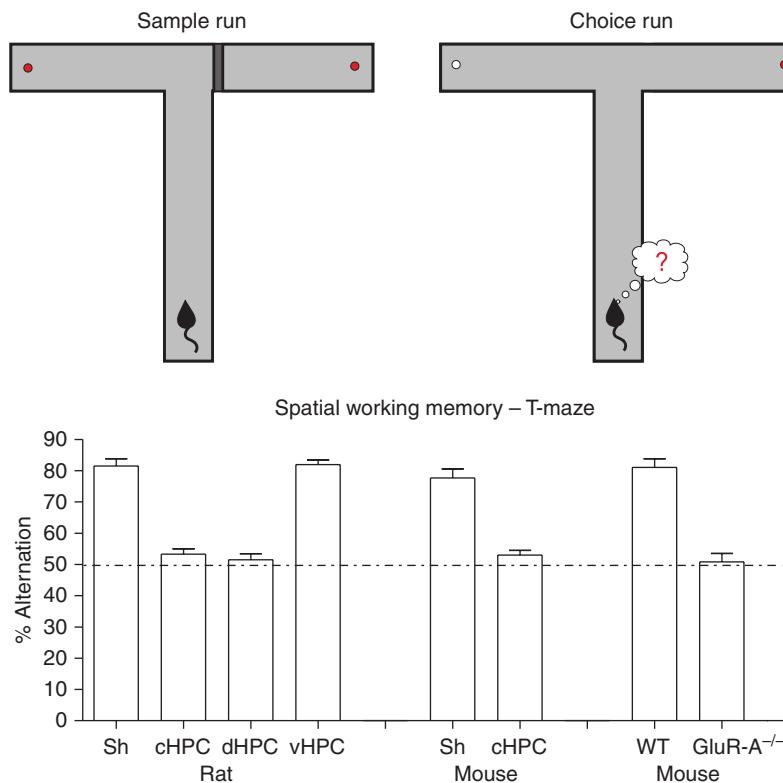


Figure 4 T-maze rewarded alternation. Hippocampal lesions and GluR-A deletion both impair spatial working memory performance during non-matching to place testing (rewarded alternation) on the elevated T-maze. *Top:* During a sample run (*left*) mice are forced into either the left or right goal arm, according to a pseudorandom sequence, and receive a milk reward. During the choice run (*right*) the animals are required to go to the opposite (previously unvisited) goal arm for a second milk reward. Many trials are run over a period of time with the identity of the arm visited on the sample run varying pseudo-randomly. *Bottom:* Mean percentage of trials on which the mouse alternated successfully (\pm SEM). *Left:* Performance of sham, complete (cHPC), dorsal (dHPC), and ventral (vHPC) lesioned rats., *Center left:* Performance of sham and complete (cHPC) hippocampal lesioned mice (unpublished), *Center right:* Performance of wild-type and GluR-A-knockout mice. (*Left*) Reproduced with permission from Bannerman DM, Deacon RM, Offen S, et al. (2002) Double dissociation of function within the hippocampus: Spatial memory and hyponeophagia. *Behavioral Neuroscience* 116: 884–901. (*Center left*) unpublished. (*Center right*) Reproduced with permission from Reisel D, Bannerman DM, Schmitt WB, et al. (2002) Spatial memory dissociations in mice lacking GluR1. *Nature Neuroscience* 5: 868–873.

both arms are rewarded to an equal extent over the many trials of a session. Further, by pseudo-randomizing the allocation of the sample runs to the left and right arms, a continuous alternation strategy is insufficient for optimal performance. Rodents with hippocampal lesions display chance levels of performance on this task. GluR-A^{-/-} mice are also impaired, and also perform at chance levels (see Figure 4, bottom panel), despite their perfectly normal spatial reference memory performance on tasks like the watermaze. Rewarded alternation is also profoundly sensitive to direct infusion of AP5 into the dorsal hippocampus.

Rodents have a natural tendency to alternate their choice of goal arm on the T-maze, in much the same way as they demonstrate win-shift behavior on the radial-maze spatial working memory task. Therefore, they often demonstrate high levels of alternation immediately (typically $>80\%$). In fact, animals do not need to be appetitively rewarded to show this behavior; and in this

case it is called ‘spontaneous alternation.’ This likely reflects short-term habituation to the more familiar arm that was experienced on the most recent sample trial. This can be illustrated with a one-trial spatial novelty preference test.

Spontaneous Spatial Novelty Preference

Conceptually, this task is equivalent to a single trial of spontaneous alternation. In the first stage, the rat or mouse is exposed to two arms of a Y-maze for 5 min. These two arms are designated the start arm and the familiar arm. The novel arm is blocked off during this initial exposure (see Figure 5, top left panel). The animal is then removed from the maze for 1 min and the door to the novel arm is opened. The animal is then placed back into the start arm and allowed to explore all three arms for two minutes (see Figure 5, top right panel). Normal rats and wild-type mice will spend more time in the novel arm

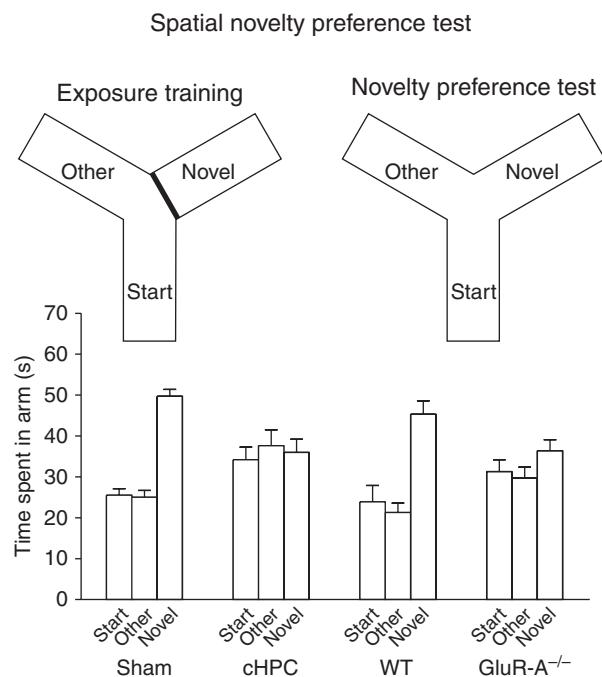


Figure 5 Spatial novelty preference. Hippocampal lesions and GluR-A deletion both impair performance on a spatial novelty preference test. Sham and wild-type (WT) mice exhibit a preference for a previously unexposed (Novel) arm of a Y-maze over two familiar arms to which they have previously been exposed (Start and Other). GluR-A knockout mice and hippocampal lesioned mice do not show a significant preference for the novel arm. Reproduced with permission from Sanderson DJ, Gray A, Simon A, et al. (2007) Deletion of glutamate receptor-A (GluR-A) AMPA receptor subunits impairs one-trial spatial memory. *Behavioral Neuroscience* 121: 559–569.

than the familiar during this test phase, reflecting their habituation to the recently experienced familiar arm(s). Hippocampal lesioned mice show no preference. Similarly, GluR-A^{-/-} mice are also impaired in this task, showing no preference for novel over familiar arms (see **Figure 5**, bottom panel), despite their normal spatial memory performance in the watermaze.

The preference that normal animals show for the novel arm during the spontaneous spatial novelty preference task likely reflects their short-term habituation to the two familiar arms during the initial exposure phase. Similarly, during win-shift behaviors on appetitive maze tasks, normal animals choose relatively more novel over more familiar arms. In contrast, spatial reference memory performance reflects the ability to form associations between spatial locations and outcomes (e.g., an escape platform or food reward). Thus, the impaired spatial working memory observed in GluR-A^{-/-} mice on the radial maze and during T-maze rewarded alternation reflects the fact that GluR-A mediates short-term habituation to recently visited spatial locations, based on a nonassociative memory for the relative familiarity of that

recently visited place. Associative, long-term spatial memory, as typified by performance on reference memory tasks, is GluR-A independent. This division of spatial memory processes into short-term, nonassociative and long-term, associative mechanisms is, in principle, similar to the dual-process memory models that have been proposed for nonspatial stimuli. Thus, although spatial and nonspatial information processing may, to some extent, be subserved by different brain regions, the same basic learning rules and models may apply.

Conclusions

In summary, converging evidence argues that the hippocampal formation is critical for allocentric spatial learning and memory. More recent evidence suggests that separate, dissociable mechanisms may underlie spatial working memory (short-term, nonassociative) and spatial reference memory (long-term, associative). The latter is unimpaired in GluR-A^{-/-} mice. In contrast, performance on spatial working memory tasks, which relies on short-term memory processes, is GluR-A-dependent and could reflect a rapidly induced, short-lasting component of synaptic plasticity; although the direction of the plasticity change (increase or decrease) remains to be established.

Future Directions

Over the last 40 years, great progress has been made in characterizing the anatomical and cellular substrates of spatial learning and memory. However, the repertoire of spatial behaviors in mammals is vast and complex and we are a long way from a complete account. For example, we know little about how different brain areas communicate to facilitate spatial navigation. Moreover, it remains to be determined whether allocentric spatial information is processed within a specialised network or whether spatial information processing is just one manifestation of a more general cognitive process that also applies to certain aspects of nonspatial processing. Answering these questions will pose some of the major challenges for behavioral neuroscientists in the twenty-first century.

See also: Genes and Behavior: Animal Models; Knock-Outs: Learning and Memory; Navigation in Virtual Space: Psychological and Neural Aspects; Neural Representations of Direction (Head Direction Cells); Place Cells.

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Cognitive Decline in Laboratory Animals: Models, Measures, and Validity

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Glossary

Aging – A process that accumulates physiological, psychological, and social changes in an organism over time.

Alzheimer's Disease – A neurodegenerative disorder named after the German psychiatrist, Alois Alzheimer, who first described the hallmarks of the brain symptoms associated with progressive dementia.

Animal model – Animal preparation used to mimic and study a normal or pathological human condition.

Construct validity – A validation approach designed to determine the degree to which an animal model incorporates relationships across multiple levels (molecular, cellular, systems, and functional) of analysis as proposed by theory.

Dementia – Dementia describes an advanced state of declining cognitive functions due to neurodegeneration.

Mild Cognitive Impairment (MCI) – A syndrome characterized by cognitive impairments that are greater than expected for an individual's age and education level but that does not interfere in everyday activities. MCI is widely considered a precursor state of Alzheimer's disease.

descending a stairway), which may reflect limitations in attentional capacities.

Research on the neuronal mechanisms mediating such age-related decline in cognitive capacity in humans is largely limited to neuroimaging. This research demonstrated that older adults engage different and often more – including more bilateral – brain regions, and often exhibit greater activity in key brain regions in order to match the cognitive performance of younger adults. These findings corroborate cognitive analyses suggesting that age-related change in cognitive capacity is not merely due to a loss of function, but that older brains process information qualitatively differently.

Such expansion of brain systems recruited by the aging brain has been suggested to reflect the additional cognitive operations, or efforts, that are required to perform a cognitive task. Greater demands on top-down control of cognitive operations, perhaps associated with a more effective suppression of competing operations, require more processing resources and thus the participation of more regions across the two hemispheres. A more pessimistic, competing hypothesis suggests that additional activity of brain regions reflects the relative inability to suppress competing cognitive activity and the de-specialization or de-differentiation of neuronal systems. Perhaps both hypotheses are correct and refer to different stages of decline, with the latter one predicting a more severe loss in cognitive capacity. This view is also supported by evidence showing that patients with Alzheimer's disease may initially activate brain regions similar to healthy subjects but then fail to deactivate these regions later during the performance of the task.

The evidence obtained from research on the cognitive functions and capacity of aged but nondemented humans – a focus of this article – provides understanding of the cognitive mechanisms and variables that need to be modeled in animals for research on underlying neuronal mechanisms. Attentional impairments, particularly resource limitations – whether resulting from impaired top-down mechanisms and inadequate suppression of competing cognitive activity or true decline in processing capacity – are key cognitive mechanisms that would need to be reproduced in animal models. Tasks for various aspects of attentional functions and capacities have been developed and validated for use in laboratory rodents. Therefore, this component of animal models can be readily implemented.

Cognitive Targets and the Nature of Models

Cognitive performance of aging, nondemented human beings is characterized by slower processing speeds, de-automation of the performance of habits, well-practiced skills and cognitive operations, and reduced attentional capacities, particularly with respect to the ability to divide attentional resources between competing tasks and to switch between tasks ('resource-reduction hypothesis'). These impairments are not restricted to situations that require coping with multiple demands explicitly for cognitive operations. For example, the age-related increase in the incidence of falling and the severity of fall-related consequences have been suggested to be due in part to the more effortful processing required by older people to perform complex movements (such as

In contrast, the literature on age-related cognitive decline in humans provides little guidance for selecting specific neuronal alterations that would be hypothesized to mediate the age-related cognitive decline discussed above. Although there is a substantial amount of data that describes a wide range of morphological and neurochemical changes in the aging brain, evidence that would directly relate such changes to specific aspects and mechanisms of cognitive decline is scarce. This situation causes a major challenge for the validation of animal models. How is one to validate an animal model when the human evidence on cognitive aging does not provide neurobiological benchmark data to directly validate the model? However, this situation is not that different from the challenges of modeling most neuropsychiatric disorders. In the absence of evidence obtained directly from the modeled condition, evidence about the basic neuronal mechanisms that mediate the cognitive functions of interest, and the theories that summarize and conceptualize this basic evidence, may be employed to validate a model.

While such an approach may, at first glance, appear disappointing relative to the modeling of ‘hard facts,’ it represents a productive scientific perspective. First, an animal that reproduces only a neuronal mechanism *per se* does not constitute a model; likewise, a cognitive task is not a model (it is a task). An animal model consists of a model of brain–behavior relationships that reproduces both neuronal and cognitive mechanisms and demonstrates precise relationships between the two levels of analysis (see **Figure 1**).

This view implies that an animal model always represents a theory of the condition to be modeled, and as such, the model itself is subject of research on basic brain–behavior relationships. This is called construct validation; it is the most useful aspect of validity and thus deserves more consideration.

Construct Validity and Other Modeling Issues

The literature examining the validity of animal models is littered with classification schemes, but rarely addresses the perennial question: which model should I choose to model age-related cognitive decline? Should I use senescent rats for my research? Or should I create a lesion of a brain system to produce impairments, or somehow produce abnormal amyloid accumulation, or tangles, or what? Construct validity suggests that these are the wrong questions to ask. The correct question would be: How can we test the cognitive functions of interest in animals? What do we know about the neuronal systems that mediate the cognitive functions of interest? How can we employ the (scarce) information about age-related changes in relevant neuronal systems to produce decline in this neuronal system and thereby cognitive decline? Stated simply, we will use existing biopsychological theories to build a model and then study the relationship between manipulations of key neuronal systems and cognitive decline. Such an approach entails the obvious risk

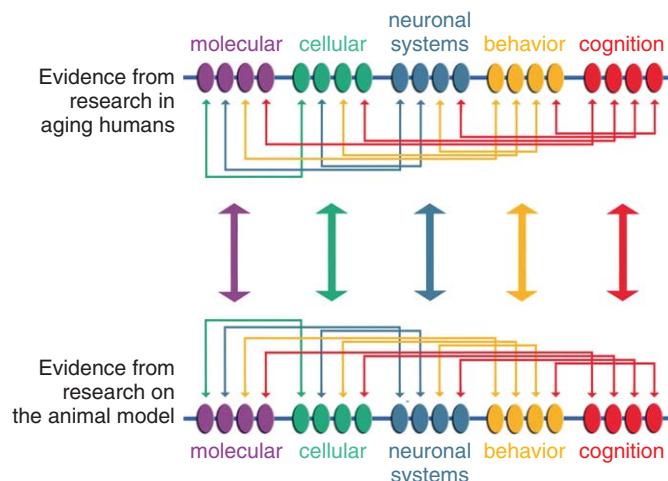


Figure 1 Research using animal models of age-related cognitive decline as well as research in aged human beings test theories about causal and correlational relationships between changes in molecular and cellular mechanisms, impairments in the regulation or integrity of brain systems, and cognitive decline. The main levels of analysis (molecular, cellular, neuronal systems, behavior, and cognition) ideally are presented in research on animal models (bottom) and in research in aged human beings (top). Each research domain establishes relationships between evidence from these levels of analyses (horizontal connections) and, importantly, informs the other domain about such potential relationships. The collective hypotheses concerning the neuronal mechanisms of cognitive decline constitute a theory. The construct validity of an animal model reflects the degree to which relationships between cognitive and neurosystems or cellular changes determined in the model map onto corresponding relationships predicted by theory on cognitive aging and/or are demonstrated in research involving aging human beings (vertical connections; the figure was modified from Sarter M (2006) Preclinical research into cognition enhancers. *Trends in Pharmacological Sciences* 27: 602–608).

that the relationships studied in the model and the new theories created based on the model do not apply to the modeled condition. However, as the model is being investigated, it will evolve in part by integrating new insights from research in humans. Conversely, as cognitive neuroscience research in humans is increasingly informed by results from research using animal models, such cross-fertilization can be particularly fruitful for the process of construct validation.

To reiterate, a model represents a bundle of theories about the brain–behavior relationships underlying the condition to be modeled, and as such it needs to evolve based on bidirectional interactions with human research. This is the essence of construct validation and it is illustrated in **Figure 1**.

Reverse Modeling

Construct validation is an open and ongoing research process that benefits not just the model but that also inspires research on the target condition. For example, evidence suggesting abnormal regulation in the cortical cholinergic input system as a major source for age-related decline in attentional capacity may sponsor efforts to generate measures of the dynamic regulation of cholinergic neurotransmission in humans. Such reverse modeling of hypothesis-driven research (the model dictates research in the condition that is to be modeled) has become more common in recent years and is driven in part by the rigor of tasks developed for the test of cognitive functions in animals. Such tasks have been increasingly adopted for parallel research in humans, thereby fostering interdisciplinary research approaches.

Reverse modeling is further motivated by the unavailability of measures of the dynamic characteristics of neuronal dysregulation in humans. Evidence concerning such dysregulation forms the basis for hypotheses that are testable in humans. For example, postmortem measures of enzymes associated with acetylcholine (ACh) synthesis or metabolism inform about the residual number of cholinergic neurons or terminals, but are not capable of revealing abnormalities in the timing, rise time, or clearance of the phasic cholinergic signals that mediate key aspects of attention. However, research in animals indicates that impaired orchestration of cholinergic signals, at the scale of seconds, may contribute to attentional decline in aging. Obviously, current imaging methods cannot detect such changes; however, studies on the effects of agonists at nicotinic ACh receptors have been shown in animals to modify the cholinergic transients that mediate attentional performance and thus these data can be employed to test hypotheses that predict precise attentional effects of such agonists in the human, including the aging human. Moreover, since brain systems activated by attentional performance have been well characterized in recent years, these hypotheses can be

expanded to predict, for example, drug-induced modulation of mediolateral prefrontal activity in situations that require shifts in attention.

Face Validity

Face validity refers to the degree to which the model, in strictly phenomenological terms, corresponds with overt features of the target. Face validity is a seductive criterion but it is of limited usefulness. Conducting research using old rats, looking overweight and featuring a ruffled coat, and exploring a maze significantly less efficiently than a young animal, seems intuitively attractive and certainly benefits from an enormous amount of face validity – both with respect to the condition of the animal and the overt performance in the maze task. Hypothesis-generating research often depends on, and is informed by, experimental approaches that are justified by face validity. In that respect, aged animals represent a highly valuable resource. However, the deduction of specific brain–behavior relationships from such approaches is a challenge. First, changes in maze patrolling behavior have been shown to result from variations in numerous cognitive and noncognitive variables, including sensorimotor, motor, and motivational variables, rendering the substantiation of changes in terms of impaired learning or memory to be an unexpectedly complicated subject. Second, the exact neuronal condition modeled by an aged animal, and therefore the neuronal basis underlying the behavioral/cognitive change, is difficult to determine. For example, it is not clear whether the brains of laboratory rats reach a sufficiently high age to spontaneously reproduce some of the changes that are considered key to the human-brain aging. The activity of choline acetyltransferase (ChAT) in the cortex of aging humans suggests a continuous thinning of cholinergic presynaptic terminals after the age of 40 (this will be discussed later in more detail). Aged rats, however, even very old ones, show only limited changes in this neuronal system and, indeed, these neurons remain able to release ACh even following strong challenges. Conversely, spontaneously aged rats exhibit numerous physiological, sensory (including retinal degeneration), and endocrinological abnormalities. The frequently used Fisher-344 rat is particularly prone to extensive age-related pathology.

Using spontaneously aged animals, the determination of the neurobiological mechanisms that are responsible for defined changes in behavioral/cognitive performance is extremely challenging. Depending on the biological variable of interest, correlations between chronological and biological aging in rats may not map onto such correlations in humans. The alternative approach examining the cognitive consequences of hypothesis-driven manipulation that influences the brains of young rats to exhibit defined features of the aged human brain affords

experimental control and the ability to generate conclusions about specific brain–behavior relationships. Collectively, face validity-based approaches assist in exploratory research, but as the field progresses and hypotheses begin to describe increasingly specific brain–behavior relationships, they are neither necessary nor sufficient for testing such hypotheses.

Predictive Validity

Predictive validity typically refers to the prediction of treatment effects in human subjects on the basis of treatment effects in animals. In this context, this issue concerns specifically the prediction of attenuation of cognitive decline, more generally, of cognitive enhancement by pharmacological treatments. Several analyses of the available evidence indicate that traditional animal models of cognitive decline failed spectacularly. These models typically combined the use of off-the-shelf or fast-and-dirty behavioral tests with unknown validity in terms of measuring the learning and memory (e.g., object-recognition tests, avoidance tasks), and/or the use of testing procedures that violate such validity, with the use of spontaneously aged animals or animals exhibiting behavioral/cognitive impairments produced by manipulations such as lesions or pharmacological (cholinergic) receptor blockade. These approaches produced an enormous number of false-positive results. The fact that these conventional approaches have remained popular represents a huge concern, in part as clinical trials on the effects of drugs in cognitively impaired subjects continue to be initiated on the basis of evidence from animal models that has been shown extensively to not predict clinical efficacy.

Mismatch between Neurobiological and Cognitive Targets and Task Validity

The rapid accumulation of evidence concerning the neurobiological markers, events, and mechanisms that are altered during nonpathological aging and the dementias (see below for a brief review) has formed the basis for the generation of a considerable number of animal models that reproduce aspects of these neurobiological abnormalities. Efforts to characterize the cognitive status of these animals and, more rarely, cognitive decline frequently utilized tasks that assess cognitive functions that are either unrelated to the neuronal system of interest or of unknown relevance for specifically revealing the cognitive impact of the neurobiological abnormality of interest. The literature provides a classic case to illustrate this issue. Several publications reported the absence of effects of forebrain cholinergic lesions on spatial learning and memory. Such a negative result is predicted by present

theory of cholinergic function, particularly in view of the limited demands on attentional stimulus processing involved in spatial learning and memory of standard maze tasks. Despite this evidence, research has continued to explore the effects of age, and the interactions between the effects of manipulations of the cholinergic system and age on spatial performance. As expected, results were negative and formed the basis for rejection of the general hypothesis that the cholinergic changes contribute in any way to age-related cognitive decline.

This example stresses the importance of a detailed justification of the selection of the behavioral or cognitive function employed to determine the consequences of a particular neuronal manipulation or process (Figure 1). In the absence of such justification, the search for age-related cognitive decline amounts to little more than an indiscriminate hunt for putative effects.

The justification for selecting a task – or of the focus on the test of a particular cognitive function – is necessarily associated with the demonstration that the selected test, indeed, measures this function. The literature has addressed the wide-ranging, serious concerns about the validity of popular behavioral tests and related testing procedures. These concerns about validity focused on traditional learning and memory tasks, such as avoidance tasks, as well as maze tasks and maze-testing procedures. Furthermore, the object-recognition task has recently gained popularity and been claimed to measure declarative memories. The popularity of the object-recognition task appears to be based largely on face validity. However, there is little proof that animals indeed recall discrete and declarative information, in terms of “I have seen this particular object before.” Furthermore, certain testing procedures, such as the presence of a putative drug treatment during massed acquisition sessions, as well as the test session introduced potential confounds associated with the state of the animal while repeatedly exploring the familiar object. The most sophisticated reproduction of a particular age-related neuronal process or event in an animal forms a useful model only in conjunction with the valid assessment of a behavioral or cognitive function that is conceptualized to be affected by this particular neuronal process or event.

Longitudinal versus Cross-Sectional Studies and Confounds Based on Neuro-Stagnation

The modeling of age-related cognitive decline benefits enormously from longitudinal approaches. Compared with cross-sectional designs, longitudinal experiments tracking age-related brain changes offer substantial experimental power due, in part, to the statistical advantage of with-in subject analyses and have the potential to

reveal the dynamic effects of aging on brain–behavior relationships. For example, we demonstrated that limited thinning of the cortical cholinergic input system does not affect rats' performance of a sustained-attention task until the animals reached about two-thirds of their maximal life span. In other words, animals' performance remained comparable to control animals until they reached the equivalent of about 65 years of human age. The exact interactions between this pre-condition and the aging process remain unknown; however, models that address the age-related emergence of attentional impairments perhaps are key to understanding how aging reveals the vulnerability of compromised or inadequately developed neuronal systems.

Such longitudinal models also offer the opportunity to control for the impact of lifelong cognitive activity on (pathological) aging. When conducting cross-sectional studies, aged animals typically have spent over 2 years without practicing any cognitive skills, experiencing adequate social interaction, and, in most cases, are housed in conditions that fail to stimulate cognitive activity prior to their first exposure to cognitive testing. Thus, it is likely that the results of cognitive training and testing at a relatively old age are robustly confounded by the negative neuroplasticity – or better, neuro-stagnation – that is a result of having been reared under socially and cognitively deprived conditions. It is not implausible that such housing conditions cause major behavioral and cognitive abnormalities, including abnormal responses to the presence of conspecifics, being handled, being exposed to new environments, and so forth. As a result, one has to be concerned that the cognitive decline observed in spontaneously aged animals in cross-sectional studies reflects interactions between detrimental housing conditions and the aging process, rather than aging *per se*. This concern may also apply to studies that tested spontaneously aged rats that were screened for robust behavioral and cognitive impairments. It would seem of interest to address the mechanisms that render subgroups of animals more vulnerable to the interactions between the aging process and extended periods spent in socially and cognitively impoverished housing conditions.

The persistent cognitive testing that characterizes longitudinal studies provides a more informative approach for studying cognitive decline when compared with cross-sectional studies. Based, in part, on the scarcity of longitudinal studies in humans as well as in animal models of cognitive decline, evidence concerning extremely fundamental questions, such as whether persistent cognitive practice and associated activation of key brain systems accelerates or in fact delays the consequences of pathological vulnerability.

Neurobiological Targets

Aging in the Absence of Dementia

Longitudinal and cross-sectional morphometric studies indicated that gray and white matter volumes shrink with age, that there are signs of dendritic regression and a widespread decrease in synaptic density primarily in telencephalic regions. Major loss of neurons is not considered to represent a general age-related event in the brains of nondemented patients. The morphological evidence is conflicting and inconclusive, reflecting the limited significance of chronological age as a predictor of neuronal (and cognitive) decline.

The neurochemical evidence likewise has not formed the basis for a general concept of the neuronal basis of age-related cognitive decline. However, considering the key role of the cortical cholinergic input system in the mediation of attentional processes and capacities, early dysregulation and subsequent disintegration of this system has long been hypothesized to contribute to cognitive decline. Immunohistochemical and neurochemical studies indicated a decrease in choline acetyltransferase (ChAT) activity in hippocampal regions, as well as in the temporal lobe of aged subjects, eventually reaching levels similar to those in subjects with Alzheimer's disease. These decreases in ChAT activity reflect loss of cholinergic terminals and perhaps neurons, or at least the loss of cholinergic markers in neurons that show age-related morphological alterations that index their increased vulnerability.

Similar to findings in aged humans, spontaneously aged animals were reported to exhibit morphological alterations in the basal forebrain, primarily neuronal shrinkage. However, robust ChAT loss was not found in aged rodents and measurements of ACh release using *in vivo* microdialysis did not suggest robust changes in basal release or in the capacity of this system to respond to challenges. Thus, with respect to this key neuronal system, the brains of spontaneously aged rats do not appear to age sufficiently. Corresponding with this evidence, the attentional performance of spontaneously aged rats remains stable until a very old age when more general behavioral decline interferes with basic task performance. Thus, as already mentioned in our discussion about the use of spontaneously aged animals (above), and at least with respect to cholinergic systems and attentional capacities, spontaneously aged rats do not appear to represent a useful animal model. Consequently, attempts to model the declining cholinergic system in humans and the associated decline in attentional capacity requires additional, hypothesis-based manipulations in rats in order to reproduce the vulnerability and functional decline of this neuronal system during aging. Such hypotheses concern, for example, developmental abnormalities in the trophic

factor support of cholinergic neurons, the particularly detrimental effects of aggregations of amyloid-beta ($A\beta$)-peptide for cholinergic neurotransmission, or the exquisite vulnerability of the cholinergic system to ischemic events and microvascular disorder.

Despite a considerable literature describing age-related changes obtained at all levels of analysis (from human blood flow and metabolic markers to markers of mitochondrial mechanisms), this evidence provides little guidance for focusing on one over the other neuronal symptom for modeling purposes. This situation has often been cited as a justification for conducting exploratory studies using spontaneously aged animals (discussed above). However, to reiterate, this situation also reflects the limited heuristic power of chronological age as a main independent variable. It may be more useful to study hypothesis-driven changes in neuronal systems in animals that either spontaneously, or as a result of an experimental manipulation, exhibit cognitive decline during the aging process.

Mild Cognitive Impairment

Mild cognitive impairment (MCI) is a psychometrically determined diagnosis of cognitive impairments in subjects older than 50 years of age. The cognitive capacity of these subjects typically is one standard deviation below the mean for the age group and affects between 15% and 35% of the general population. Several studies indicated that MCI represents a precursor of Alzheimer's disease, as the majority of subjects develop dementia within a decade. Research on MCI and animal models of this intermediary stage may generate particularly useful, fundamental insights into the dynamic processes of pathological brain aging and cognitive decline.

As we repeatedly focused on the role of cholinergic systems in the mediation of attentional performance and age-related decline in attentional capacities, it is worth noting that evidence indicates that basal forebrain (BF) cholinergic neurons are not normally regulated in MCI. The number of cholinergic neurons expressing TrkA receptors, a high-affinity receptor for nerve growth factor (NGF), is markedly decreased in MCI patients compared to normal age-matched noncognitively impaired subjects. NGF-mediated signaling via TrkA receptor is crucial for the development, maturation, and function of cholinergic neurons. Despite the absence of cholinergic cell loss in MCI, the number of TrkA-positive neurons in the BF of subjects with MCI was found to be as low as in patients with Alzheimer's disease. Furthermore, TrkA receptor downregulation correlated with cognitive decline in MCI subjects.

The critical role of TrkA receptors for the maintenance of the aging cholinergic system is substantiated by the finding that, in mice lacking this receptor, cholinergic

neurons do not fully mature and begin to atrophy during the early weeks of postnatal life. Furthermore, in mice with segmental trisomy of chromosome 16, which models aspects of Down's syndrome, decreases in BF TrkA immunoreactivity predict behavioral and cognitive impairments. Collectively, these studies suggest that loss of TrkA receptors disrupt maturation of cholinergic neurons and eventually lead to degeneration of these neurons and cognitive impairments.

The mechanisms that lead to the suppression of TrkA-mediated NGF signaling, and the ontogenetic regulation of this defect have remained completely unclear. However, the evidence collectively suggests that an animal that reproduces early disruption of TrkA suppression and that exhibits age-related decline in cholinergic signaling and associated attentional performance may be an extremely useful model. Such a model may also assist in revealing the developmental nature of the mechanisms that evoke age-related cognitive decline, including the age-related mechanisms that interact with such a pre-existing condition to evoke such decline.

Alzheimer's Disease

Plaques and tangles

Substantial evidence suggests that cerebral deposition of amyloid precursor protein (APP) product, $A\beta$ -peptide, plays a critical role in the pathogenesis of Alzheimer's disease. $A\beta$ is suggested to be an early pathological biomarker for Alzheimer's disease and associated cognitive and behavioral deficits. Genetic linkage studies suggested the involvement of a genetic mutation in APP gene on chromosome 21 in familial Alzheimer's disease. Approximately 20 pathological mutations in APP have been identified. Mutations in APP do not account for all cases of familial Alzheimer's disease. A large number of genetic linkage studies have identified mutations in Presenilin 1 on chromosome 14 and Presenilin 2 on chromosome 1. Presenilins are associated with the formation of active site of γ -secretase complex that is involved in the production of $A\beta$. Finally, a variant of a gene coding for protein involved in $A\beta$ clearance, that is, the presence of apolipoprotein E (ApoE) $\varepsilon 4$ allele, represents a risk factor for nonfamilial (or sporadic) Alzheimer's disease. It also needs to be noted that there is substantial evidence linking abnormal APP processing with cholinergic decline, including the regulation of the high-affinity choline transporter, the capacity of which is critical for ACh synthesis.

Transgenic mice overexpressing APP, such as TgAPP22 mice, exhibit amyloid plaques and deficits in standard laboratory tasks for the measurement of learning and memory. Likewise, mice overexpressing Presenilin-2(PS2) reproduce essential neuropathological features. More recent strategies led to the development of

double/triple transgenic mice to mimic the multiple neuropathological markers of Alzheimer's disease, including double transgenic mice that co-express human PS1 and APP, or triple transgenic mice harboring a PS1_{M146V}, APP_{Swe}, and tau_{P301L} transgenes. These latter mutants also exhibit cholinergic deficits and cognitive impairments.

Clearly, these molecular advances offer a rich set of neurobiological targets for the development of animal models. It is unexpected, therefore, that research using these animals has rarely demonstrated a precise relationship between the degree, density, or amount of neuropathological product and specific aspects of cognitive decline. As we discussed above, such relationships are key to a productive use of these models, including for predicting the efficacy of potential treatments. The widely held assumption that reproducing the neuropathological markers alone represents a complete animal model, and that reducing, for example, A β levels or plaque burden *per se* is sufficient to predict improvement of the cognitive status of patients interferes with the important test of the hypothesis that the presence of these markers is causally responsible for the severity of cognitive decline.

Cerebrovascular disorder

Cerebrovascular pathology is now increasingly recognized as a causative factor for all dementias, including Alzheimer's disease. At least 20% of subjects diagnosed with MCI develop vascular dementia supporting the general notion that acceleration of cognitive decline and its underlying neuropathology involve vascular dysfunction. Decreases in cerebral blood flow as a result of transient ischemic attacks (mini-strokes) are proposed to act as a significant causal factor for the emergence of cognitive decline in Alzheimer's disease. Vascular lesions in cortical associational regions predict the severity of dementia, and Alzheimer's disease has even been conceptualized as a vascular disorder with neurodegenerative consequences.

Forebrain cholinergic neurons innervate and dilate cortical microvessels directly via muscarinic or nicotinic receptors, as well as indirectly via a local cortical nitric oxide transmission. Cholinergic lesions produce decreases in cerebral blood flow. Decreased cholinergic activity impairs cortical perfusion and metabolic activity and thus enhances the risk for ischemic events. Furthermore, as a result of cholinergic cell loss in advanced Alzheimer's disease this predicts a reduced capacity for cerebral microvascular dilation and thus an increased vulnerability for ischemic events. Ischemic events and microvascular disorder have been suggested to trigger the production of excitotoxic and inflammatory mediators that may reduce further cortical cholinergic activity and cholinergic innervation. Moreover, ischemic attacks associated with microvascular disorders may contribute to the accumulation of A β and neurofibrillary tangles. These highly

reciprocal interactions between the consequences of abnormally regulated cholinergic system and impaired cerebrovascular function, perhaps as a result of mini-infarcts occurring at the time of onset of the dementia, may trigger essential pathological cascades.

Given the predominance of models focusing on the aggregation of A β and tau, the modeling of cerebral microvascular disorders, including the demonstration of relationships between the severity of such disorders and age-related cognitive decline, appears to be a pressing research subject. We demonstrated that intracarotid administration of microspheres produces embolism and microvascular disorder and impairments in attentional abilities in rats. It would be important to employ these and other models to study the age-related consequences of mini-infarcts, perhaps in interaction with abnormal protein aggregations and decline in the regulation of key neuronal systems.

Developmental Animal Models of Age-Related Cognitive Decline

Why is it the case that only 15–35% of the aging population develops MCI? Is it really the case that lifelong cognitive activity protects against age-related cognitive decline, or do such correlations in fact reflect self-selection for sustained cognitive activity based on a healthier and thus more efficacious brain early in life?

Although chronological age is a robust predictor for cognitive decline, it appears unlikely that major neuronal events manifest fairly rapidly because human beings reach 50 or 60 years of age, resulting in MCI or even dementia. Rather, it appears likely that age-related decline of brain systems mediating cognitive functions are a result of vulnerabilities that result from neurodevelopmental aberrations, perhaps interacting with later cardiovascular and cerebrovascular, infectious, or immunological mechanisms to trigger age-related cognitive decline. Additionally, head traumas and vascular events are risk factors and as such may interact with developmentally established imperfections in neuronal wiring, synaptic mechanisms, dendritic organization, and so forth to evoke age-related cognitive decline.

These considerations suggest that efforts to model age-related cognitive decline may need to integrate manipulations that cover the entire life span, perhaps beginning prenatally and involving the implementation of multiple risk factors throughout adolescence and adulthood. Perhaps a single event during neurodevelopment is sufficient to produce MCI-like cognitive decline in aged animals, or perhaps a multitude of variables will need to interact over longer periods of time to establish age-related vulnerability. As we stressed above, the scarcity of longitudinal studies combined with models that rarely

focused on the demonstration of specific, selective, and age-related relationships between neuronal and cognitive mechanisms (**Figure 1**) and the indiscriminate use of behavioral tests with limited or unknown validity in terms of measuring a particular cognitive capacity – have collectively contributed to the rather sobering view that despite a sizeable literature there is little evidence to begin addressing the very basic, mechanistic questions about the bases of age-related cognitive decline.

The famous Nun study demonstrated that the density of ideas and the complexity of the grammar in the autobiographies written by the sisters in their early 20s predicted their cognitive status late in life. If pathological aging begins early in life, then the modeling of this condition likewise needs to begin with neuronal manipulations or events that occur early in life.

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See also: Aging and Cognition; Brain Aging: Structural Imaging Biomarkers of Risk of Alzheimer's Disease; Cholinergic Systems in Aging and Alzheimer's Disease:

Neurotrophic and Molecular Analysis; Memory and Aging, Neural Basis of; Value of Animal Models for Predicting CNS Therapeutic Action.

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Communication of Emotions in Animals

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Glossary

22-kHz vocalization – Ultrasonic calls emitted by rats in dangerous and aversive situations with the peak sound frequency close to 22 kHz (usually 20–25 kHz) and call duration from 100 to 3000 ms. Rats receiving these calls show a number of defensive responses, including freezing, escape, and hiding.

50-kHz vocalizations – Ultrasonic calls emitted by rats in social and appetitive situations with peak sound frequency close to 50 kHz (usually 40–60 kHz) and call duration from 20 to 70 ms. Rats receiving these calls show affiliative responses, including approach and play.

Cholinergic system – Any of the brain systems consisting of neurons synthesizing acetylcholine as the neurotransmitter and releasing it at the terminals of their axons. The tegmental cholinergic system pertains to the group of neurons in the laterodorsal tegmental nucleus, which projects to the forebrain limbic regions.

Mesolimbic dopamine system – A brain subsystem of neurons synthesizing dopamine as the neurotransmitter and releasing it at the terminals of their axons. The mesolimbic dopamine system originates from the ventral tegmental area and projects to the forebrain, and predominantly to the nucleus accumbens.

researchers agree that emotional states enhance the organism's interaction with the environment – by prolonging certain internal states, amplifying experiences, and thus allowing for better evaluation of salient stimuli and situations critical for survival. Emotional states are also critical for anticipatory capabilities, which allow the animal to organize its behavior in light of potential events rather than actual events. Although the evolutionary appearance of emotional states seems to be understandable, the question arises: why do animals express their emotional states and inform other individuals, including their opponents, about their state? One may argue that hiding an organism's emotional state rather than demonstrating it would be a more beneficial solution. Although animals may overtly express their emotional states or show an expressive suppression that masks their inner states, the former phenomenon is overwhelmingly dominating, as emotional expression has a highly adaptive value.

Situations Promoting Emotional Expression

The expression of an internal state might have limited biological value without the presence of other individuals of the same or other species. Conversely, the presence of other individuals has a catalytic effect on the expression of these states. Expression of emotional states was probably an effective tool in promoting, creating, and facilitating social interactions among animals and building and enhancing biologically successful social groups. Thus, the expression of emotional states gained biological significance as a social phenomenon and, as such, is imminently associated with communication among animals. Although not all communicative signals are emotional expressions, the vast majority are. Darwin strongly emphasized the communicative aspect of emotions. It is worth mentioning that both the expressive repertoire of the displaying animal, as well as the effects of that expression on receivers and their receptivity and acuity coevolved and were mutually interdependent.

The phylogenetically oldest situations promoting such a communication would be in reproductive contacts, while raising offspring, and when interacting with predators. Effective expression of internal states is equally adaptive for infants, their mothers caring for them, and during defense against predators (although, in the latter

Charles Darwin, in his book entitled *The Expression of Emotions in Man and Animals*, published in 1872, provided the first modern biological explanation of the expression of affective states in animals and its evolution and homology among species. Although Darwin's ideas and explanations were based on limited observational evidence, new data and facts reinforce and support most of Darwin's hypotheses, which were largely ignored by researchers for almost a century. Darwin was convinced that not only observable expressions of emotions ('expressive movements') but also the underlying brain processes ('states of the mind') were subjected to the evolutionary process. He argued that certain emotional expressions are innate, and formulated one of his principles about the 'direct action of the nervous system,' that is, that the activity of the central nervous system produces and directly regulates mechanisms of these expressions.

Emotional states have evolved in animals over a long period of time as biological adaptations increasing the likelihood of survival and successful reproduction. Most

situation, it is usually the expression of emotional state by adults and expressive suppression by infants.

Expression of emotional states in animals may be classified into interspecific and intraspecific communication. Interspecific communication involves communicating signals directed to the predator or potential predator, while intraspecific communication may be further subdivided into a form of dyadic communication in which only two individuals of the same species communicate with each other, or as a group communication in which one or more organisms express their state to many individuals – usually to the whole social group or colony. Despite some similarities in biological functions of these expressions, the dyadic and group situations differ from each other and the relevant emotional expressions may also differ. For instance, in some forms of defensive or appetitive behavior, the presence and size of the social group have an enhancing effect on emotional expression.

The expression of an emotional state may be autocentric (i.e., serving the immediate individual needs of the displaying animal) or allocentric (i.e., the entire social group may benefit from the display). Autocentric expression of emotions is displayed in situations such as defending against an aggressor, forcing an opponent to retreat, soliciting sexual activity, or begging for food, and occurs mostly in dyadic communications. Allocentric expression of an emotional state may be demonstrated by alarming postures or vocalizations or some cooperative behaviors, generally occurs during group communication, and is usually dependent on the presence of many individuals.

Biological Functions of Emotional Expression

Interspecific communication involves mostly agonistic interactions, such as deception, threat, warning, and intimidating displays directed to the predator or to another, usually larger, animal posing a potential danger. For example, rodents will readily express their emotional state of fear or anxiety toward a cat, dog, or human. Occasionally, interspecific communications can be appetitive in nature, as between a human and a dog or among some household animals of different species who live together with the human.

Biological functions of intraspecific communication could be subdivided into numerous categories, including dyadic and social group interactions. Each function may contain, to a varying extent, an emotive component (emotional expression), that is, information about the emitter's internal emotional state and its valence. These functions include: (1) a locating function that might be the oldest form of communication, in which an individual signals its presence and enables its localization; (2) an identifying

function that allows for individual recognition and facilitates guiding one animal to another; (3) a monitoring function that facilitates watching the environment for potential danger, in which one animal relies on the signals from other animals with regard to relative safety; (4) a conative function, which mobilizes or recruits the recipient(s) to action in a general way or activates their attention; (5) a calming function – which extinguishes unnecessary activation or commotion in a general way; (6) an alarming function that informs about an actual or potential danger and promotes defensive responses; (7) an agonistic function, which has a breaking-contact role and promotes escape, withdrawal, or dispersion; (8) an appeasement function that reduces the possibility of (conspecific) attack; (9) an affiliative function, which signals approach, promotes conspecific contacts, and attracts individuals to each other; (10) a phatic function, which maintains connections among individuals, enhances cohesiveness within the social group, plays a reassuring role as to the close presence of the group members, and/or makes them aware of their mutual presence and proximity; and (11) a hedonic function, which informs about joyful situations and is most frequently (but not exclusively) observed during ludic behavior (playful behavior) of juvenile and adolescent animals. It has been noted that the most frequent signals used in animal communication pertain to negative affect (fear, threat, anxiety, etc.).

Most of these functions serve the entire social group, and emotional expressions of individuals are addressed to other members of that group. Ethological studies of many mammalian species have shown that emotional expression of an individual organism is subjected to the phenomenon of emotion contagion, an adaptive mechanism by which emotional state may be quickly transferred across the social group, with many other members of the social group repeating the emotional expression of the initial signaler and changing their behavior. This phenomenon is particularly clearly observed in recruiting or alarming situations but is not restricted to these situations. Emotion contagion may also have a calming function. In some situations, emotion contagion may lead to an overreaction of the group in a form of panic behavior or outbreak of agonistic interactions.

Forms and Physiological Channels of Emotional Expression

There are a large number of possible forms of emotional expression used by animals, with nearly every sensory modality utilized in this process. Signals can be conveyed by visual, olfactory, gustatory, tactile, acoustic, or even electric discharge displays (in fish). Forms of emotional expression include posturing, expressive movements, production of odors or pheromones, or release of other

chemicals, nonvocal acoustic, or vibrational signaling (sounds produced by friction, beating the ground or water surface, or own chest, as in gorillas), emission of vocalizations (by blowing air through an orifice), or even electrical phenomena (although mammals do not use electric discharges for communication, electroreception has been documented in monotremes). Mammals have the most diversified and versatile forms of emotional expression and communication as compared to any other taxon. Most mammalian species rely on two or more of these physical forms for unambiguous communication, involving a combination of olfactory, visual, tactile, and vocal signals.

Emotions are not expressed by unchanging structural attributes of organisms, such as bright spots of coloration (badges) on the animal's body, although such nonbehavioral signals do serve as intraspecies communication and may acquire, for instance, warning value in some situations. However, emotional expressions can be communicated by a number of physiological channels, which include somatomotor, hormonal, and autonomic responses. Somatomotor responses include expressive whole-body posturing (kinesis) – particularly, position and movements of head, ears, and extremities; active facial expression; and vocal expression (vocalization). Several mammalian species pay particular attention to facial expression, position of eyelids and eyebrows, eye movements, and direction of the gaze, which might be more important than body posture. For example, looking straight into an individual's eyes may have aggressive and intimidating properties (dogs, monkeys, the human, etc.), while gaze directed in another direction may point out a source of danger or object of interest. Autonomic responses involved in the expression of emotional states include changes in breathing pattern, skin coloration, and appearance of the eyes (e.g., size of pupils, drooping eyelids, exophthalmos, etc.); piloerection; tremor; external secretions (salivating, drooling, lacrimation (tearing), sweating, producing odors, etc.); and micturition and defecation. Many autonomic expressions play an important role for animal communication (e.g., piloerection along the arched back of a cat) but a lesser role in human behavior (e.g., bulging blood vessels on a forehead).

Despite the numerous forms of emotional expression, precise communication is limited, particularly as to its quantitative value, except in vocal communication.

Vocal Communication and Emotional Expression

Utilization of vocal signals has numerous advantages over any other forms of communication, and vocal communication is the most widespread, complex, and elaborate

form of emotional expression. Mammals have developed a sophisticated system of sound production by the vocal apparatus (the larynx) and by modulatory and articulatory mechanisms of supralaryngeal sound modification. There are several biological reasons for this development.

Sound has many advantages over any other forms of communication, particularly in emotional communication, which frequently takes place in biologically dangerous situations. Sound can travel over a relatively large distance and its reception is not dependent on daylight. The transmission rate of acoustic signals is practically immediate and signals could be terminated instantly. Sound can also travel in water, in underground tunnels, and in dense vegetation (albeit for a shorter distance). Sound fades away quickly, leaving no permanent trace. Acoustic signals can vary greatly in sound frequency (pitch), signal duration, and loudness. Acoustic signals can also vary immensely in temporal pattern (e.g., frequency-modulation pattern and repetition rate). The combination of all of these parameters offers an unusually diverse freedom for coding signals. Most mammalian species use both changes of acoustic sound parameters as well as temporal patterning in coding their signals. As a result, mammalian vocal signaling and vocal emotional expression represent the richest collection of coded signals with an almost infinitive number of possible outcomes.

Evolution has favored different vocal outcomes and codes, depending on the species – their physiological and anatomical features, the environment of their habitation, and biological and ecological conditions. Although most mammalian species emit sounds within the human hearing range (sonic range of 20 Hz to 20 kHz), some mammals can utilize infrasounds (below 20 Hz, such as elephants, whales, and tigers), or ultrasounds (above 20 kHz, such as bats or rodents). Many species can produce sounds spanning from low frequencies up to the kHz range (humpback whales) or from sonic to ultrasonic frequencies (e.g., juvenile rats). Whales produce the loudest vocalizations among animals because they need to communicate over the longest distances.

Production of vocal sounds (i.e., those produced by the respiratory system) may have many mechanisms. It is believed that primates and whales have attained the highest sophistication of sound production among mammals. Most mammalian vocal sounds are laryngeal in origin, but sounds of toothed whales are produced by a specialized nasal system (phonic lips and nasal sacs), and thus are not homologous with those originating from the laryngeal system of other mammals. The mechanism of laryngeal sound production is also not uniform. Most mammalian species use vibration of vocal folds; however, baleen whales have a modified larynx without vocal folds. Finally, rodents use a stabilized larynx as a whistle without vibration of vocal cords. Vocal folds are used to

narrow the orifice for airflow, and in this way the vibrating air column produces sound that can reach high ultrasonic frequency. It is important to point out that all of these mechanisms of sound production are governed and regulated by complex control mechanisms of the central nervous system.

Neural Substrate for Vocal Emotional Communication

It has been known for more than half a century that the brainstem and limbic system are responsible for the initiation and control of species-specific vocalizations. This system is also a main substrate for the generation and regulation of affective states with concomitant behavioral manifestations, including vocal expression of these states. Despite enormous variability in emitted vocalizations, the neural substrate for their production is well preserved across mammalian species. The main components of the brainstem and limbic system remain very similar with only slight changes across mammalian species, including the human.

The attention of researchers was focused initially on the brain structures involved in the initiation of vocalization and concomitant affective state as a result of early studies with electrical stimulation of various brain regions in awake animals. In 1928, Swiss physiologist Walter R. Hess published the first description of an affective expression induced by electrostimulation of the cat's brain, and then fully mapped the brain system responsible for control of autonomic functions and release of affective manifestations and expressive vocalization. This system for vocal expression included the septal, hypothalamic-preoptic, amygdala, fornix, periaqueductal gray, and other brainstem regions. The highest level of the limbic system from which vocalization could be induced by direct stimulation appeared to be the cingular gyrus, but only in monkeys.

Subsequent studies of emotional expression and affective vocalization suggested a descending hierarchical organization of brain control of this expression. The lower level of the central nervous system in the brainstem (reticular formation, structures of pons and medulla) contained motor nuclei controlling vocalization and respiratory movements. The periaqueductal gray, hypothalamus, and limbic system contributed to motivational/emotional aspect of this expression. Finally, the cingular gyrus, a limbic cortical area, was suggested to be involved in the voluntary initiation of vocal expression.

Direct stimulation of the brain with chemical agents (chemostimulation) and the development of behavioral neuropharmacology in the early 1960s brought new insight into the organization of limbic structures in the regulation of vocal expression of internal states. Several

pharmacological agents (mostly cholinergic, e.g., pilocarpine, tremorine, carbachol, arecoline, eserine, and d-tubocurarine) were reported to have properties of inducing different species-specific vocalizations in the cat following central application. Thus, the central cholinergic system has been implicated in the initiation of species-specific vocalization as an emotional expression.

Comparable responses to cholinergic muscarinic stimulation were discovered in rats, except that these animals emitted ultrasonic vocalizations. Following a thorough mapping of the cat and rat brains for vocal responses induced by intracerebral injection of carbachol, a predominantly muscarinic agent, a similar brain system was identified to that known from studies utilizing electrostimulation. This system, termed the medial cholinoreceptive vocalization strip, extended from the tegmentum and the periaqueductal gray, through the medial hypothalamic-preoptic regions and the bed nucleus of stria terminalis, up to the lateral septum. The main difference between results of electrostimulation and chemostimulation was that, while results of the former studies pointed to the descending system to the brainstem, the latter revealed an ascending system from the tegmentum to the forebrain and identified a specific transmitter, acetylcholine.

In recent years, intracerebral application of dopaminergic agents into the nucleus accumbens induced a different type of species-specific vocalization in the rat and implicated a second neurotransmitter in the initiation of vocalization. It appeared that another ascending tegmental system, the mesolimbic dopamine system, is involved in vocal expression of positive affective states. The laboratory rat became the best-studied mammalian species with regard to the organization of the neurochemical systems regulating emission of vocalization and vocal expression of affective states.

Neurochemical Control of Vocal Communication of Emotions

Rats are highly social animals and have developed a complex system of vocal communication. They use predominantly ultrasonic vocalization as a defensive measure against numerous predators. Adult rats emit two different types of ultrasonic calls, termed 22 kHz calls and 50 kHz calls. It has been observed that 22 kHz vocalizations are mostly associated with aversive situations and retreat behavior, and 50 kHz vocalizations with appetitive situations and approach behavior. It has been suggested that 22 kHz calls evolved as antipredator alarm behavior, while 50 kHz vocalizations are affiliative calls promoting approach and cooperation among conspecifics. As 50 kHz calls were observed to be particularly abundant in juvenile rats during play behavior, it was also suggested that

these calls represent a rodent primordial counterpart of human laughter.

Numerous recent studies strongly suggest that 22 kHz vocalizations are associated with negative states and expression of negative affect, and that 50 kHz vocalizations express positive states and positive affect. These two types of calls have substantially different acoustic structures, guaranteeing an unambiguous recognition of these categories of signals by recipients. In addition, rats can emit only one of these types of calls at a given time and mixed vocalizations are almost never observed. Only in rare situations of behavioral ambiguity may rats emit both types of calls.

Neuropharmacological studies of rats have led to the determination of two neurotransmitter systems responsible for initiation and emission of affective calls. Chemical stimulation of structures along the cholinergic vocalization strip with cholinergic muscarinic agonists invariably induced 22 kHz vocalization. It has been suggested that the ascending cholinergic pathways from the laterodorsal tegmental nucleus to the basal forebrain, medial hypothalamus, and septum that release acetylcholine at their terminations will initiate negative emotional states that may be expressed by 22 kHz alarm vocalization. On the other hand, application of dopaminergic agents (amphetamine, quinpirole, etc.) into the nucleus accumbens, a structure known to be associated with reward, appetitive processes, and anticipation of reward, invariably induced 50 kHz vocalizations. The ascending mesolimbic dopamine system from the ventral tegmental area to the basal forebrain, olfactory tubercle, and nucleus accumbens that releases dopamine at its terminals was suggested to initiate positive emotional state, which may be expressed by 50 kHz affiliative vocalizations. Several neurochemical and cellular studies further suggested that these two ascending systems work in a mutually opposite manner; that is, an increase in activity of one of these systems will cause a decrease in the activity of the other, and vice versa. Thus, acetylcholine and dopamine seem to be specific neurotransmitters in initiating negative or positive states and vocal expression of these states. Such results could not be obtained using intracranial electrical stimulation, which was not specific to any neurotransmitter system and often induced mixed behaviors.

Although the neurochemical control of emotional expression and ultrasonic communication has been studied mostly in rats (and partially in cats), the acetylcholine–dopamine balance between the ascending tegmental systems is most likely common to all mammalian species, including the human. The ascending direction of tegmental projections indicates that these systems are involved in changes of the general and affective states in animals; the descending pathways from the limbic system to the brainstem motor nuclei will then initiate the actual motor acts for vocalization. It is also important to mention that there are several other

neurotransmitters involved in the control of emotional states and vocal expression; however, acetylcholine and dopamine seem to be critical for the initiation of these states as a first and necessary step in emotional expression.

Emotional Communication as an Interactive Process

In agreement with the theory of communication, the expression of emotions is meaningful only in the context of the interaction between a sender and a receiver. The emitted signal has to contain encoded semiotic value (a message); however, it is meaningful only if the receiver can decode the signal and respond to it appropriately. In early ethological studies, such communication was usually understood as a unidirectional process and discussed in terms of costs and benefits to the sender as compared with the receiver. Recent studies indicate that expression of emotional states is a dynamic and interactive process between senders and receivers. For example, in dyadic interactions, vocal signals are produced not only to influence the behavior of the other animal; the sender also continuously responds and adjusts its behavior according to the behavior of the receiver. Affective communication can be interpreted here as an interactive management/assessment process, and not as a unidirectional act of sending information to the receiver. Thus, emotional expression may not only inform other conspecifics about the affective state of the sender, it also probes the affective state and responses of the receivers. This process involves both sending and gaining information from other individuals and may have a feature of continuous dialog probing the state of other companions. As a result, the sender and the receiver make predictions about future behavior and can reach adequate decisions. These observations provide a better understanding of the process of emotional expression and its adaptive value in a social group.

Finally, the question arises as to which part of the brain is responsible for the process of assessment and decision making as to the outcome behavior, including vocal expression of emotions. Although not understood in detail, this process is likely to occur at many levels of the central nervous system, and may involve the acetylcholine–dopamine balance in the ascending tegmental system. Thus, the interaction between acetylcholine and dopamine may take part in the process of decision making and selection of optimal behaviors in the context of emotional communication.

See also: Emotions; Evolution of Emotions; Fear, Anxiety, and Defensive Behaviors in Animals; Human Fear and Anxiety; Neural Bases of Defensive Aggression; Neural Substrates of Conditioned Fear and Anxiety; Neurobiology of Offensive Aggression; Offensive and Defensive

Aggression; Physical and Emotional Pain; Subjective Experience and the Expression of Emotion in Man.

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Depression

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Glossary

Diagnostic and Statistical Manual (DSM-IV) of the American Psychiatric Association

A manual published by the American Psychiatric Association, providing diagnostic criteria for mental disorders. It is used by mental health professionals, researchers, psychiatric drug regulation agencies, health insurance companies, pharmaceutical companies and policymakers to standardize diagnostic classification.

Endophenotype – The term ‘endophenotype,’ in a psychiatric context, was coined by Gottesman and Shields in the early 1970s, where they described it as an internal phenotype that emerges from the pathway between genes and the disease state. Fundamental to the concept is the assumption that the genetic basis of variations of the given endophenotypes between patients and control subjects are fewer than those involved in the manifestation of a complex disorder *per se*. Thus, employing endophenotypic approaches provides a means for identifying the genetic basis of specific clinical phenotypes, in addition to aiding the analysis of the phenotypic consequences of genes being turned on or off.

Intracranial Self Stimulation (ICSS) – Also called brain-stimulation reward, this refers to the phenomenon in which animals will perform an operant task to receive an electrical current delivered through electrodes implanted in certain parts of their brains, such as the lateral hypothalamus.

Long-term potentiation (LTP) – The long-lasting improvement in communication between two neurons that results from stimulating them simultaneously. LTP is widely considered one of the major cellular mechanisms that underlies learning and memory.

laboratory testing. In clinical practice, many tools have been developed and validated to better diagnose depression and the efficacy of treatment strategies in humans. These range from the *Diagnostic and Statistical Manual (DSM-IV)* of the American Psychiatric Association to the various rating scales such as the Hamilton depression scale. Further, clinicians also rely on self-reporting from patients for the diagnosis in depression and it goes without saying that no such tools are available when we shift to animal models. Indeed, many of the human symptoms of depression as described in the DSM-IV (such as recurring thoughts of death or suicide or having excessive thoughts of guilt) are impossible to be modeled in rodents. The question therefore remains impenetrable as to whether we can ever know whether a rodent is depressed. It is clear that evolutionary progression has enabled humans with a much more elaborated cerebral cortex than rodents that facilitates integration of complex psychological concepts also relevant to human depression, such as self-esteem and the ability to perceive the future, that are absent in rodents. Nonetheless, there are also many fundamental physiological and behavioural responses that have been evolutionarily conserved between species, in order to regulate homeostasis. Therefore, largely through inference, we can exploit these latter responses to elucidate phenotypes relevant to emotional behaviors. Moreover, it is becoming clear that increased interaction between clinical and basic sciences is allowing new insights into the generation of tractable, valid, and translational animal models.

An ideal animal model of depression would have identical causative factors, symptomology and treatment modalities to the disease. Despite these hurdles a number of diverse animal models of depression have been widely utilized and many show substantial construct face and predictive validity (i.e., antidepressant administration reverses the behavioral parameters assessed). These animal models are often referred to as tests of antidepressant-like activity given that they have all been validated since the introduction of clinically approved medications. This also renders the predictive validity of the models unclear until a novel acting compound from preclinical testing is successfully applied in man. The distinction between a model and a test is not always made clear and as a result, sometimes a test is called a model. A model comprises both an independent variable, known as the inducing manipulation, and a dependent variable that is a behavioral/neurochemical readout, whereas a test simply

Introduction

Depression is one of the most serious illnesses in today's society. Moreover, it is a heterogeneous disorder with symptoms manifested at the psychological, behavioral, and physiological level and which lacks a clearly defined and specific pathology, thus providing great difficulty in attempting to mimic the disorder in the laboratory. Most attempts have focused on using rats and mice for

comprises the latter variable. As the underlying pathophysiology of mood disorders is poorly understood, this has had a knock-on effect in choosing independent variables. In recent years, the increased clinical experimental evidence has, however, provided the preclinical researchers with more information to design the independent variable and therefore garner more information about the underlying aetiology of mood disorders. This has also been aided by the relatively recent focus on an endophenotype-based approach to study psychiatric disorders.

Animal models of antidepressant-like activity and depression

The Forced Swim Test

The forced swim test (FST) is the most widely used tool for assessing antidepressant activity preclinically. This is a model of antidepressant activity as opposed to depression *per se*. The widespread use of this assay as a model of antidepressant activity is largely a result of its ease of use, reliability across laboratories, and ability to detect a broad spectrum of antidepressant agents. The test is based on the observation that rats, following initial escape-oriented movements, develop an immobile posture when placed in an inescapable cylinder of water. If they are replaced in the testing apparatus 24 h later, they resume this posture quickly. The immobility is thought to reflect either a failure of persistence in escape-directed behavior (i.e. behavioral despair) or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli. If antidepressant treatments are given between the two exposures, the subjects will actively persist engaging in escape-directed behaviors for longer periods of time than after vehicle treatment. Investigators have been able to distinguish specific behavioral components of active behaviors, namely climbing and swimming behavior which have been able to selectively distinguish noradrenergic (increasing climbing) from serotonergic (increasing swimming) antidepressants. In mice, one exposure is sufficient to generate a stable immobility readout that can be countered by acute pretreatment with antidepressant agents. One major drawback of the FST (as with many antidepressant-sensitive paradigms) is the fact that short-term antidepressant treatments reverse the immobility, whereas in the clinic it can take weeks for the same antidepressants to elevate mood. As with many behavioral models motor performance can also affect behavior in this test and needs to be controlled for by running parallel locomotor activity assays.

The Tail Suspension Test

Since its introduction over 20 years ago, the tail suspension test has become one of the most widely used models for assessing antidepressant-like activity in mice. As is the case with the FST, it is a model of antidepressant activity and not a model of depression as there is no distinction between readout and inducing manipulation. The test is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail, will develop an immobile posture. Various antidepressant medications reverse the immobility and promote the occurrence of escape-related behavior. Although similar in construct to the closely related FST, there are distinct differences between the actions of drugs in both tests and they can offer complimentary information on a drug. Overall, the tail suspension test is a useful test for assessing the behavioral effects of antidepressant compounds and other pharmacological and genetic manipulations relevant to depression.

Olfactory Bulbectomy

The bilateral removal of the olfactory bulbs of a rat (hamsters and mice also have been used) results in a complex constellation of behavioral, neurochemical, neuroendocrine, and neuroimmune alterations, many of which are correlated with changes observed in major depression. To many investigators the bulbectomy model seems to be an obscure test because the rationale for its use as an animal model has been often questioned based on construct and etiological validity arguments. However, this model has one of the best portfolios for the prediction of known antidepressant compounds following repeated administration and is reliable between laboratories. The most consistent behavioral change caused by bulbectomy is a hyperactive response in a novel, brightly lit open field apparatus, which is reversed almost exclusively by chronic, but not acute, antidepressant treatment. Reasons for this time-dependent reversal are not fully understood but have generated much interest of late.

Learned Helplessness

The learned helplessness paradigm is based on the fact that following repeated uncontrollable shocks, animals demonstrate escape deficits that are reversible by antidepressant agents. The behavioral deficits are sensitive to a broad spectrum of antidepressant compounds usually following short-term treatment. The major drawback of the model is that most of the depression-like symptomatology does not persist beyond 2–3 days following cessation of the uncontrollable shock. Controlling for alterations

in pain sensitivity and motor performance must be carried out as they can alter activity in this test.

Chronic Mild Stress

The chronic mild stress (CMS) model consists of exposing rodents to series of mild unpredictable stressors during a prolonged period (usually >2 weeks). This stress regimen induces many long-term behavioral, neurochemical, neuroimmune, and neuroendocrine alterations resembling those dysfunctions observed in depressed patients. Primarily, there have been two major antidepressant-sensitive readouts characterized in the CMS model: (1) CMS depresses the consumption and preference for sucrose solution and (2) CMS decreases brain reward function as assessed using intracranial self-stimulation (ICSS). Both measures are correlated with anhedonia, one of the core symptoms of depression as defined in the DSM-IV. These anhedonia-like behaviors have generally been shown to be reversed by chronic, but not acute, treatment with several classes of antidepressants. Although the paradigm has been described as a model with a high predictive, construct, and etiological validity, it has very poor reliability and CMS-induced effects could not be reproduced in many laboratories.

Other Stress-Based Models

As the chronic mild stress model has fallen out of favor due to the lack of cross-laboratory reliability, other approaches to develop chronic stress-based models of depression have emerged.

Restraint Stress

One of the most consistent findings from neuroimaging studies in depressed patients is that there is a deficit in hippocampus function, whereas the amygdala is hyperactive. Building on these findings Chattarji and colleagues have developed a model of repeated stress in rats and mice, in which both of these findings are recapitulated. Following 10 days of repeated restraint stress there were deficits in hippocampal LTP, and dendritic atrophy and debranching in CA3 pyramidal neurons were observed, whereas there was increased arborization in the amygdala. Further studies with this paradigm may help to shed light on the mechanisms underlying the changes in these two important regions in depression.

Social Defeat Stress

There has been a resurgence of interest in social defeat stress as a model of depression. Behavioral and neurochemical alterations have been assessed following

exposure of mice to social defeat and continuous contact with the aggressor for 10 consecutive days, resulting in a long-lasting social aversion, even to unfamiliar mice. The authors suggest that given that chronic stress can lead to depression in vulnerable humans that this paradigm could represent a model of depression. This hypothesis is given some credence as chronic, but not acute antidepressant, nor anxiolytic (chronic or acute), administration reversed this social aversion.

Models of the Early-Life Stress

Experience of stressful events during childhood has long been thought to contribute to the pathophysiology of emotional disorders, and recent research findings describe how early life trauma and neglect exert a profound and pervasive influence on risk for depression and anxiety disorders. Indeed, it appears that early life trauma may not only increase risk for these disorders in adulthood, but may also precipitate illness onset, increase co-morbidity among disorders, and alter the efficacy of treatments for these conditions. In rats postnatal maternal separation can produce lasting increases in emotional behavior and stressor reactivity together with alterations in neurotransmitter systems implicated in emotionality, including corticotrophin-releasing factor, serotonin, noradrenaline, and glutamate.

Models Based on Drug-Withdrawal-Induced Anhedonia

Reward deficits associated with withdrawal from drugs of abuse can represent an animal model of the symptom of 'diminished interest or pleasure (anhedonia)' with construct, convergent, and predictive validities. Recent studies showed that amphetamine withdrawal is characterized by decreased breaking-points under a progressive ratio schedule for a sucrose solution reinforcer. Under the progressive ratio schedule animals are required to increase their operant responding for a fixed reward until they reach a break point which determines the maximal amount of effort the animals will expend to procure the desired rewarding stimulus, thus the break point provides an objective measure of the subjects motivation. Amphetamine withdrawal is also associated with decrements in anticipatory and motivational measures for sexual reinforcement and elevations in brain reward thresholds in rats. In certain situations, antidepressant compounds have been shown to attenuate the withdrawal-induced reward deficits in ICSS thresholds.

Endophenotype-Style Approaches

The traditional models of depression mainly rely on a small number of final readouts following a lesion or stressor. However, given the lack of novel antidepressants being derived, recent studies have attempted to use a more focused approach to study psychiatric disorders in preclinical laboratories, which attempts to assess only one symptom, or marker, of the disease. This approach aims to utilize characteristic endophenotypes of diseases and model them independently rather than the whole syndrome. Fundamental to this endophenotype-based concept is the assumption that the genetic basis of variations of the given marker between patients and control subjects are fewer than those involved in the manifestation of a complex disorder *per se*. This has the benefit of simplifying a complex disorder, such as depression, into individual behaviors, which are more easily measurable in both patients and laboratory animals. Moreover, this approach is akin to preclinical testing, where only one variable is generally measured. Such endophenotypes of depression that can be easily modeled in animals include psychomotor alterations, hedonic deficit (lowered response to rewarding and pleasurable stimuli), appetite and weight alterations, circadian and sleep disturbances, and cognitive deficits. However, care must be taken when using this approach for concerns of pseudo-specificity may arise, as even those endophenotypes considered as core symptoms in depression can, and usually are, present in other diseases.

Genetic Approaches

There has been an upsurge in the use of mice as models of psychiatric disorders, due to the ability to generate genetically modified mice. Such mice provide insight into receptors where there is a lack of pharmacological agents and have aided discovery of numerous potential targets underlying depression, which may give rise to novel antidepressants. However, care must be taken when using knockout mice, as compensatory changes can occur due to the life-long ablation of a protein and it may in fact be such alterations that result in the behavioral phenotype. More recently, inducible and site-specific knockouts have been generated, which enable the role of proteins to be assessed in adult mice negating the compensatory effects. Similar strategies can also be used to knock-in specific genes, which lead to an overexpression of the protein. The development and refinement of more mice behavioral models has also expanded the strength of genetically modified mice and as such they represent a powerful tool with which to study the role of specific proteins in depression.

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Selective Breeding in Animal Models of Depression

In order to discriminate potential genetic influences on depressive-like behavior, several attempts have been undertaken using selective breeding programs of animals based on the individual responsiveness in animal models of depression. To this end, rats were selectively bred for susceptible to learned helplessness and for high and low level of immobility in the FST. Other genetic models have been developed based on an underlying alteration in the function of both cholinergic (Flinders Rat) and serotonergic neurotransmitter systems. One mouse model that has been derived from animals bred for spontaneous high or low immobility scores in the tail suspension test, referred to as helpless or nonhelpless mice. In addition to increased immobility in the TST, helpless animals also had an increased immobility in the FST, differences in sleep architecture, disturbance in hypothalamic–pituitary–adrenal, and alterations in serotonergic function. Two other lines of mice and rats (HAB and LAB mice and rats) were selectively bred for their differential sensitivity to anxiety behavior on the elevated plus maze and also have been postulated as an animal model of depression.

It should be noted that any two genotypes that differ markedly in a trait can serve as the basis for a genetic inter-cross that is informative for gene mapping purposes. There is always the possibility that, in undergoing such selected breeding programs, the effects seen may be influenced by genetic drift. This would result in independently selected lines having qualitatively different behavioral responses. The comparison of different common strains of mice and rats has also yielded some potential genetic models of depression; among the most widely used are the Wistar Kyoto rat and the BALB/c mouse both of which have an elevated response to stress and altered behaviors in the FST.

Conclusions

Presently, with the advances in techniques to study mood disorders in humans, there is an opportunity to incorporate such findings into preclinical research. Additionally, the increased awareness of the need for cooperation between psychiatrists and behavioral neuroscientists has led to a more endophenotype-based approach to study single symptomatic clusters. Furthermore, genetic approaches are giving detailed insights into changes that occur in molecular cascades relevant to depression-like behaviors that are used to measure antidepressant action. Initially, such efforts will provide a greater understanding of the pathophysiology of major depression which, in the long term we hope, will lead to novel, faster, and more universally effective antidepressants.

See also: Animal Models of Bipolar Disorder; Emotion–Cognition Interactions; Genes and Behavior; Animal Models; Maternal Deprivation; Mouse Genetic Approaches to Psychiatric Disorders; Regulation of the HPA Axis by Acute and Chronic Stress; Stress and Emotionality; Value of Animal Models for Predicting CNS Therapeutic Action.

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Fear, Anxiety, and Defensive Behaviors in Animals

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Glossary

Anxiety disorders – A group of disorders involving inappropriate or dysfunctional responses to potential threat.

Anxiolytic and panicolytic drugs – Drugs that reduce anxiety (often focusing on Generalized Anxiety Disorder (GAD)), or Panic Disorder.

Danger learning – Associative learning when a stimulus or situation is paired with pain, or with cues such as cat fur/skin odor that are highly predictive of the presence of a predator. Such stimuli commonly support one-trial learning.

Defensive aggression – Aggression triggered by the attack of a predator or conspecific. In rats and mice, defensive attacks are generally targeted to the head of the attacker, vulnerable sites where the potential for damage may cause the attacker to interrupt or cease its attack.

Defensive behaviors – A group of unconditioned response patterns elicited by threat stimuli. These are conservative across mammalian species and are modulated by features of the threat stimulus and the situation in which it occurs. In the rat and mouse, they include flight, freezing, risk assessment, defensive threat, and defensive attack.

Enabling stimuli – Features of a situation that influence the probability of success of a particular defensive response. As a result of evolution, these have a modulatory role in determining the type of defense seen when a threat is encountered.

Fear/anxiety distinction – A differentiation between responsiveness to a clearly present threat, typically associated with active responses such as escape or avoidance (fear) versus that to an ambiguous or poorly defined threat, typically associated with worry, rumination, and interference with normal activities. It is often (mistakenly) conceptualized as involving normal (fear) versus pathological (anxiety) reactions.

Defensive behaviors are a group of responses or response patterns that have evolved because they are adaptive in enabling animals to deal with threats to survival. A crucial feature of this adaptiveness is the match between defensive behavior and important features of the threat stimulus and the situation in which it is encountered.

Threat Stimuli

Threat stimuli are traditionally divided into three classes: predators, conspecific attackers, and dangerous features of the environment. All three of these are, to varying degrees, factors in the survival of most vertebrate animal species, with predators representing a particularly important, and highly variable, class of threat stimuli. Evidence from a number of bird and mammal prey species indicates that these animals can differentiate aerial, terrestrial, and even subterranean predators, and are capable of signaling to conspecifics – animals of the same species – which type of predator may be present. Some prey species also appear to be able to discriminate the alarm signals of a nonconspecific made in response to the appearance of these disparate types of predators. These discriminant abilities and the signals with which they are associated form part of the defensive repertory of such animals.

Conspecific threats are from another animal of the same species. Despite a commonly held misconception to the effect that fights between animals of the same species seldom result in harm or death, conspecific attack can be lethal, even among animals such as ungulates that are not predators and do not have strongly developed weapon systems. However, conspecific attack may involve particular features such as targeting of bites and blows to relatively nonlethal sites, or reduction of attack when the opponent signals submission or defeat, that tend to reduce the lethality of within-species fighting. These features are especially common among more social species in which proximity of related animals enhances the genetic maladaptiveness of damage or death from conspecific attacks.

Dangerous environmental features such as fires, floods, and earth movements may threaten individuals of any species. Somewhat paradoxically, these tend to have less influence on the complexity of defensive behaviors, as most are either relatively easily avoided (e.g., bodies of water or high places for terrestrial animals), or, almost impossible to avoid except for flying animals, and sometimes not even then (e.g., pyroclastic flow or noxious fumes from an erupting volcano).

Defensive Behaviors

These examples of threat stimuli point to the first requirement for a successful defense: It must involve detecting, and to some degree discriminating, important

features of the threat stimulus. This involves a strong tendency to attend to potential threat in the environment; typically labeled ‘vigilance’ in the context of field studies, or ‘risk assessment’ in laboratory work. These behaviors tend to be measured in terms of specific orientation and movement characteristics, in which the subject first orients toward any danger signals that may be present (e.g., an unexpected movement or sound) and may even approach it, often utilizing a movement pattern that reduces the likelihood of itself being detected as it attempts to approach and investigate the potential danger. In laboratory rodents, this takes the form of low back or stretched approach movements in which the animal alternates periods of immobility with brief forward movements.

After a threat stimulus has been detected, the defensive behaviors that appear to be common to most mammalian species include flight, often followed by hiding if a place of concealment or protection is available; freezing (cessation of movement); defensive threats to the threat stimulus; defensive attack to the threat stimulus; and, especially in highly social species, alarm cries or other signals of the presence of the threat. Other specific behaviors may be related to or comprise components of one or more of these general tactics. For example, the explosive startle reaction measured in tests of conditioned startle responses may function to surprise and even briefly stun an attacker, providing an opportunity for the subject to escape. Defensive burying is likely to have different functions with different types of threat stimuli. With noxious or potentially toxic stimuli, such as a shock prod or a decaying carcass, burying may interpose a barrier that reduces the dangerousness of the stimulus. That this is its major function, however, is brought into question by findings that rats not only bury novel stimuli in their home environment, but also routinely dig them up again. An additional function of defensive burying may simply be that it throws things – dirt or other substrate – at potentially dangerous objects. If the object responds, for example, by movement when hit, then it reveals itself as an animate rather than an inanimate object, thereby confirming its potential as a threat. Defensive threat and attack occur when the danger is imminent. In several rodent species and in domesticated cats, both defensive threat and defensive attack may be differentiated from other forms of aggressive threat and attack by both the actions involved and by the targets on the body of the opponent where weapons (teeth, claws) are aimed. Although less well studied in other animals, a number of species from ungulates to primates have been shown to display different attack patterns and weapon use as a reaction to threat stimuli, as compared to those shown in offensive, conspecific aggression.

The Effectiveness of Defense

In order to evolve, individual defensive behaviors must be effective against the type of threat against which they are utilized. For some threat stimuli, this may be very difficult, and the concept of effective may reflect only a small increment in success against a dismal baseline: if a small mammal is caught by a larger and more powerful predator, the overwhelmingly likely outcome is its death. Any defense that reduces the probability of that outcome, albeit even by 5%, is effective in comparison. The finding that alley cats generally fail to attack wild rats if the latter weigh more than about 200 g suggests that rats of such a size, but not smaller, are capable of damaging the attackers enough to reduce their adaptive potential. Defensive attacks may be adaptive through related conspecifics, even if most of the defensively attacking rats are themselves killed in the process.

If defensive threat/attack or a prodigious startle causes the predator to back off or release its grip on the prey while freezing is ineffective, then the former behaviors are not only more effective than no defense, they are also more effective than freezing, to that particular type of threat stimulus. Conversely, flight may be highly effective against approaching environmental dangers such as a slow-moving flood, against which defensive threat/attack or startle would be useless or counterproductive. The centrality of the relationship between particular types of threat and individual defensive behaviors has resulted in the evolution of relatively specific connections between characteristics of threat stimuli and situation, on the one hand, and the type of defense emitted, on the other. This is manifest as a high probability of that particular response to that type of threat/situation combination. Some important threat/situation characteristics are discussed in the following.

Defensive Distance

Defensive distance, the physical distance between predator and prey, strongly impacts the type as well as the intensity of defense offered. Whereas a distant predator may elicit only continued vigilance with freezing or flight as the distance between predator and prey decreases, defensive threat and then defensive attack (alternatively startle, depending on the prey species) may occur as contact between the two becomes imminent. All of these activities are oriented toward the predator, or directly away from it (flight), but they are clearly distinguishable on the basis of well-defined features of the behavior itself.

Threat Ambiguity

Many threat stimuli are not discrete, obvious, or clearly localized, at least not until it is too late for any specific antipredator defenses to have a good chance of success.

This situation is exacerbated by predators' use of stealth or crypsis in order to reach striking distance to the prey without being detected. As early detection typically produces a sharp reduction in the success of the hunt, mammals of prey species spend a substantial proportion of their time budgets attempting to detect danger, utilizing a pattern of scanning the environment, focused listening, or sniffing, to the detriment of nondefensive activities such as eating, grooming, or care of young. Among animals that live in groups, behaviors of other group members may be observed for indications that the latter have detected danger: In some highly social species, such as meerkats, sentinels rotate the duties of scanning for danger. These activities, labeled vigilance or risk assessment are strongly associated with threat stimulus ambiguity. In laboratory settings, they are particularly prominent when a subject encounters ambiguous or non-specific stimuli potentially associated with threat, such as novel or unexpected places, objects, sounds, and smells. Ambiguous stimuli often elicit approach, investigation, sniffing or other information-gathering activities, with relatively rapid habituation of these behaviors if no actual danger is found. Stimuli with a more specific association with danger, such as the odor of a predator, elicit a similar pattern but one that is much less likely to show rapid habituation.

Enabling or Expediting Stimuli

If evolution is responsible for the development of defensive behaviors, animals showing adaptive defenses, that is, defenses that are appropriate to the situation, have survived and reproduced more successfully than those that did not. These behaviors should represent the most effective defense possible, given not only the particular threat encountered, but also the specific features of the situation in which the encounter takes place. Some examples are given as follows: the presence of an exit feature from a threat situation is crucial in order for flight to be effective. This is not a matter of definition or circular reasoning. Rapid locomotion away from a threat (flight) is physically possible in any fairly large enclosure, but it is not effective against most animate attackers without a way for the subject to actually escape the enclosure. When escape is not possible, other defenses such as freezing and defensive threat/attack have been shown to be more effective in reducing damage to the subject. Other evidence for an association between flight and an escape route is that blocking of an existing escape route may rapidly elicit a switch from flight to freezing in rats. A place of concealment or protection can open up new defensive possibilities: hiding, either alone or in combination with facing out of the hiding place and threatening the oncoming predator. Rodent subjects, in particular, often utilize

even blind tunnels to face outward, confronting their attackers with their most effective weapons, the teeth.

Orientation to such features can be clearly demonstrated. When the silhouette of a predator was displayed overhead, gerbils that had established a place preference during a 15-min exploratory period in the test situation showed a flight path that maximized the distance to the threat stimulus, minus the distance to the preferred area. Animals moved involuntarily to a new location show high-level exploration of the new site. This reflects the importance of information on the presence and location of defense-relevant features such as an escape route or a hiding place, should the new location also contain danger. Notably, when a new site is opened adjacent to a familiar area, such exploration is more tentative and delayed.

An additional expediting or enabling stimulus is the presence of conspecifics. This is necessary for alarm cries to fulfill the function of alerting relatives to the presence of danger, and alarm cries, at least in rats, do appear to depend on the presence of conspecifics. Cries for help, suggested by anecdotal evidence in primate infants or young, might similarly be expected to be facilitated by the presence of familiar adults.

These findings suggest that unconditioned defensive behaviors, elicited and modulated by a host of features of the threat stimulus and situation, are the product of evolution, reflecting differential survival/reproductive success that they afford to individuals that display them appropriately. Because the types of stimuli or factors that provide a direct threat to the bodily well-being of animals of different species are relatively consistent, as are the factors that make specific defenses effective, it is a reasonable hypotheses, for which one set of tests will be later described, that a corresponding consistency may be seen between eliciting stimuli/situations, and specific defenses, across mammalian species. These relationships are outlined in **Table 1**.

Learning and Defense

The relationship between features of the threat stimulus/situation and the form of defense is not one of physical necessity. Moreover, it is often not a matter of learning: in studies using laboratory rodents, all of the relationships described here have been demonstrated in animals that have never had experiences that might have produced such learning. Some studies have shown specific defenses in rat pups, by 12–18 days of age, and by shortly after weaning rats appear to show a relatively wide range of appropriate defenses. Other findings arguing against a learning interpretation of the core relationships between threat stimuli/situations and defensive behaviors include the difficulties of changing the form of an elicited defensive behavior by either lack of reinforcement or by

Table 1 Defensive behaviors as a function of the discreteness or ambiguity of threat, defensive distance, and presence of particular enabling stimuli

<i>Source of threat (and distance)</i>	<i>Enabling Simuli</i>	<i>Behavior</i>	<i>Typical Outcome</i>
Discrete	"Way out" available	Flight	Escapes
Discrete	No means of escape	Freezing	Reduces attack
Discrete	Conspecifics nearby	Alarm cry	Warns conspecifics
Discrete	Hiding place available	Hides	No detection/access
Discrete (close in)		Defensive threat	Threatens attacker
Discrete (contacting)		Defensive attack	Hurts attacker
Discrete (contacting)		Startle	Startles attacker
Uncertain (potential)		Risk assessment	Localize, identify threat
Uncertain (potential)	Substrate	Defensive burying	Elicit animate movement

specific and immediate punishment. These findings do not, of course, suggest that learning has no impact on defensive behavior, a view that is clearly incorrect in terms of the rapidity of associative learning utilizing predators or predator odors as unconditioned stimuli.

Danger Learning

Defensive behaviors show robust and rapid conditioning to stimuli, particularly situational stimuli, when pain is used as the unconditioned stimulus. Many studies of this phenomenon have focused on defensive immobility behaviors, variously labeled crouching or freezing. Such immobility is robust following shock, and also when the subject is replaced in that situation 24 h later, demonstrating one-trial conditioning. The intensity of the response increases as a function of intensity of a (single) shock, but is reduced by previous familiarity with the situation in which the shock is given (latent learning). A particularly important point is that the discriminability of the shock source can change the form of the conditioned defense, evaluated during a later presentation of the CS: highly discriminable shock sources (objects) produce predominant avoidance of that object, whereas shock from a poorly discriminable source (cage flooring) produces a conditioned immobility in the shock situation.

The presentation of a noncontacting cat to a rat that has never previously encountered a cat elicits freezing. Such freezing shows robust one-trial contextual conditioning, indicating that pain, or even contact with the threat source, is unnecessary to support learning. Mice also show a range of defenses when confronted by a mouse-predator, a rat; a paradigm embedded in a number of studies of mouse defensive behaviors, in the Mouse Defense Test Battery. Although even quiet predators may elicit some degree of defensiveness, primarily risk assessment or avoidance, more active predators, such as an approaching cat, rat, or lion, elicit a more intense defense, typically one that is relevant to the action of the predator, for example, flight to an approaching US. Partial predator stimuli (cat fur/skin odor) also elicit risk assessment,

freezing, and, when clearly localizable, avoidance. There may be a relationship between the degree to which a partial predator stimulus elicits risk assessment and the degree to which that stimulus supports, as a US, one-trial aversive or danger conditioning. Cat fur/skin odor has repeatedly been demonstrated to support one-trial conditioning, whereas cat feces or urine, or trimethylthiazoline, a synthetic chemical derived from fox anal gland extracts, all elicit some degree of defensiveness but may not elicit risk assessment and do not appear to support one-trial learning.

Contextual danger learning, such as occurs when a predator or other strong threat is encountered in a particular context, may be evolutionarily adaptive because it results in enhanced defensiveness in situations where danger is likely. It is also possible that stressors might produce a general enhancement of defensiveness as reflecting an increased level of danger. Both exposure to a cat, and to cat fur/skin odor stimuli, have been shown to enhance anxiety-like behaviors in rats in a number of anxiety models, up to 21 days later. This effect is not limited to predator or partial predator stimuli. Stressors such as pair-housing with a more dominant animal or imposition of an array of stressors on a chronic basis have been shown to enhance durations of defensive burying, and freezing. Conversely, early experience of stress-reducing events may be capable of reducing defensiveness; for example, offspring of high-licking mothers showed less defensiveness in both defensive burying tests, and when serving as intruders in resident-intruder tests.

In summary, although defense patterns, at least in rats and mice, are not based on specific experience, they may become conditioned with a single pairing or trial. This very rapid, one-trial conditioning has been demonstrated with painful stimuli, or the actual presence of a predator, or some, but not all, partial predator stimuli serving as the US. The crucial factor in the ability of a threat stimulus to support one-trial conditioning appears to be the degree to which it may predict the presence of danger. In addition, exposure to threat stimuli such as predators or dominant conspecifics, as well as chronic exposure to other types of

stressors, may result in long-term enhancements of emotionality, manifest as increases in both individual defensive behaviors and in more general anxiety measures.

Defensive Behaviors in People

Laboratory and, especially, field studies make it clear that many of these defensive behaviors are included in the defensive repertory of most mammals, not just rats and mice. This brings up the question of the mammalian species most interesting to the vast majority of neuroscientists: humans. If defensive behaviors are to serve as models for investigating and understanding the biology of emotional response to aversive stimuli, it is essential to evaluate whether these systems show strong parallels in human responsiveness to threat. In attempting to determine whether there are meaningful human parallels to these behaviors, the obvious solution is to examine human defenses in the same contexts of eliciting and enabling stimuli that modulate the defenses of rodents.

The most focused attempt to determine how people react in such situations provided brief scenarios that sketched out threat stimulus and situational characteristics, and required subjects to choose their first response to each such situation. Subjects were given a list of possible behaviors, but also allowed to fill in their first choice when this was not on the list. Because most people have had very limited exposure to nonhuman predators, whereas virtually everyone has had some or much personal or vicarious (via TV and films) exposure to human attack, the scenarios described actual or potential human, rather than predator, attack.

When the first response chosen in each such scenario was analyzed in terms of the same range of factors (threat stimulus and situational characteristics) that strongly determine the form of attack in laboratory animal studies, the similarities in these relationships were striking. Over three-quarters of the responses chosen were similar to those identified in animal research, with the remainder reflecting human abilities presumably not available to nonhuman animals, such as negotiate. Significant and sometimes extremely high correlations were found between stimulus/situational characteristics and the defensive behaviors with which they were associated. As an example, the correlation between ambiguousness of stimuli and risk assessment was nearly +0.90. These results have been replicated in published studies based on subjects in several different cultures, each of which utilized a relatively large number of subjects. Comparisons between the results of the studies show only limited evidence of cultural differences on the choice of responses to the different threat stimuli and situations.

The data from all of these studies were consistently consonant with a view that people utilize many of the same defensive behaviors as do rodents, and that major features of threat stimuli and situations that are determinative of defensive responses for rodents have parallels in human behavior. These findings must be taken in the context of scenarios designed to avoid some real complications arising from cognitive and technological differences between human and nonhuman animals, such as an omission of guns or other weapons. Nonetheless, they provide strong support for a view that not only individual human defensive behaviors, but also the patterns of their responsivity to important antecedent and surrounding situations, have some important similarities to those of nonhuman mammals.

Defense and Anxiety

Etymological analyses of the terms ‘fear’ and ‘anxiety’ suggest that, while both describe reactions to threat or danger, they are different in terms of their eliciting stimuli; the behaviors they encompass; and the degree to which they are regarded as normal and adaptive. Sometimes described as free-floating, anxiety is a response to an ambiguous or poorly defined danger, and its manifestations tend to be largely subjective, involving extended worry or rumination. Although these are extremely unpleasant to the individual, they are typically without a definitive outcome in terms of specific action patterns, except for the negative impact they may have on more productive life activities. This combination, of a poorly defined cause and a response that is regarded as relatively unproductive, contributes to a view that anxiety is a less adaptive response than is fear, seen as a normal response to danger.

The foregoing analyses of defensive behaviors suggest that this common differentiation between fear and anxiety is rooted in biology. Ambiguous threat stimuli elicit risk-assessment responses that have a strong parallel in worry and rumination, that is, cognitive efforts to understand an ambiguous or poorly conceptualized problem. Moreover, characterizations of anxiety in terms of ‘apprehensive expectation’ are clearly congruent with the vigilance aspect of defensiveness in potential threat situations. Finally, the notion of jumpiness or physiological hyper-reactivity fits well with observations that risk assessment is associated with muscle tension, likely as a preparation for action should the results of the assessment demand it. Conversely, defensive behaviors to clearly present and differentiable threat, such as flight or avoidance, fall squarely within the range of activities associated with fear and can be seen as potentially effective solutions to the danger presented.

The existence of a biological basis for the differentiation of fear and anxiety does, however, call into question the common view that fear is more normal and adaptive than anxiety. Risk assessment may be the best-possible response when an individual is confronted by an ambiguous threat or difficulty, as it is aimed at identification or understanding of the threat source. This suggests that both fear and anxiety may be normal if they occur in response to appropriate situations and decline in intensity in accord with the degree to which the situation becomes less dangerous. Pathology may occur when the response is out of proportion to the danger, inappropriate to the danger, or fails to decline with reductions in the danger. These conditions may hold for behaviors classifiable as either fear or anxiety, although many problems in human life are so complex, ubiquitous, and difficult to analyze that they provide particular occasion for the elicitation of anxiety. In addition, a history of stressful experience such as may also be common in human life, can also adversely affect cognitive functioning, such that early and prolonged stress would be expected to negatively impact the quality of risk-assessment processes, promoting anxiety in situations with which normal individuals might cope. Indeed, the notion of coping includes cognitive strategies as an important component.

Analyses of defensive behaviors, with their emphasis on differences in both the stimulus/situational elicitors of these behaviors, and in the form and function of the individual behaviors themselves, are also relevant to a consideration of the wide range of ways in which anxiety disorders are expressed, that is, their specific defining symptoms or characteristics. While response similarities in behaviors used as the basis for animal models of anxiety, and behavioral aspects of relevant psychopathologies are often dismissed as face validity, it might be noted that the differentiations among anxiety disorders are typically based on behavioral symptoms that have some parallels to these defensive behaviors. Findings of considerable specificity of response by particular defensive behaviors, to drugs effective against generalized anxiety disorder (GAD), or, panic, raise the possibility that these defensive behaviors are the output of neural systems that are differentiable in terms of hodology, neurotransmitter involvement, or some combination thereof. This possibility, in turn, opens new avenues for pharmacological research, as well as for behavioral treatments that reflect the normal functions of individual defenses.

Responsivity to Anxiolytic Drugs

If, as these findings suggest, defensive behaviors may be embedded in anxiety disorders, then they should respond to antianxiety drugs. It is notable that there are substantial differences in responsivity to particular classes of drugs for different anxiety disorders, and the possibility of specific involvement of various defensive behaviors in these disorders suggests that the latter may also show differential drug response. In particular, drugs effective against GAD have been compared to those that reduce panic attacks: Drugs effective against GAD consistently reduce risk assessment, and defensive threat/attack, whereas drugs that are effective against panic disorder consistently reduce, and propanic drugs consistently enhance, flight. The ability of drugs effective against GAD to reduce risk assessment supports a view that risk assessment is a focal feature of GAD. It is also notable that excessive rumination is more associated with women than men, and that females are overrepresented in GAD by about 2 to 1. The effect of anti-GAD drugs on defensive threat and attack is less obvious, and may relate to the inclusion of irritability or hyperreactivity as a factor often associated with GAD.

In contrast to GAD, a well-developed hypothesis of panic disorder has a focus on flight as a core motivation and response tendency. Because of this hypothesis, a number of studies have focused on the relationship between flight and panic, demonstrating that antipanic drugs and drug schedules increase flight; propanic drugs and drug schedules decrease flight; and drugs without effect on flight also have no effect on panic; all of which provide consistent evidence for the view that defensive behaviors may provide both a conceptual model for analysis of anxiety disorders, and a useful experimental model for the evaluation of drug effects on these conditions.

See also: Fear Conditioning; Fear: Potentiation and Startle; Human Fear and Anxiety; Neural Bases of Defensive Aggression; Neural Substrates of Conditioned Fear and Anxiety; Neural Substrates of Unconditioned Fear, Defense, and Anxiety.

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F

Fear Conditioning

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Glossary

Activity burst – The burst of activity elicited by the aversive unconditional stimulus in fear-conditioning experiments, primarily characterized by jumping and/or rapid locomotion.

Circa-strike defensive behaviors – The behaviors that occur when the animal is attacked by a predator. These behaviors evolved to facilitate escape and are characterized by rapid locomotion, jumping, and counter attacks aimed at the predator.

Conditional response – The behavioral response elicited by the conditional stimulus after a conditional association has been formed with the unconditional stimulus.

Conditional stimulus – An initially neutral stimulus that comes to elicit a behavioral response due to a conditional association with a biologically significant stimulus.

Fear conditioning – A general term applied to procedures in which an aversive unconditional stimulus is paired with conditional stimuli and fear-related conditional responses are measured.

Freezing – The complete absence of all movement except that necessitated by breathing. As the primary postencounter defensive response in rodents, it evolved to prevent detection by predators as well as attack, because movement acts as a releasing stimulus for predatory attack.

Pavlovian conditioning – A learning process where an initially neutral stimulus enters into a conditional association with a biologically significant stimulus and comes to elicit a behavioral response. It is also referred to as ‘classical conditioning.’

Postencounter defensive behaviors – The behaviors that occur when the animal has detected the presence of a predator. These behaviors evolved to prevent detection and, if the animal has already been detected, decrease the probability of attack.

Predatory imminent – The perceived proximity of an animal to predatory attack. This is characterized by a continuum from low predatory imminent, where no predators have been detected, to high predatory imminent, where the animal is currently being attacked. Prey species, such as the rodent, have evolved specific behaviors that optimize survival along this continuum.

Pre-encounter defensive behaviors – The behaviors that occur when an animal is potentially in danger but a predator has not been detected. In general, these behaviors evolved to avoid predator detection and to facilitate escape but still allow for other adaptive behaviors such as foraging for food.

Species-specific defensive reaction – The behavioral responses that evolved to optimize survival in the face of environmental threats that a particular species is likely to encounter in its native habitat.

Unconditional response – The innate behavioral response elicited by the unconditional stimulus.

Unconditional stimulus – An inherently biologically significant stimulus that elicits an innate behavioral response.

Historical Background

The modern study of fear conditioning is based on the assumption that fear is a defensive motivational system that evolved to optimize survival in the face of environmental threats. Activation of this fear system produces highly predictable changes in behavior and physiology and the magnitude of these changes are proportional to the level of fear. A thorough understanding of the parameters which produce fear, combined with the predictability of fear responses, has allowed researchers to delve deeply into its underlying neural mechanisms.

The modern view that fear produces predictable and functionally organized behavioral responses in proportion to the level of fear, was not widely held until the past few decades.

Early studies conducted during the middle of the last century dominated by the theoretical framework of Neil Miller and O.H. Mowrer focused more on how fear is able to modify behavior and less on fear itself. The dominant view for decades of research was that painful stimuli, such as an electric shock, could be used to punish behavior and that termination of signals for punishment could serve to reinforce a particular behavior. These researchers used instrumental avoidance learning procedures in which they made avoidance of shock contingent on an arbitrarily chosen behavior. For example, the animal should be able to learn to run on a running wheel, shuttle between two chambers, rear, or press a lever to terminate fear-producing cues. This view stressed the acquisition of arbitrary behaviors on a trial-and-error basis.

In the early 1970s this prevailing view began to come under fierce assault, most notably by Robert Bolles. In a highly influential paper entitled ‘Species-specific defensive reactions and avoidance learning,’ he questioned the basic theoretical assumptions of instrumental avoidance learning as well as the empirical evidence upon which it was based. First, he argued that fear evolved as a way to defend against predators and that learning the proper response to predators through trial and error is a highly ineffective strategy to avoid being eaten. “Survival is too urgent, the opportunity to learn is too limited and the parameters of the situation make the necessary learning impossible.” A momentary failure to avoid death has much more severe consequences than a temporary failure to obtain food and the learning rules should therefore be different. Bolles pointed out that some avoidance responses, such as flight, could be rapidly and efficiently acquired, whereas others, such as lever-press avoidance, required thousands of trials to achieve mediocre effectiveness. Rapidly acquired avoidance responses, Bolles argued, were part of the rat’s innate defensive behavioral repertoire and did not require reinforcement to be expressed. These behaviors, which he termed ‘species-specific defensive reactions’ (SSDR), evolved to optimize survival in the face of environmental threats that rats are likely to encounter in their native habitat.

The shaping of optimal defensive behaviors, in the SSDR view, occurs at the species level through natural selection, rather than at the individual level through reinforcement or punishment of a particular behavior. In a prey species, such as the rodent, the experiences of countless predator-prey interactions, passed on in the genes of those who avoided predation, supersede the experience of the individual. The precise nature of defensive behavior, that is, its shape, vigor, and duration, is therefore genetically determined and largely impervious

to change by reinforcement contingencies. These behaviors occur because the animal is frightened, not because they have been reinforced by escaping or avoiding pain. The huge number of trials required for some avoidance responses to be acquired suggests that, while instrumental avoidance learning can occur under some conditions, the primary responses to fearful situations are SSDR’s.

In 1988, Fanselow proposed a modification of SSDR theory, which he termed ‘predatory imminence theory.’ It provided a formulation for how different defensive behaviors are expressed under different conditions. Predatory imminence, defined as the perceived proximity of an animal to predatory attack, determines the level of activation of the fear motivational system and this, in turn, determines which defensive behavior will be expressed. In this model, defensive behavior is not only species specific, but also specific to the level of threat. Thus, certain behaviors evolved to optimize survival along a continuum of predatory imminence.

The main points along this continuum are referred to as pre-encounter, postencounter, and circa-strike behaviors. ‘Pre-encounter’ behaviors occur in situations where the animal is potentially in danger but no predator has been detected. These behaviors, such as thigmotaxis (running along walls), preference for the dark and stretched approach (slow, cautious forward movement), minimize the chances of detection by predators, while still allowing other adaptive behaviors such as foraging. ‘Postencounter’ behaviors occur when the animal has detected the presence of a predator. This can be triggered by innately aversive stimuli such as predator odors or by stimuli that have been paired with previous predatory attack. The primary postencounter behavior in rodents is freezing, which helps prevent attack by making the animal less visible to predators, which rely largely on movement for detection of their prey. Additionally, since movement acts as a releasing stimulus for attack, a detected but frozen prey is less likely to be attacked. ‘Circa-strike’ behaviors occur when the predator is actively attacking the animal. The primary circa-strike behavior is an activity burst, which can involve jumping and/or fleeing from the predator. Defensive biting, often aimed at the nose of the predator, may also occur if no escape is available.

Predatory imminence-SSDR (PI-SSDR) theory has proven to be a powerful theoretical framework for predicting and explaining fear-related behavioral changes and it now forms the basis for the study of fear conditioning. Integrating PI-SSDR theory with Pavlovian conditioning theory provides a complete picture for how animals acquire and express fear. Pavlovian, or classical, conditioning refers to a learning process where a neutral stimulus, the conditional stimulus (CS), becomes associated with a biologically significant stimulus, the unconditional stimulus (US). The US is able to produce a response on its own, which is referred to as the

'unconditional response' (UR). After one or more pairings of the CS and US, the CS comes to elicit a conditional response (CR). Pavlov emphasized that the CR requires a dependent, or conditional, relationship between the CS and the US. Thus, the term conditional is the correct and the preferred term, rather than conditioned, which is unfortunately frequently used. In laboratory studies of fear conditioning, the CS can take many forms, with tones, lights, and the conditioning chamber itself being the most common. The US is a painful or startling stimulus such as an electric footshock or loud noise. The CR is a conditional fear response which produces species-specific behavioral and physiological responses according to the level of fear.

Distinguishing what constitutes the UR to the shock versus the CR to cues associated with the shock can be clarified by PI-SSDR theory. In contrast to other Pavlovian paradigms, the UR and the CR are quite different in fear conditioning. As the US is equivalent to attack from a predator, the UR is a circa-strike SSDR, that is, an activity burst. As presentation of the CS is equivalent to detecting a previously encountered predator, the CR is characterized by postencounter defensive behavior such as freezing.

The strength of the CS-US association determines the magnitude of the conditional fear response, which, in turn, determines the amount of freezing. Measuring the

percentage of time the animal spends freezing therefore provides a behavioral measure of the CS-US association. Conditional fear also produces a number of other changes, such as increased blood pressure, alterations in heart rate, analgesia, release of stress hormones, and potentiation of the startle response. The percentage of time an animal spends freezing has become the most commonly used measure of fear.

Pavlovian Fear-Conditioning Procedures

The basic fear-conditioning procedure involves pairing an aversive US with an initially neutral CS. Fear conditioning can be conducted using a wide variety of different parameters that can be tailored to suit the particular needs and theoretical questions of a given experiment. Different CSs can be used; these are usually tones, white noise, lights, and/or the conditioning chamber itself. Different aversive USs can also be used, although electric footshock is the most common. The number of US presentations can be varied to adjust the overall level of conditioning and the CS and US can be presented in different temporal relationships. A brief review of the more standard fear conditioning procedures will be presented below and are outlined in [Figures 1 and 2](#).

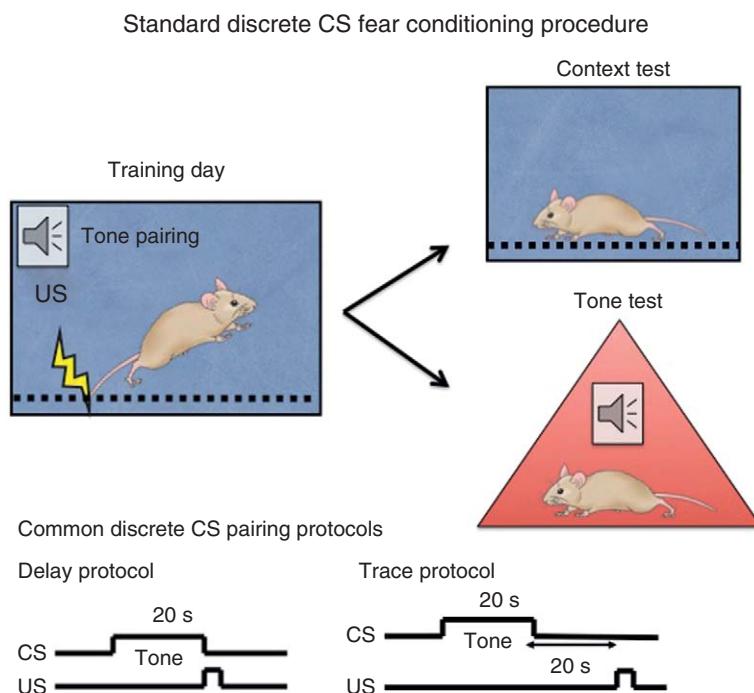


Figure 1 Standard discrete conditional stimulus fear-conditioning procedure. On the training day, the animal receives one or more pairings of the discrete CS with the shock US. Testing consists of returning the animal to the original conditioning chamber for a context test and to a novel chamber for the tone test. The order of tone and context tests can be counterbalanced. Delay and trace conditioning are the most commonly used parameters in discrete CS fear conditioning.

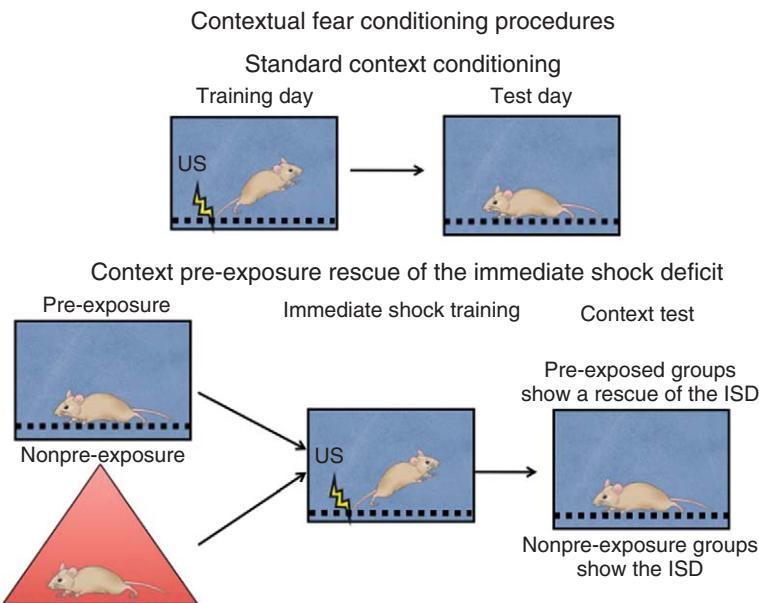


Figure 2 Contextual fear conditioning procedures. The most standard form of context conditioning is to place the animal in the conditioning chamber and then present one or more footshocks. Context fear is then tested by returning the animal to the conditioning chamber for a context test. The context pre-exposure rescue of the immediate shock deficit usually involves two groups: one which is pre-exposed to the training context and one which is not. Both groups receive a single footshock immediately (15 s or less) after being placed in the conditioning chamber. Animals that were not pre-exposed show the immediate shock deficit because they were unable to form a contextual representation to associate with the immediate shock. Animals that were pre-exposed show the context pre-exposure rescue of the immediate shock deficit, that is, they are able to associate the contextual representation formed during the pre-exposure with the immediate shock and acquire contextual fear.

Fear-conditioning procedures can be broken down into three different phases: acquisition, consolidation, and expression/retrieval. The acquisition phase refers to the formation of the CS-US association on the training day. Consolidation is a process that occurs immediately after training and involves solidification of the CS-US association into a lasting memory trace. When the animal is reexposed to the CS on the test day it retrieves the CS-US association, which triggers the expression of fear.

Discrete CS Procedures

Simple stimuli from a single sensory modality, such as a tone, a white noise, or a light, are referred to as 'discrete CSs.' In a discrete CS procedure, the animal is placed in the conditioning chamber and the CS is presented in a precise temporal relationship to the US. The most common procedure is delay conditioning where termination of the CS coincides with the onset of the US. In this terminology, the 'delay' is the time between CS onset and US onset. Delay conditioning tends to produce very robust conditioning to the CS. In trace conditioning, the onset of the US is separated from CS termination by a 'trace' interval. This tends to produce weaker conditioning to the CS and, as discussed below, relies on different neuroanatomical circuitry than delay conditioning. See

[Figure 1](#) for an outline of discrete CS procedures and [Video Clip 1](#) for an example of a tone test.

Context Conditioning

Context conditioning involves placing an animal in an experimental chamber and administering one or more footshocks. Freezing can be measured immediately after the shock(s) to assess short-term acquisition of the context-shock association or, more commonly, the animal is returned to the conditioning chamber at a later time for a context test. Despite the apparent experimental simplicity of this procedure, the mechanisms underlying context conditioning are quite complex and still hotly debated.

The CS in context conditioning is thought to be a configural or unified contextual representation of the multimodal cues in the conditioning chamber. As the animal explores the context, the sights, sounds, smells, textures, and spatial information bind together into a complex CS. It is this complex CS that becomes associated with shock. Forming the contextual representation requires that the animal spend time exploring the context. If the shock is administered immediately after placement in a conditioning chamber, no contextual fear is acquired. This finding, referred to as the 'immediate shock deficit,' occurs because the unified representation of the context has not been formed and, therefore, there is no CS for the shock to

become associated with. The immediate shock deficit can be rescued if the animal is pre-exposed to the conditioning chamber prior to the immediate shock training. This pre-exposure allows the animal to form the contextual representation, which it can then subsequently retrieve and associate with the shock. This view of context conditioning stresses the importance of two separable processes: first the animal must form the contextual representation and then it associates this representation with the shock (see **Figure 2** for an outline of context conditioning procedures and **Video Clips 2a and 2b** for illustration of the context pre-exposure rescue of the immediate shock deficit).

Associative Competition among CSs

One important issue in Pavlovian fear conditioning is that conditional stimuli compete with one another for association with the aversive US. In a discrete CS procedure, for example, the animal acquires conditional fear of the conditioning chamber in addition to the discrete CS. In practical terms, this means that context and discrete CS fears need to be tested separately. This is achieved by returning the animal to the conditioning chamber for a context test and placing it in a novel context to test fear to the discrete CS. Testing in a novel context, which should produce very little conditional fear, allows for a pure assessment of discrete CS fear. This practice is a standard fear-conditioning procedure, as shown in **Figure 1**. It allows for the assessment of two kinds of memory in the same animal, which, as discussed in more detail below, has proven to be an important tool in understanding the neurobiological mechanisms of learning and memory.

Extinction of Conditional fear

After conditional fear has been acquired, repeated presentations of the CS in the absence of the US will eventually lead to a loss of the conditional fear response. This process, referred to as ‘extinction,’ is considerably slower than the original acquisition. A great deal of evidence suggests that it is a form of new learning rather than forgetting of the original CS–US association. Three extinction phenomena, which involve return of the conditional fear response, illustrate this point: (1) An extinguished conditional fear response can be ‘reinstated’ by a single presentation of the US without the CS. (2) The context in which the extinction occurs is critical in the loss of the fear response. If the CS is presented in a different context than where the extinction occurred the CR returns. This is referred to as ‘renewal.’ (3) The conditional fear response can also return spontaneously over time in a process called ‘spontaneous recovery.’ These three return-of-fear effects argue that the original fear is still intact after

extinction but its expression is repressed. A great deal of current research is aimed at determining the mechanisms of extinction due to its clinical relevance as a model of exposure therapy in patients with anxiety disorders.

The Neuroanatomy of Fear

The underlying neurobiological mechanisms of fear conditioning have been intensively studied over that last few decades. Researchers have employed a staggering array of interdisciplinary techniques ranging from lesioning specific brain regions to recording neuronal activity in awake-behaving animals to conducting detailed microscopic analysis of brain slices. Synthesis of a huge number of studies from many different laboratories has led to the formulation of a canonical pathway for conditional fear.

Restricted anatomical lesions produced by electrolytic or excitotoxic stimulation have been one of the most important tools in understanding fear conditioning. If lesioning a particular region prevents the acquisition and/or expression of conditional fear, then that region is likely to be a critical part of the conditional fear pathway. Microinjection of drugs into specific brain regions has also become an indispensable tool as it allows for precise timing and for the use of different pharmacological agents.

Electrophysiological recording can be used to correlate neuronal activity with behavior in awake-behaving animals. Staining techniques, such as immunohistochemistry and *in situ* hybridization, have been used to map patterns of gene expression. Individual neurons express a huge array of enzymes, receptors, ion channels, etc., and each brain region is defined by a unique expression pattern. This information has been combined with genetic techniques where a particular gene can be altered or deleted and the effects of this manipulation can be assessed. This technology has been extensively developed in mice and therefore a great deal of fear-conditioning work is now conducted in genetically altered mice. The increasing sophistication of genetically engineered mice is allowing for targeted genetic manipulations of fear-conditioning pathways to an extent that was not possible with more traditional techniques.

The Canonical Fear Pathway

Conditional fear depends on multiple brain regions, which can be divided into three main categories: sensory input structures, sites of CS–US convergence, and output structures (see **Figure 3** for a simplified diagram of fear-conditioning circuitry). The amygdala is the central hub in the acquisition and expression of conditional fear. The amygdala is divided into a number of important

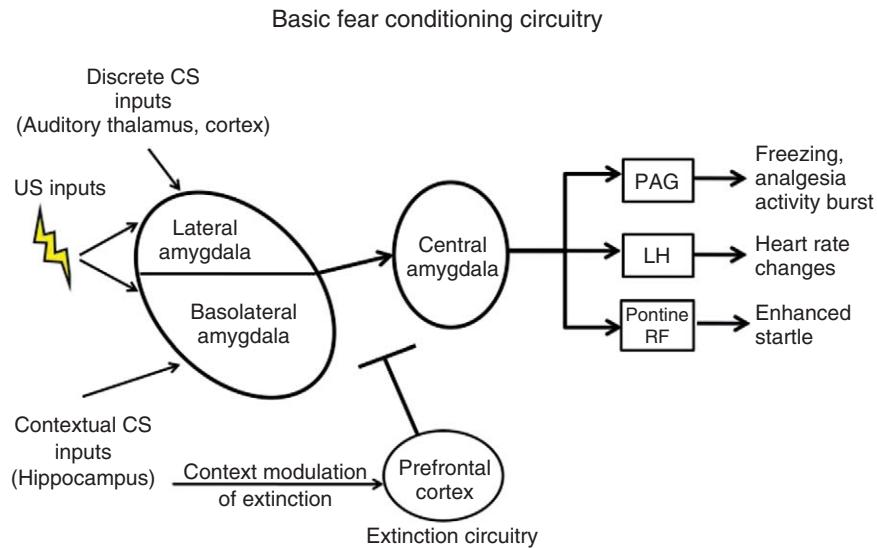


Figure 3 Basic fear conditioning circuitry. This diagram outlines a simplified view of the most important neuroanatomical pathways in fear conditioning. CS and US information converges in the amygdala. During training, co-activation of CS and US inputs strengthen the weak CS input such that after training the CS can activate the amygdala on its own. This drives activity in the central amygdala, which subsequently drives activity in downstream fear output regions such as the periaqueductal gray (PAG), lateral hypothalamus (LH), and pontine reticular formation (pontine RF) to produce conditional fear responses. During extinction, where the CS is presented in the absence of the US, prefrontal circuitry comes to inhibit the activity of neurons in the amygdala. This is a form of new learning that is under complex regulation by contextual information provided by the hippocampus.

subregions. The lateral (LA) and basolateral amygdala (BLA) regions receive sensory input from throughout the brain, which includes both CS and US information. From here, output information flows through the central nucleus of the amygdala (CeA) which projects to fear output regions such as the periaqueductal gray (PAG), lateral hypothalamus, and the pontine reticular formation. The PAG is a complex structure that plays a critical role in coordinating defensive behavior. The dorsolateral portion of the PAG is necessary for the activity burst response to the shock, whereas the ventrolateral portion is necessary for freezing and analgesia. The lateral hypothalamus mediates the cardiovascular aspects of fear, such as increased blood pressure and changes in heart rate. The pontine reticular formation is critical in fear-mediated potentiation of the startle response.

Acquisition of conditional fear is thought to depend on associative synaptic plasticity in amygdala neurons that receive both CS and US inputs. The US produces strong activation of the LA and BLA, which then activates the CeA to produce the UR, that is, the activity burst. CS inputs to the amygdala are initially too weak to produce activation of the CeA; however, CS-US pairings result in the strengthening of the CS input pathways to the amygdala. Thus, after conditioning the CS alone is capable of activating the CeA to produce fear responses. In addition to being a site of plasticity itself, a great deal of work from the laboratory of Jim McGaugh and others has shown that the amygdala also enhances memory storage in other brain regions.

Input Structures

Auditory inputs

Extensive work conducted in the lab of Joe LeDoux and many others has shown that auditory inputs to the amygdala come into the LA through two routes: directly from the auditory thalamus, that is, the medial geniculate nucleus, and more indirectly through the auditory cortex. Lesioning or inactivating these regions can impair the acquisition of conditional tone fear. These inputs differ in the rate, stability, and pharmacology of LTP induction and may also convey different forms of auditory information. The cortical input provides more processed sensory information that may be important, for example, in the proper timing of fear responses. Fear conditioning potentiates responding to the auditory CS in both of these regions, but this potentiation lags behind the plasticity at sites of CS-US convergence in the amygdala.

Hippocampus

The hippocampus receives highly processed sensory information from throughout the cortex. It is important for both episodic memory, that is, autobiographical memory for unique personal experiences, as well as for spatial navigation. Although a thorough discussion is outside of the scope of this article, a combined view of these functions of the hippocampus is that it allows for the encoding of multimodal sensory events, that is, episodes, and is able to connect these events into a complex and flexible cognitive map of the environment. In fear conditioning, the

hippocampus is critically important for the acquisition and expression of context fear. The hippocampus sends outputs to the BLA through the ventral angular bundle and plasticity at these synapses is necessary for hippocampus-dependent forms of conditional fear.

Synaptic plasticity at multiple subregions within the hippocampus is important for the formation of the contextual representations. Electrophysiological recordings in awake-behaving animals have shown that hippocampal neurons, referred to as ‘place cells,’ fire in specific locations in a given environment. The unique firing pattern of a population of these place cells can therefore tell an animal which environment it is in and exactly where it is in that environment. This may represent the neural correlate of the contextual representation and the strengthening of synapses which convey this information to the amygdala may be the site of the context–shock association.

Similar to human amnesic patients, hippocampal lesions produce a profound retrograde amnesia for context–shock associations formed just prior to the lesion. Context conditioning that occurred a few weeks or more before the lesion, however, is still intact. Based on this kind of evidence, researchers believe that the hippocampus is a site of temporary memory storage and that these memories are slowly consolidated in a distributed cortical network over time.

The role of the hippocampus in tone fear is quite complex. Strongly conditioned delay tone fear does not require the hippocampus. Strengthening of the auditory inputs to the amygdala appears to be sufficient. Trace fear conditioning, utilizing trace intervals of greater than about 10 s, does require the hippocampus. The exact role of the hippocampus in trace fear conditioning is still unknown; however, an increasingly plausible possibility is that it is involved in encoding and retrieving an episodic-like memory of the tone–shock pairing.

Alternate Pathways in Fear Conditioning

The canonical pathway discussed above is considered to be the primary pathway that mediates fear conditioning under normal circumstances. A great deal of emerging evidence, however, suggests that there are alternate sites of plasticity that can mediate fear learning when these primary structures are compromised. Extensive overtraining, for example, can overcome the loss of the amygdala. Compensation for loss of the hippocampus in contextual fear conditioning usually requires only a few extra context–shock pairings. Due to this, pretraining lesions of structures in the primary pathway tend to produce much less severe impairments than posttraining lesions. This discrepancy is thought to occur because the primary pathway actively inhibits learning in these alternate pathways. If learning occurs in the absence of an intact primary pathway, that is, with pretraining lesions, then the alternate pathways are able to compensate. If learning occurs in the

presence of primary pathway, which inhibits compensatory learning in the alternate pathways, but is tested in the absence of the primary pathway, that is, after a posttraining lesion, then compensation is largely absent. The exact identity of these alternate pathways and the mechanisms by which the primary pathway inhibits these alternate pathways is still unknown; however, researchers are rapidly identifying potential candidates.

Extinction Pathways

Not surprisingly, there is a great deal of overlap between the structures that are necessary for the acquisition and expression of conditional fear and for the structures involved in its extinction. Extinction results in the active inhibition of fear expression in a context-dependent manner. The ventromedial prefrontal cortex (vmPFC), particularly the infralimbic region, provides inhibitory modulation of the amygdala. The context specificity of this modulation is provided by interactions of the vmPFC with the hippocampus. Plasticity in the hippocampus, vmPFC, and amygdala are all required for the long-term memory of the extinction session. Expression of extinction requires vmPFC-mediated inhibition of the amygdala and proper contextual modulation of extinction requires hippocampal activity.

Fear Conditioning in Humans

Studies in humans have found that the underlying mechanisms of conditional fear are highly conserved between rodents and humans. An elegant study from the Antonio Damasio’s lab looked at fear conditioning in patients with selective bilateral damage to either the amygdala or the hippocampus. They found that the amygdala patients were unable to acquire the conditional autonomic fear response, yet they were perfectly aware that the CS predicted that shock was going to occur. In contrast, the hippocampal patients were able to acquire the CR, but they had no conscious awareness that the CS predicted the shock. Another study conducted in Larry Squire’s lab, which compared trace versus delay fear conditioning, found that successful trace conditioning required conscious awareness of the CS–US association, whereas delay conditioning did not. Thus, the amygdala is critically important in conditional fear responses just as it is in rodents. Furthermore, these studies suggest that the hippocampal contribution to trace fear conditioning may be mediated by its ability to support conscious awareness of CS–US contingencies, perhaps through retrieval of an episodic memory of the conditioning trial. Functional magnetic resonance imaging studies of fear conditioning show a striking consistency with the animal literature. Amygdala activation is consistently seen and

hippocampal activation has been observed with procedures designed to approximate context conditioning. Extinction is associated with vmPFC activation, just as it is in rodents, and the hippocampus seems to show specific activation when the extinction is context dependent.

Proper fear responses are critical to survival; however, improperly regulated and excessive fear responses can be highly debilitating. Specific phobias, anxiety disorders, and posttraumatic stress disorders are just a few examples of clinical disorders in which dysregulation of fear mechanisms is thought to occur. Our ability to treat these disorders depends critically on how well we understand the neurobiological basis of fear.

See also: Active Avoidance and Escape Learning; Animal Models of Learning and Memory; Fear, Anxiety, and Defensive Behaviors in Animals; Fear: Potentiation and Startle; Human Fear and Anxiety; Neural Basis of Classical Conditioning; Neural Substrates of Conditioned Fear and Anxiety.

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Fear: Potentiation and Startle

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Glossary

Acoustic startle circuit – The acoustic startle response is mediated by three synapses: first, at the cochlear root neurons, which then synapse onto neurons of the nucleus reticularis pontis caudalis (PnC), and these then project to motoneurons in the spinal cord.

Acoustic startle response – A characteristic cross-species reflex characterized by a regular pattern of muscle contraction and extension that is elicited by an abrupt, intense auditory stimulus. Acoustic startle is easily and objectively quantified in rodents and the human and is modulated by a variety of environmental conditions including conditioned fear and anxiety.

Anxiolytic drug – A drug that reduces the behavioral signs of anxiety.

Bed nucleus of the stria terminalis (BNST) – An area of the forebrain closely associated with the amygdala (indeed, it has been referred to as part of the ‘extended amygdala’). The BNST is thought to be responsible for many of the behavioral signs of anxiety.

Conditioned fear – A hypothetical central emotional state brought about through Pavlovian conditioning in which a conditioned stimulus (CS), such as a light, is consistently paired with an aversive unconditioned stimulus (US), such as shock. Conditioned fear is inferred from a variety of behaviors (e.g., freezing, potentiated startle, changes in heart rate and blood pressure, defecation, and analgesia) that are elicited by the CS after CS-US pairings.

Fear-potentiated startle – A measure of conditioned fear in which fear is defined as elevated startle amplitude in the presence versus the absence of a stimulus that was previously paired with shock.

Light-enhanced startle – A measure of unconditioned fear in rodents. Startle amplitude is larger when it is elicited in a constant, high level of illumination than when it is elicited in the dark. Light-enhanced startle is selectively blocked by anxiolytic drugs and is thought to depend on the bed nucleus of the stria terminalis. A similar phenomenon has been observed in the human. Human participants show enhanced startle amplitude in the dark.

In the last few decades, considerable emphasis has been placed on examining the neurobiological correlates

of fear and anxiety. Through all of this work we have not only come to understand the principal neural circuitry and physiology of fear and anxiety, but we have also developed a solid understanding of the methods that are used to produce and measure these emotions in the laboratory. One such method is the potentiation of the acoustic startle response. In this measure, fear or anxiety are inferred from an increase in the amplitude of the acoustic startle response. This measure, initially described by Brown, Kalish, and Faber, is derived in part from clinical observations that anxious individuals often exhibit exaggerated startle reactions to sudden, loud noises. While several studies followed this initial observation, it has been largely through the efforts of Michael Davis and colleagues that startle potentiation has become one of the most well-understood and valuable measures of fear and anxiety.

Pavlovian Conditioned Fear and the Fear-Potentiated Startle Procedure

In the typical Pavlovian conditioned-fear procedure, a neutral stimulus – such as a light – is paired with a mildly aversive stimulus – such as a footshock. After a few of these pairings, the light conditioned stimulus (CS) comes to elicit a variety of behaviors that are indicative of learned fear. In the fear-potentiated startle procedure, learned fear is assessed by measuring the acoustic startle response in the presence and absence of the CS after it has been paired with shock. Conditioned fear is operationally defined as elevated startle amplitude in the presence versus absence of the CS. It is important to note that facilitation of the startle response by the CS is the measure of conditioned fear. The CS does not elicit startle nor is the startle stimulus ever paired with shock. Instead, it is assumed that the conditioned response (CR) elicited by the CS is a state of fear. This state of fear potentiates the startle response that is elicited by the acoustic startle stimulus.

Fear-Potentiated Startle Is a Valid Measure of Conditioned Fear

Fear-potentiated startle is observed following training in which the CS is explicitly paired with shock and not when the CS is explicitly unpaired with shock or when CS and shock are presented randomly. Thus, fear-potentiated

startle is not the result of pseudoconditioning or shock-induced sensitization but instead reflects the learning that occurs with the association of the CS and shock. Moreover, fear-potentiated startle, like other measures of conditioned fear, has been measured over long training-to-testing intervals and has been measured following fear conditioning with visual, auditory, olfactory, tactile, and contextual CSs. Fear-potentiated startle has also been used to examine extinction of conditioned fear, as well as second-order conditioning and conditioned inhibition of fear indicating that it is a reliable measure of conditioned fear that is sensitive to experimental manipulations of learning.

It has been more than three decades since Davis and Astrachan touted fear-potentiated startle as an “easy and economical way to study classical [fear] conditioning.” In this time we have learned that fear-potentiated startle has many unique attributes that distinguish it from other measures of conditioned fear. These unique attributes rest squarely with the attributes of the acoustic startle reflex and because of them, studies using fear-potentiated startle have contributed a great deal to our understanding of behavioral and neural correlates of conditioned fear (and anxiety, see below).

Fear-Potentiated Startle and the Temporal Aspects of Conditioned Fear

Acoustic startle is a highly characteristic and rapid pattern of muscle flexion and extension that is elicited by an abrupt, intense acoustic stimulus. The latency of acoustic startle can be as short as 8 ms measured electromyographically in the hind leg of rats and last less than 200 ms. In the context of fear-potentiated startle, this allows for a momentary assessment of conditioned fear at any point during the CS. Capitalizing on this, Davis, Schlesinger, and Sorenson examined the temporal specificity of conditioned fear following conditioning with various CS + shock intervals. Separate groups of rats were given fear conditioning in which the interval between the onset of the light and the onset of the 500-ms shock (the inter-stimulus interval – ISI) was 0, 25, 50, 100, 200, 800, 3200, 12 800, or 51 200 ms. In the fear-potentiated startle test that followed, the brief (50 ms) startle-eliciting stimulus was presented at each of the nine ISIs on separate trials for all rats. Thus, for each group, the startle stimulus was presented at the time the shock had occurred in fear conditioning as well as at shorter and longer ISIs. Their results clearly demonstrated the temporal specificity of conditioned fear: each group of rats showed the greatest fear-potentiated startle at the CS + shock interval that was used in fear conditioning and much less fear-potentiated startle at intervals on either side of the conditioning interval. As Davis and colleagues point out, these results suggest that rats learn to time the occurrence of the shock,

even at very short intervals. Thus, a mechanism for timing must be part of the neural circuit for fear. Such timing experiments would be very difficult, if possible at all, with other measures of conditioned fear where responses are slower to develop or persist for a time after the offset of the CS.

In another examination of timing in fear conditioning, Falls and Davis were able to use fear-potentiated startle to document the transition from fear expression to fear inhibition in a serial feature-negative discrimination procedure. Rats were given fear conditioning in which a light was paired with foot shock. On some trials, the light was immediately preceded by a noise, and the combination was not paired with shock (noise → light no shock). As a result, the noise acquired the ability to inhibit fear to the light: fear-potentiated startle on light trials was significantly greater than on noise → light trials. Somewhat unexpected, however, was the observation that startle was also potentiated by the noise: startle amplitude in the presence of the noise alone was similar in magnitude to startle amplitude in the presence of the light CS. Thus, it appeared that the noise had acquired the ability to both elicit fear when presented by itself (perhaps through a process of second-order conditioning) and inhibit fear when presented in combination with the light. To examine this further, Falls and Davis used procedures similar to those used by Davis and colleagues to examine the temporal transition of fear to the inhibition of fear. Rats were trained for conditioned inhibition, and in the subsequent fear-potentiated startle test, startle was elicited at various time points during the noise and light on noise → light trials. The results showed that the fear to the noise was present until its offset, at which time it inhibited fear to the light. The ‘trace inhibition’ by the noise was subsequently confirmed when it was found that trace fear conditioning to the noise-conditioned inhibitor (a procedure in which the shock is presented after the offset of the noise at the time when fear inhibition is thought to occur) was weaker following conditioned-inhibition training. Similar to the temporal specificity of conditioned fear, the ability to momentarily assess conditioned fear at any point during the CS has allowed the use of fear-potentiated startle to explore aspects of conditioned fear not easily assessed using other measures.

Fear-Potentiated Startle and Pharmacology of Conditioned Fear

Fear-potentiated startle has a nonzero baseline and, as a result, has proven useful as a model for screening drugs. In fear-potentiated startle, conditioned fear is measured within the subject by comparing startle amplitude in the presence and absence of the CS. Unlike other measures of conditioned fear, manipulations that affect conditioned fear can be easily dissociated from those that affect the

behavioral measure of fear (i.e., startle amplitude). A number of drugs reduce fear-potentiated startle, most of which do so without affecting startle amplitude. For example, drugs that are anxiolytic in the human, such as the serotonin 1_A partial agonist buspirone, the γ -aminobutyric acid-A (GABA-A) agonists diazepam, or morphine and bupenorphine, as well as novel anxiolytics such as metabotropic glutamate agonists, nonpeptide bombesin receptor antagonist, and secretin reduce fear-potentiated startle without the confounding influence of reducing startle amplitude in the absence of the CS. Thus, the nonzero baseline that is inherent in the fear-potentiated startle procedure (i.e., startle amplitude in the absence of the CS) serves as an important measure for evaluating the nonspecific (e.g., sensory or motor impairing) effects of drugs.

However, there have been some reports in which putative anxiolytic drugs reduced both fear-potentiated startle and startle in the absence of the CS. In the fear-potentiated startle procedure, conditioned fear is defined as elevated startle amplitude in the presence versus the absence of the CS. It is assumed that fear is more or less restricted to the time during which the CS is present and that startle in the absence of the CS reflects a baseline of little or no fear. However, under certain circumstances, startle may also be modestly potentiated in the absence of the CS, and this potentiation of baseline startle may reflect contextual fear that may also be sensitive to anxiolytic drugs. Guscott, Cook, and Bristow showed that following CS and shock fear conditioning, rats demonstrated a robust fear-potentiated startle to the CS as well as potentiation of startle in the absence of the CS. However, if rats tested in an apparatus that was different from the training apparatus, they did not show potentiation of startle in the absence of the CS despite showing robust fear-potentiated startle to the CS. Interestingly, although the GABA-A partial agonists FG8205 and bretnazene attenuated fear-potentiated startle regardless of the apparatus the rats were tested in, they reduced startle in the absence of the CS (i.e., baseline startle) only in rats trained and tested in the same apparatus. This suggests that, under some circumstances, startle in the absence of the CS may be potentiated by contextual fear and that this fear is also sensitive to anxiolytic drugs. Hence, the nonzero baseline may not necessarily reflect startle in the absence of fear, and care should be taken to fully evaluate possible baseline changes when measuring fear-potentiated startle.

The Neural Circuit for Acoustic Startle and Fear-Potentiated Startle

A great deal is known about the neural circuit for acoustic startle, making it unique among measures of conditioned fear. A combination of anatomical, lesion, and stimulation

experiments have shown that acoustic startle is mediated by three synapses: first, at the cochlear root neurons, which then synapse onto neurons of the nucleus reticularis pontis caudalis (PnC), and these then project to motoneurons in the spinal cord. Although other studies have suggested that startle may also involve projections from the posteroverentral cochlear nucleus to an area ventral and medial to the ventral nucleus of the lateral lemniscus and projections to the PnC, more restricted lesions of these areas failed to interfere with acoustic startle suggesting that they are not obligatory synapses in the acoustic startle reflex.

Numerous studies have shown that the amygdala is essential for the acquisition and expression of conditioned fear and several studies have shown that lesion or pharmacological inactivation of the amygdala also interferes with fear-potentiated startle. It is widely believed that CS and shock information converge at the lateral nucleus of the amygdala and that fear conditioning is mediated by changes in neurons in lateral or basal nuclei of the amygdala. The major output of these amygdala nuclei is through the central nucleus of the amygdala. Using a combination of anatomical, lesion, and stimulation techniques, studies have shown that the central nucleus projects directly to the PnC and these projections mediate fear-potentiated startle. The direct projection from the central nucleus to the PnC may involve the release of corticotropin-releasing factor (CRF) because CRF containing cells of the central nucleus projects directly to the PnC and micro-injection of the CRF antagonist, α -helical CRF, into the PnC blocks fear-potentiated startle without affecting startle amplitude. Indirect projections appear to involve the midbrain in an area identified as the superior colliculus/mesencephalic area. A combination of anatomical, collision, and lesion studies suggests that synapses in this area serve as a relay between the central nucleus and the PnC. Thus, areas in addition to the central nucleus of the amygdala may participate in fear-potentiated startle. Through all of this work one thing is clear: as a result of the firm understanding of the acoustic startle pathway, we have come to understand the pathways through which fear modulates acoustic startle making the ‘fear-potentiated startle circuit’ one of the most well understood in the vertebrate brain.

Fear-Potentiated Startle in Rodents, Nonhuman Primates, and the Human: Translational Potential of Fear-Potentiated Startle

Acoustic startle is a ubiquitous cross-species reflex that is easily measured in rats, mice, monkeys, and the human. Fear-potentiated startle has also been examined across these species. The startle has been examined in inbred, genetically modified mice, and has been used for high-throughput examination of conditioned fear in

mutagenized mice. Fear-potentiated startle has also been examined in nonhuman primates and, like fear-potentiated startle in rodents, is reduced by anxiolytic drugs. However, it is its use in the human that has truly highlighted the translational power of the fear-potentiated startle procedure. Startle is reliably increased in the presence of fear-eliciting stimuli and the human shows reliable fear-potentiated startle following fear conditioning in which a light is explicitly paired with shock. The human also shows fear-potentiated startle to a light that signals the threat of shock even if shock is never explicitly paired with the light.

The Human Appears to Show Consistent and Robust Contextual Fear-Potentiated Startle

In a series of experiments, Grillon and colleagues have shown that following fear conditioning in which a CS and shock are presented explicitly unpaired (i.e., temporally discontiguous), or in an unpredictable fashion, the startle response in the absence of the CS is potentiated. In these instances, potentiation in the absence of the CS is thought to reflect context conditioning or a more sustained form of potentiation that reflects learning around the unpredictability of shock. Interestingly, it is the context potentiation (i.e., the sustained potentiation) that is most sensitive to anxiolytic drugs. For example, chronic citalopram (daily for 2 weeks) did not affect fear-potentiated startle to a CS that was previously paired with shock. However, chronic citalopram reduced the startle potentiation in the absence of the CS in participants who were given random presentations of the CS and shock (i.e., contextual fear, sustained potentiation). Similar results were obtained with the benzodiazepine alprazolam and the β -blocker propantheline. Interestingly, contextual fear may also highlight differences among clinically anxious populations. Individuals with posttraumatic stress disorder (PTSD) or panic disorder show normative fear-potentiated startle to a CS that was previously paired with shock and exaggerated contextual fear following unpredictable shock. In contrast, participants with generalized anxiety disorder showed normative fear-potentiated startle to both the CS and context. Together, these results suggest that startle responses to CS paired with shock and to a context may represent different aversive states and may be pharmacologically distinct. Once again highlighting the translational value of potentiated startle, rodent-potentiated startle experiments have also suggested that there are distinct aversive systems in the brain. These systems may distinguish fear to discrete CS and fear (or anxiety) that is more generalized and sustained (see below).

Anxiety, Fear, and Unconditioned Potentiation of Startle

Unconditioned Potentiation of Startle as a Model of Anxiety

As mentioned at the outset, fear-potentiated startle was developed in part from the observation that clinically anxious individuals often show exaggerated startle reactions. Acoustic startle is not only used to measure conditioned fear, it is also used to measure unlearned aversive states – such as those produced by fear-arousing pictures or pain and those produced by ‘anxiety’ or anxiogenic drugs. The recognition that startle is potentiated by these other aversive states led Walker and Davis to investigate whether unconditioned stimuli (US), such as bright lights, could also potentiate startle. If so, such unconditioned potentiation could be used to investigate unlearned fear while holding on to many of the advantages of fear-potentiated startle. In a series of experiments, they demonstrated that when rats were placed in a constant high level of illumination, they showed higher startle amplitude than when they were placed in the dark. This so-called light-enhanced startle effect was a function of the level of illumination and was attenuated by the anxiolytic buspirone. Since this initial report, light-enhanced startle has also been demonstrated in mice and has been shown to be blocked by a variety of anxiolytic drugs. Light-enhanced startle has been used to examine anxiety associated with nicotine withdrawal as well as sex differences in anxiety. It is worth noting that the human, as a diurnal animal, instead shows dark-enhanced startle.

The Neural Substrates of Anxiety-Potentiated Startle

Lesions of the bed nucleus of the stria terminalis (BNST) abolish the anxiogenic effect of intracerebroventricular CRF and, like the central nucleus of the amygdala, the BNST receives projections from other amygdalar nuclei and projects to the brain regions mediating behavioral responses to fear, including the PnC. This led Walker and Davis to examine whether the BNST mediated the light-enhanced startle effect – an effect, like CRF, that produces a sustained potentiation of startle. Rats were given either BNST, central nucleus of the amygdala, or sham lesions and tested for light-enhanced startle or fear-potentiated startle to a light CS. As expected, lesions of the central nucleus abolished fear-potentiated startle to the light CS. Central nucleus lesions did not affect light-enhanced startle. In contrast, lesions of the BNST abolished light-enhanced startle but did not affect fear-potentiated startle. This double dissociation of the role of the central nucleus of the amygdala and the BNST

in conditioned and unconditioned fear has led to the hypothesis that the central nucleus of the amygdala mediates fear to discrete, predictable stimuli whereas the BNST mediates sustained fear, or anxiety, to long-duration, unpredictable threats. Following this initial observation, experiments have shown that lesions of the BNST in rats also abolish fear to long duration CSs as well as contextual stimuli.

Recalling the effects of anxiolytic drugs in human fear-potentiated startle, it is interesting to speculate that the unpredictable nature of unpaired or random CS and shock presentations engages the BNST which, unlike the central nucleus of the amygdala, may be more sensitive to the effects of anxiolytic drugs. Following this further, given that individuals with PTSD or panic disorder show exaggerated sustained fear, it is also interesting to speculate that these disorders may involve dysregulation of the BNST.

Conclusion

The acoustic startle response has utility in both basic and clinical science. For more than 50 years, scientists have studied the acoustic startle and have used it as a tool to investigate the behavioral, pharmacological, and neuroanatomical correlates of conditioned fear and anxiety. Owing to the insights and creativity of these scientists, we now have a solid understanding of startle potentiation. Future studies in rats, mice, monkeys, and the human are certain to build on this foundation and create an even deeper understanding of conditioned fear and anxiety.

See also: Aging and Cognition.

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Feeding

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Glossary

Behavioral satiety sequence – A sequence of behavior that is characteristic of the transition from feeding through satiation to satiety. In rodents it is typically associated with a short period of activity followed by grooming and inactivity. A similar behavioral sequence is observed in many other mammals, including the human.

Conditioned satiety – A phenomenon in which sensory characteristics of a food that are consistently associated with the energy value of that food come to influence consumption during *ad libitum* feeding.

Conditioned taste aversion – A phenomenon which leads to reductions in consumption of food that was previously associated with illness. A wide variety of manipulations, including those that may have rewarding effects in other contexts, can lead to conditioned taste aversions.

Devaluation (of a food reward) – Any process that leads to reduced motivation to consume a particular food can be described as resulting in devaluation of that food.

Diet-induced obesity – An increase in body weight, most of which can be attributed to adipose tissue, that results from increased caloric intake of a palatable diet.

Hunger – A motivational state in which feeding behavior is very likely, providing that an appropriate food is available.

Meal – A short period of time during which feeding behavior occurs at high intensity. Meals are operationally defined using a ‘meal criterion.’ The characteristics of a sequence of meals include measures of meal size, duration, inter-meal interval, and feeding rate.

Meal criterion – The operational definition(s) used to define a meal. The definition will normally comprise a time between consumption of food items which, when exceeded, signal that the next occurrence of feeding will be part of the following meal. Optionally there may also be a requirement that a meal involves consumption of more than some minimum amount of food.

Pair feeding – A behavioral paradigm that is used to determine whether the effects of a particular manipulation on body weight depend on changes in energy intake or energy expenditure.

Satiation – The process that occurs during an extended period of feeding and leads to its cessation. Satiation normally develops in response to a combination of

cognitive and pre-ingestive cues associated with feeding.

Satiety – A state in which feeding behavior has become unlikely because of recent consumption of food. Satiety is usually maintained by a combination of postingestive and postabsorptive cues.

Sham feeding – A preparation, most usually in rats, in which consumed foods or liquids drain from the stomach through a surgically prepared fistula. This paradigm can be used to eliminate postingestive cues and thus assess their role in influencing food intake. Most commonly, sham feeding leads to very substantial increases in intake.

Introduction

The amount of food eaten in a test situation is almost certainly the most widely used behaviorally dependent measure in behavioral neuroscience. It is necessarily employed in the context of studies where the primary interest concerns the neural circuits or physiological mechanisms underlying food intake and energy balance. However, it is also often used as a ‘control’ condition in studies of other motivational systems, especially of those that involve self-administration of drugs. Finally, food is the most frequently used positive reinforcer in studies of learning and memory. Even in such studies, the amounts that are consumed make it useful to have some appreciation of the properties of feeding behavior.

In rodents, and many other commonly used laboratory animals, feeding has a complex behavioral structure. Feeding behavior – at least when food is easily available – takes place several times a day in clearly defined meals. Small laboratory rodents, such as rats and mice, may take 10–20 such meals during a 24-h period. Feeding is also subject to strong circadian influences which can entrain other behavior as well as physiological responses, such as hormone secretion and neurotransmitter synthesis.

Feeding behavior is organized and modulated by a distributed neural network that includes structures such as the parabrachial nucleus and nucleus of the solitary tract within the brainstem, medial and lateral hypothalamic systems, as well as ventral and dorsal components of the striatum and a number of cortical areas. These different brain areas rely on a wide range of neurotransmitter

systems and receive peripheral inputs that signal both short- and long-term correlates of energy reserves. The distinction between appetitive and consummatory components is often made for motivated behaviors such as feeding. Although it may be simplistic, it does reflect some important differences in the underlying neural and physiological systems. The distinction is followed here, with consummatory behavior referring to the behavioral processes that are engaged once an animal is in close proximity to, and consuming, food, whereas appetitive behavior relates to those components which might be expressed as foraging in a natural environment. In the laboratory, the study of appetitive behavior typically involves behavioral responses that have been conditioned by food reward. For example, an animal may react to discrete or contextual cues that predict the presence of food (Pavlovian responses) or may be performing responses that have, in the past, led to food reward (instrumental responses). This dichotomy, although somewhat artificial, does reflect important differences both in underlying mechanisms and in the relevant behavioral paradigms.

Consummatory Behavior

'Simple' Measures of Food Intake

The simplest paradigm that can be used to investigate feeding is to allow an animal to feed for a fixed period time and record the mass of food eaten by the end of that period. However, even in this apparently simple case, a host of decisions must be taken that will influence the amount of food that is eaten and modify the relative effect of any experimental manipulation that is being studied. Is the animal to be deprived of food before the test? Will it be presented with its habitual food? If a different food is to be used, will it be novel, or one to which the animal has been exposed previously? Often an animal will be deprived of food for a period of time before an intake test. The chosen time period is frequently 24 h or greater for rats. Following withdrawal of food for such a period, the animals will eat rapidly for a protracted period. The factors that determine intake in this situation will be very different from those that operate when food is freely available. Intake will be at close to ceiling levels, which means that the measure will be quite insensitive, especially to manipulations that tend to increase feeding behavior. Excessive periods of food withdrawal should also be avoided on welfare grounds. The motivation to feed can be increased in a number of other ways, most obviously by supplying food that is more attractive than the maintenance diet. When increases in feeding are expected, it will be common practice to use nondeprived animals. Some manipulations, such as intra-accumbens administration of γ -amino butyric acid (GABA) agonists,

lead to very robust increases in intake. Other manipulations, such as the administration of noradrenaline into the paraventricular nucleus (PVN) of the hypothalamus, generate increases in feeding which are harder to demonstrate. For example, simply presenting 'satiated' rats with a weighed, fresh supply of their maintenance diet is often sufficient to stimulate intake of a 1–2 g over the following 30 min. This will be quite sufficient to mask a noradrenaline-induced increase in feeding. Merely presenting the rats with fresh pellets 30–60 min in advance of testing and then presenting the weighed portion of these pellets following drug administration will provide a much more sensitive measure of the drug effect.

Accurate measurement of food intake will require assessment of the degree of spillage which may well be treatment dependent. In addition, laboratory rodents will often respond to the presence of a more novel food with defensive burying.

The degree of food intake may be enhanced in a variety of ways. Where there is no wish to vary the dietary constituents, then adding water to a standard, dry laboratory diet will often be sufficient to make a very palatable mash. Rats presented with a short daily period of free access to mash made in this way will consume between one-third and one-half of their daily caloric intake within 30–40 min, even when the mash is presented during the light phase of the photoperiod when intake is otherwise minimal.

There has been considerable interest in the idea that changes in some neurotransmitter systems associated with feeding, especially serotonin, may modify diet selection. Studies investigating this idea typically allow animals a choice between pure macronutrients such as casein (protein), lard (fat), and either a simple or complex carbohydrate (e.g., glucose or polyose, respectively). One important issue, which should never be ignored, is the extent to which differences in texture, water content, and taste – rather than macronutrient type – are responsible for any observed changes in intake.

Responses to cues previously associated with food may also have a powerful influence on the amount that is consumed during a test session (see section titled 'Pavlovian influences'). Effects of this type were first explored systematically by Weingarten. He showed that if hungry rats were periodically presented with food after presentation of conditioned stimulus, such as tone, they would soon learn to approach the receptacle into which the food was delivered. If, at a later test session, they were given access to food when sated then they would eat more if the tone was sounded during the test session. More recent experiments have demonstrated that the effect is selective to the stimulus associated with food delivery and not to another stimulus that was not associated with food delivery. It is also clear that the stimulus is not simply generating an approach response to food that then leads to

consumption. If the animals have learnt to expect food in a different place, although this has not previously been associated with presentation of the stimulus, they will nevertheless approach and eat food. This suggests that the conditioned stimulus has a broad effect that increases the motivation to feed. It is important to be aware that effects of this kind may occur inadvertently during repeated simple tests of food intake.

Long-Term Feeding Studies

Chronic increases in food intake may be increased by modifying the diet. In rodents, and many other mammals, provision of a diet relatively rich in fat and simple, sweet-tasting sugars will increase total caloric intake. This may be accomplished by either using a preprepared commercial diet in place of regular chow, or by providing additional food types in a ‘cafeteria’-style diet (e.g., a separate container of lard and a bottle of sucrose solution in addition to regular chow and water). When a preprepared diet is used, then further increase in caloric intake – and body weight – can be achieved by giving access to a high-calorie fluid such as Ensure. It is often argued that diet-induced obesity, especially when it also involves choice among dietary constituents, offers a better model of common human obesity than genetically manipulated animals such as the *ob/ob* mouse.

In some feeding studies, a critical question will be the extent to which changes in body weight over a period of some days have resulted from changes in energy intake and energy output. Energy intake should be approximated by the energy content of the food eaten during the relevant period, although there may be changes in the efficiency of digestion and absorption, especially when intake is being restricted in some way. Energy output may be estimated directly using indirect calorimetry or other techniques. However, an alternative way of tackling this question is to use a pair-feeding paradigm.

A typical experiment of this type might use three groups: the first receives the manipulation in question (perhaps a drug treatment or brain manipulation that increases body weight), a second group receives the same manipulation but has its food intake restricted to that of a third control group which receives no active treatment. Such an experiment may have a number of potential outcomes. One possibility is that the degree of weight increase in the two experimental groups is very similar and higher than that seen in the control group. This would indicate that the mechanism underlying weight increase is one that depends on a reduction in energy output. It might reflect a lowering of the metabolic rate, but it is also possible that reductions in voluntary activity, perhaps induced by a sedative or muscle-relaxant effect of a drug, might be responsible for such an outcome. Direct behavioral observations should help to separate

these options. An alternative outcome might be that the weight of the pair-fed and control groups is similar, and lower than that of the experimental group given unrestricted access to food. This would strongly suggest that increased food intake is responsible for the increase in body weight produced by this manipulation.

The same kind of design can be applied to a treatment that is associated with a reduction in body weight. In such cases there will be a single drug-treatment group and two control groups, one of which receives *ad libitum* access to food and the other of which has its food intake matched to that of the drug-treated group. Although these designs may seem ideal for separating the effects of energy intake and output, the results from a particular experiment may be ambiguous in several ways. If the experiment is under-powered and the data of the pair-fed group is intermediate between the treatment and control groups, then the conclusion drawn will depend on which of the (nonsignificant) paired comparisons is judged to be more important. A frequent outcome, especially when the experiment is conducted over a longer time period, is that there may be differences between the pair-fed and unrestricted group which become less evident with time. Several points are relevant in interpreting such an outcome. First, energy intake cumulated over the study period may remain substantially different and sufficient to explain the observed weight differences. Second, there may be gradually emerging adaptive changes to perceived energy deficits that occur at either a behavioral or physiological level. These may be exacerbated by the very different daily intake patterns that emerge as the experiment progresses.

Detailed Measures of Consummatory Behavior

The consummatory phase of eating may be studied at several levels. Observations at the macro-level often involve recording some variant of the behavioral satiety sequence. This technique, which originated with Richter in the 1930s, and was refined by Bolles and Antin in the 1960s before the development of the present form of analysis, is especially useful for distinguishing the development of normal satiety from reductions of eating as a result of illness, pain, or stress induced by an experimental manipulation. For rodents, the method utilizes relatively broad behavioral categories, such as feed, groom, inactive, and rear. These may be recorded continuously, typically from prior recording of the experimental sessions. An alternative method, which provides very similar data, is to utilize live sequential one-zero recording from a complete experimental cohort of subjects. Although the sequence will be variable from one individual to another, common features include an initial period of intense eating followed by increased activity (both locomotion around the cage and rearing), grooming, and – provided

the observation period is long enough – a period of inactivity. One conventional, though arbitrary, indication of the onset of satiety is the point at which the proportion of time devoted to eating is less than that spent in inactive behavior.

A second, macro-level technique is the recording of free-running meal patterns. Again this may be achieved in several ways. One method, deriving from Kissileff's development of a pellet-delivering eatometer, allows a rodent to take standard, grain-based pellets (rat: 45 mg; mouse: 10 or 20 mg). Intake of pellets and water should be monitored with good temporal resolution (at least to a fraction of a second). Such a system will allow accurate assessment of the instantaneous feeding rate as well as the microstructure of feeding and drinking bouts. However, it will be less easy to manipulate dietary constituents, and if this is required then it may be better to use a recording system that depends on repeated automatic weighing (usually via built in strain gauges) of food- and water-containers. Temporal resolution will be significantly worse with such systems, and estimation of instantaneous feeding rate is not possible.

Once a record of intake has been obtained, it will require further analysis to extract meal patterns. An initial choice of a bout or meal criterion is required to define individual meals (**Figure 1**). The meals can then be characterized in terms of their size, duration, intra-meal feeding rate, and inter-meal interval. Although time-based meal criterions are most commonly used, some authors also impose a minimum size for a meal. One recent development has been to combine meal-pattern analysis in free-feeding animals with the recording of the behavioral satiety sequence. These studies have confirmed that the sequence is not an artifact of regularly presenting animals with a palatable, easily eaten food. They have also suggested that the most appropriate criterion for meal termination should include consideration of both feeding and drinking responses.

Microstructural analysis of licking patterns is frequently used when a liquid food has been provided. These techniques, especially as developed by Davis, Smith, and colleagues, are conceptually similar to meal-

pattern analysis, although they operate at a finer level of analysis. Changes in palatability and caloric load produce distinct patterns of change in the resulting licking-bout analysis.

Another technique which holds out the promise of distinguishing between hedonic and reward-related effects of a manipulation is the taste-reactivity task. This paradigm, developed by Grill and Berridge, is best known for its supportive role in the incentive sensitization theory of addiction. Animals, typically rats, are prepared with an intra-oral catheter through which more or less palatable solutions can be introduced in measured volumes. The animal's responses are scored and can be divided into negative and positive hedonic components. This relatively pure measure of hedonic responses to food-related stimuli can then be compared with the reward-related aspects that are measured using more conventional instrumental tasks (see section titled 'Instrumental tasks').

Appetitive Behavior

Pavlovian Influences

A variety of Pavlovian procedures may be used to study appetitive components of feeding behavior. They share the common feature that, during training, food is presented in combination with a conditioned stimulus without regard to the animal's current behavior. When the conditioned stimulus is discrete and signals the imminent availability of a small reward, animals will usually display an orienting reflex to the stimulus before moving toward the likely source of food. As training proceeds, the orienting reflex will often become less pronounced, especially when the relationship between stimulus and food is entirely predictable. An animal may also direct behavioral responses toward the conditioned stimulus. These are usually referred to as autoshaped responses and their form is strongly related to the relevant consummatory behavior. Under some circumstances these responses may be easily measured, as when the stimulus is a lever inserted briefly into the cage and is pressed, or a light is illuminated in an aperture where the animal may make a nose poke.

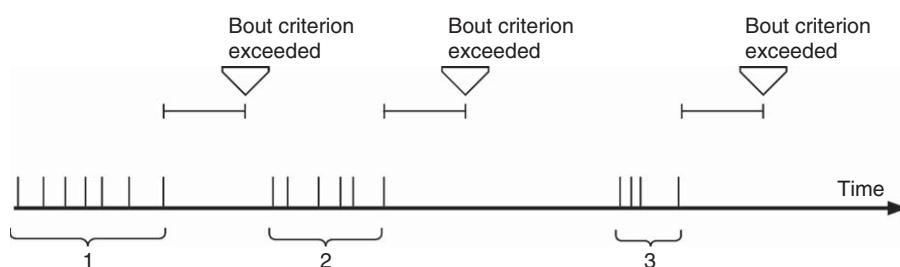


Figure 1 Applying a bout criterion to a series of individual feeding responses (short vertical lines). In this case, the process would generate three meals of sizes 7, 6, and 4.

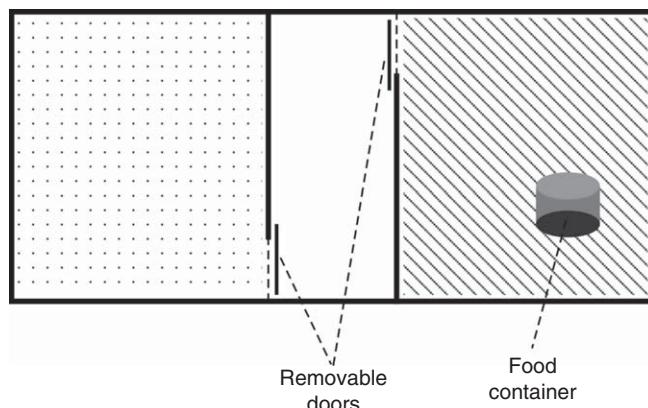


Figure 2 A typical two-chamber conditioned place-preference chamber. During the training the doors are left in place. They are removed during testing and the animal can then move from one chamber to the other through the central corridor. The food container is also removed during testing.

Contextual stimuli may also be used to condition approach responses. One classic version is the conditioned place task. It uses a two-compartment chamber, each with distinct contextual cues. During training, animals are confined to one of the two chambers (Figure 2). Food is made available in one and not in the other. During test sessions, animals are free to enter either compartment. They are likely to spend a greater proportion of time in the food-associated chamber. This learnt preference is susceptible both to reversible revaluation (e.g., the preference increases with an increasing level of food deprivation) and to more permanent devaluation (e.g., induction of a conditioned taste aversion to the particular food reward associated with the reinforced chamber). Testing is always carried out in the absence of food. One consequence is that the response is susceptible to extinction both within and across test trials. Place-preference tasks are susceptible to differences in initial preferences for the different compartments and the decision as to whether to fully randomize the assignment of animals to possible training conditions, or to train against any initial preference, is an important one.

Instrumental Tasks

Food is a potent reinforcer and many different instrumental responses have been used to estimate components of feeding motivation. In most of these paradigms, food is provided, so they do not provide pure measures of appetitive responding during the test session.

One example – historically important, and still in use today – is running speed to a food reward at the end of a straight alley. Running speeds are sensitive to the value of the reward that is provided. For example, providing a larger-than-expected food reward will produce a clear increase in running speed on the next few trials, while a smaller-than-expected reward will have the opposite

effect (the Crespi Effect, described in 1942). The decrease in running speed over a series of trials can provide a sensitive index of satiation. For example, a drug treatment that decreases satiation will have little effect on running speed in early trials but enhance it, relative to a control condition, later in the session.

Lever-press, or nose-poke, operant responses also provide useful measures of feeding motivation. The great majority of studies use animals that are mildly food deprived and tested in short sessions. Ratio or interval schedules may be used. Fixed or variable ratio schedules will tend to produce high response rates that are sensitive to feeding motivation whereas interval schedules are less useful in this regard. However, response rate on a ratio schedule will also be sensitive to other factors such as motor impairment or sedation induced by a particular experimental manipulation. An alternative approach is one in which the number of responses required for reward gradually escalates during the session. Animals on such progressive ratio schedules will eventually stop responding. The ratio at which they stop is termed the breakpoint, and will be less sensitive to extraneous effects of an experimental manipulation than the response rate. The operant requirement may escalate through either an arithmetic or a geometric series (Table 1). Food consumption prior to the breakpoint is likely to be higher with an arithmetic sequence, and hence the potentially confounding effects of satiation may be greater.

Any of the schedules described so far will result in food consumption and hence initiate satiation. A purer measure of appetitive behavior may be obtained by using a modified second-order schedule. Responding can be sustained for an extended period, especially at the beginning of a test session, if a conditioned stimulus is associated with food delivery and lever pressing is initially only rewarded with presentation of this stimulus. Schedules

Table 1 Performance measures on two progressive ratio schedules

<i>Breakpoint (B)</i>	<i>Schedule 1</i>	<i>Total presses</i>	<i>Schedule 2</i>	<i>Total presses</i>
1	1	1	1	1
2	4	5	4	5
3	7	12	6	11
4	10	22	9	20
5	13	35	12	32
6	16	51	15	47
7	19	70	20	67
8	22	92	25	92
9	25	117	32	124
10	28	145	40	164
11	31	176	50	214
12	34	210	62	276
13	37	247	77	353
14	40	287	95	448
15	43	330	118	566
16	46	376	145	711
17	49	425	178	889
18	52	477	219	1108
19	55	532	268	1376
20	58	590	328	1704

Break points, operant requirements and total lever presses for an arithmetic (1) and a geometric schedule (2). On Schedule 1 the operant requirement increments by 3 for successive rewards. On Schedule 2 the schedule is determined by the relationship $5 \times e^{b \times 0.2} - 5$. Schedule 1 is slightly harder at low breakpoints but Schedule 2 is much harder at high breakpoints.

of this kind can be used with rats and mice and can easily provide a measure of responses rate, over an initial non-food-reinforced period of 5 min in a 30-min test, which remains stable from one test session to another. This allows comparison of the effects of a several related manipulations (e.g., different drug doses) using a within-subject design.

Several other paradigms, including conditioned reinforcement and Pavlovian to instrumental transfer, deserve consideration if the research question concerns the extent to which conditioned stimuli may energize a feeding response. For example, in Pavlovian to instrumental transfer, animals undergo a two-component training process in which, separately, they acquire a Pavlovian association between a stimulus and food delivery and also learn to perform an instrumental response for that food. In the test sessions, which may be carried out in extinction or with food reward provided, instrumental response rates are measured in alternating periods during which the conditioned stimulus either is, or is not, present. Pavlovian to instrumental transfer is measured as the extent to which responding is facilitated in the presence of the conditioned stimulus.

Choosing an Appropriate Model

A number of factors will determine the appropriate animal models of feeding for a particular study. Only

rarely will the results from a single test paradigm be sufficient to determine the behavioral mechanism of action of a manipulation that affects food intake. The better approach will be to choose a test battery which reflects the neural and behavioral mechanisms of interest. Detailed behavioral analysis is likely to be rewarding in many circumstances. In addition to maximizing scientific utility, it will be important to consider how animal welfare can be enhanced by considering the principles of refinement and reduction. For example, long periods of food deprivation – which may also be scientifically undesirable – should be avoided and within-subject designs will frequently be more powerful than a between-subject approach and will also minimize the number of animals that are required for a particular study.

See also: Cognition: Attention and Impulsivity; Cognition: Learning and Memory: Pavlovian; Control of Food Intake; Depression; Drug Addiction; Gastrointestinal Peptides and the Control of Food Intake; Genes and Behavior: Animal Models; Hormonal Contributions to Arousal and Motivation; Incentive Motivation and Incentive Salience; Motivation; Motor Function and Motivation; Neural Systems of Motivation; Obesity and Binge Eating Disorder; Pleasure; Psychoneuroendocrinology of Stress; Stress and Energy Homeostasis; Value of Animal Models for Predicting CNS Therapeutic Action.

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Genes and Behavior: Animal Models

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Glossary

Congenic – A line of species that is genetically identical except at a selected locus.

Epigenetics – Changes in gene function independent of changes in DNA sequence.

Gene – A sequence of DNA that is the basic unit of inheritance.

Heterosis – When offspring from a cross of two inbred lines have greater fitness or performance compared to the parental lines.

Heterozygous – Containing two different alleles at a given locus.

Homologous recombination – The swapping of genetic materials that have similar sequences.

Homozygous – Containing two identical alleles at a given locus.

Mutation – A change in DNA sequence.

Phenotype – The observable expression of genotype.

Polygenic – A phenotype dependent upon two or more genes.

Polymorphism – An alternate allele that is expressed with some degree of regularity.

Synteny – Conservation of gene sequences and chromosomal order across species.

Transgene – A segment of exogenous DNA that has been incorporated into the host genome.

Today, it is clear that the relationship between nature and nurture, or for the purpose of this article genes and environment, is a reciprocal relationship. Perhaps this relationship is best exemplified by Escher's 1948 lithograph, Drawing Hands. In this famous work, a hand emerging from a sketch is drawing another hand, which in turn is drawing the aforementioned hand. There is neither a beginning nor an end to this image; each hand cannot exist without the other and together they complete the picture. Substitute genes for one hand and environment for the other, and substitute behavior for the complete image and the relationship between genes, environment, and behavior are well represented. While we are born with our individualized genetic code, this alone is not sufficient to dictate behavior. Genes are only influential if their code is translated into proteins, and both internal factors and external factors such as the environment can drive gene transcription and translation. If genes solely drove behavior, then identical twins would have identical personalities and behaviors, and if environment solely drove behavior, adopted children would be as similar in behavior as identical twins. Clearly, neither is the case and the modern scholar recognizes this and the importance of understanding the relationship between genes, the environment, and behavior.

This article examines how animal models have fostered a greater understanding of the influence that genes have on behavior. This topic is too expansive to be covered in a single article as books have been written on it. Thus, it is impossible to cover every important study done in the field of behavioral genetics. Instead, examples of different behavioral genetic approaches and how these approaches have been successfully implemented to address a research issue are presented to illustrate the power of these approaches. Numerous animal systems have been used in behavioral genetics studies, with three of the more common species used being *Drosophila* (fruit fly), *Caenorhabditis elegans* (*C. elegans*, nematode), and mice (*Mus musculus*). The following sections briefly examine the *Drosophila* model and the *C. elegans* model, and examine the mouse model in more detail.

Introduction

Behavioral genetics is well intertwined with the history of humankind. The domestication and selective breeding of dogs to assist in day-to-day activities is an excellent example of the application of behavioral genetics. Even with the long history of humankind systematically using genetics to improve the quality of life, whether through domestication of dogs or the development of more bountiful lines of livestock, science and philosophy once debated whether nature versus nurture dictated behavior. The position, often associated with John Locke, that the mind was a tabula rasa was in contrast and conflict with the idea favored by Descartes that behaviors or ideas were innate. Over the years, nature has sometimes been replaced with genes, as individuals favoring the nurture argument felt that an underlying motivation in the study of behavioral genetics was to demonstrate that behavior was written in the genes and thus immutable.

Behavioral Genetic Techniques

Strain Surveys

Multiple approaches with different strengths and weaknesses have been employed to study the genetics of

behavior. One approach is to examine the effects of naturally occurring genetic variation on behavior and a complementary approach is to induce genetic mutations and observe behavioral changes. Comparisons of inbred strains are one method to examine the effect of natural genetic variation on behavior. For example, in mice, 20 consecutive generations of inbred mating result in lines that are genetically identical. Thus, if environment is held constant, differences across strains should reflect genetic influences on behavior. Because genetics is held constant within an inbred strain, differences between strain members should reflect environmental influences. Strain comparisons can be used to determine if behaviors are genetically correlated. Using the strain mean performance from multiple lines of inbred mice, correlations across behaviors can be calculated. A significant correlation between behaviors indicates that common genes are involved in the behaviors.

Selective Breeding

In addition to comparisons between inbred strains, outbred strains can be selectively bred to generate lines that differ in phenotype and genotype. For example, if it is observed that some members of an outbred strain perform at a high level for a given phenotype while other members of the strain show a low level of performance, the high performers can be inbred and the low performers can be inbred. The resulting lines will be genetically different and will also differ on at least the phenotype of interest. These lines can then be used to identify which genes segregate with the phenotype and to identify if other behaviors segregate with the targeted phenotype, which would suggest some linkage between the genes of the targeted behavior and the co-segregating behavior.

Quantitative Trait Loci Analysis

Inbred strains that differ on a given phenotype and lines developed by selective breeding of opposing phenotypic responses can be used in recombinant strain studies and quantitative trait loci (QTL) studies to identify the underlying chromosomal regions or genes associated with a targeted phenotype. In QTL analysis, homozygous subjects from different populations that differ in a phenotype of interest are crossed, resulting in an F1 generation. Although the F1 generation is a combination of two homozygous genotypes, all F1 individuals are genetically identical. Crosses between F1s or between an F1 and one of the parental lines will produce an F2 generation segregation of genes including genes associated with the phenotypic trait. Offspring are then tested for the presence or absence of the targeted phenotype.

Known markers such as microsatellite DNA and restriction fragment length polymorphisms (RFLPs)

along with restriction enzymes can be used to map targeted genes. Restriction enzymes are found in bacteria, which use them to cut invading DNA into pieces. Since 1978, when Nathans, Arber, and Smith were awarded the Nobel prize in medicine for the discovery of restriction enzymes, thousands of restriction enzymes have been identified. These enzymes recognize specific nucleotide sequences, called restriction sites, and cut the DNA at those sites. The resulting pieces of DNA will vary in length depending on the location of the restriction sites. The RFLPs can be mapped to chromosomes. If the quantitative trait is associated with an altered nucleotide sequence at a restriction site, then the presence of the targeted phenotype would be associated with the absence of a given RFLP. Using a previously existing map, the chromosomal location of the RFLP can be located and the trait can be associated with a finite region of a given chromosome. Microsatellites are short segments of nucleotides that are repeated multiple times. These sequences can also be mapped to chromosomes and if a phenotype of interest is associated with altered microsatellite sequence, the absence of a microsatellite can be used to map a phenotypic trait to a chromosomal region.

Random Mutagenesis

Just as naturally occurring mutations can be used to map genes to behavior, induced mutations, either random or targeted, can be used to link genes to behavior. In random mutagenesis, a mutating agent such as radiation, N-ethyl-N-nitrosourea (ENU), or ethylmethanesulfonate (EMS) is administered to males of the species. The males are bred with females and offspring are tested for altered behavior. Offspring with altered behavior are bred to test if the altered behavior breeds true, which would suggest the presence of a mutation. If there is a mutation that breeds true, the location can be mapped.

Genetic Engineering

Transgenic mice

An alternative to linking a defined behavior to an unknown gene is identifying the function of a known gene through genetic engineering. Examples of such approaches are transgenic techniques and knock-out techniques. A transgenic animal is one that has had foreign DNA inserted into its germ line. The first animal with successful transfection of the germ line is referred to as the founder. The founder is then bred with a strain of interest to produce heterozygous animals, and this becomes the genetic background of the transgenic animals. Homozygous transgenic animals are produced via mating of animals that are heterozygous for the transgene. Transgenic animals, wild types, and sometimes heterozygotes are tested for behavioral phenotypes of interest and

for any behavioral changes that could confound interpretation of the effects of the transgene on behavior.

Other factors, in addition to expression of the transgene, can produce behavior changes. For instance, phenotype expression can be influenced by where the transgene lands in the genome, that is, expression of a transgene may be influenced by the chromosomal location. The location of the insertion can also alter behavior by affecting expression of an endogenous gene. Finally, expression of a transgene may lack temporal and spatial control. However, new techniques in genetic engineering allow expression of the transgene to be under the control of promoters of gene transcription that have spatially restricted expression and promoters that can be temporally activated.

Knock-out mice

In contrast to transgenic animals that have a foreign gene inserted, knock-out animals, as the name suggests, have an endogenous gene rendered nonfunctional. A genetic construct for a gene of interest is created that has a change in the sequence that will result in either production of a nonfunctional protein or no production of the protein at all. The knocked-out gene is inserted into an embryonic stem cell which has two functional copies of the gene of interest. Through homologous recombination, the knock-out sequence is taken into the embryonic stem cell genome. Because the gene of interest is knocked out throughout the genome early in embryonic development, some knock-out animals show developmental lethality. To circumvent this problem, conditional knock-outs can be used to restrict the knock-out temporally and spatially using the Cre-loxP system, for example, to excise the gene. Cre is a recombinase bacteriophage protein that excises DNA flanked by loxP sites and ligases remaining DNA. A transgenic animal is created with the Cre gene and a spatial or temporal promoter that can be used to regulate expression. Another line is created with the targeted gene flanked by loxP sites. The presence of Cre will excise, and thus knock out, the targeted gene when the promoter is introduced. The following sections examine three animal models, *C. elegans*, *Drosophila*, and mice, commonly used in behavioral genetics studies that employ some of the techniques just reviewed.

Animal Models

C. elegans

At first pass, the *C. elegans* may be considered a reduced preparation in that it has only 959 cells, about a third of which are neurons, but this species has been important for understanding both genetics and behavior. The *C. elegans* genome shares approximately half its genes with the human genome. The homology between the human

genome and the *C. elegans* genome is important because genes in *C. elegans* have been linked to complex processes such as learning and aging. For example, EMS mutagenesis was used to create and isolate eight mutant strains of *C. elegans* with increased life spans. Following this discovery, it was found that a single recessive mutation of the gene *age-1* increased longevity 1.4- to 1.7-fold. Interestingly, the increased longevity was associated with a fivefold decrease in fertility, suggesting that in wild-type *C. elegans*, increased fertility would come with the tradeoff of decreased longevity. Subsequent work from multiple investigators has identified over 40 single-gene mutations associated with increased life span. One factor heavily associated with increased longevity was a decrease in susceptibility to stressors such as free radicals and radiation. These important studies advanced the field of aging research by furthering the understanding of how the interaction between environmental stressors and genetics alters aging.

Drosophila

The *Drosophila* is well suited for behavioral genetics work. The *Drosophila* has a short gestational period and it is easy to maintain large *Drosophila* colonies because of their small size and low maintenance costs. The sequencing of the *Drosophila* genome in 1998 combined with the ability to rapidly generate mutants and study complex behaviors, such as courtship and learning, has facilitated identification of genes associated with these behaviors. Many of the identified genes have homologous genes in the human genome. The naming of *Drosophila* mutants and associated genes has often been colorful to say the least. For example, the Ken and Barbie mutant of either sex lacks external genitalia.

The pioneering geneticist Seymour Benzer contributed greatly to the understanding of genes and how mutations contribute to alterations in behavior. His work with *Drosophila* identified an amazing number of mutations that affected behaviors and processes such as locomotion, circadian rhythms, mating, learning, and longevity. In a classic behavioral genetics study, Dudai, Jan, Byers, Quinn, and Benzer identified a mutant with learning deficits. The offspring from the EMS-treated male *Drosophila* were classically conditioned by pairing an odor with a shock. One line showed a deficit in odor preference that was not related to sensorimotor deficits and was subsequently named *dunce*. The associated gene *dnc* was later sequenced and shown to code for cyclic adenosine monophosphate (cAMP) phosphodiesterase; the *dunce* mutation had lower levels of cAMP phosphodiesterase resulting in higher levels of cAMP. Research on the *dunce* mutation in many laboratories has been instrumental in understanding the role of cAMP in memory not only in flies but also in mammals.

Mice

The mouse has become the gold standard in animal studies of behavioral genetics. Multiple factors have contributed to the popularity of the mouse model. These factors include the availability of numerous behaviorally characterized strains of inbred mice, the well-developed techniques for manipulating the mouse genome, the reasonable balance between gestational period length and complexity of behavioral repertoire, the simultaneous sequencing of the mouse and human genome, and the high degree of homology and synteny between the human and mouse genome. Human and mouse genes show a 99% homology and a 96% conservation of syntenic regions.

History of mouse genetics

The history of the relationship between human, mouse, and genetic research is well described in the book *Mouse Genetics, Concepts and Applications* by Lee Silvers. As discussed by Silvers, domestication and even reverence for mice dates back to the ancient Greeks and Romans, but it was an interest in mice with unusual appearances in Asia that led to the emergence of businesses that developed numerous mutant lines through selective breeding. This interest in fancy mice reached Europe and America in the 1900s, and the interest and entrepreneurship of a mouse fancier, Abbie Lathrop, led to the development of many of the lines of inbred mice currently used in research today. Miss Lathrop was a retired school teacher who began to breed fancy mice in 1900. By the time of her death, it was estimated that she had up to 11 000 mice breeding on her farm. She provided early geneticists such as William Castle at Harvard University and Leo Loeb at the University of Pennsylvania (with whom she ran experiments and published on the genetics of cancer) with lines of mice, many of which are currently used in research such as C57BL/6 and C57BL/10 mice.

As director of the Bussey Institute at Harvard University, Castle, along with his students, began studies on the inheritance of traits in the fancy mice. It is easy to consider Castle as the founder of modern-day mouse genetics as many of his students went on to become prominent geneticists. Castle and his students realized the utility of lines of inbred mice for genetic research, and one of his students, Clarence Little, went on to start the Jackson Laboratory in Bar Harbor, Maine, which today is known as both a center of mouse genetic research and a vendor of mouse lines. The Jackson Laboratory lists over 200 lines of inbred mice alone. Currently, there are multiple sources of inbred and outbred mice that include B and K Universal, Charles River Laboratories, Harlan, and Taconic. Of note, Gregor Mendel perhaps would have been the first mouse geneticist if Bishop Anton

Ernst Schaffgotsch of Austria had not forbidden him to continue breeding experiments on mouse coat color.

It is beyond the scope of this article to review all the important studies that have employed mouse genetics. The following sections will provide examples of some of the tools in mouse behavioral genetics by reviewing select studies that used inbred mice or genetically engineered mice to study the link between genes and behavior. We start with an examination of inbred strains used to examine genetic correlations in behavior; this is followed by QTL analysis in the mouse, ENU mutagenesis in the mouse, and finally, an example of problem solving with genetically engineered mice.

Mouse strain surveys

A series of experiments in the laboratory of Jeanne Wehner at the Institute for Behavioral Genetics, University of Colorado examined the performance of 12 inbred strains of mice and seven F1 crosses of select inbred strains across six behavioral tasks (open field activity, y-maze activity, acoustic startle response, prepulse inhibition of the acoustic startle response, fear conditioning, and Morris water maze performance). The rationale for the study was twofold. First, complex behaviors are most likely polygenic traits and thus comparisons across strains allow investigators to determine if behaviors involve common genes, that is, if behaviors are genetically correlated. Second, transgenic and knock-out mice generated to examine the function of a gene in a behavior may be influenced by the genetic background in which the mutation is expressed; thus, knowledge of background phenotype will facilitate selection of the background strain for the mutation.

Although several behaviors were genetically correlated, the behaviors that were not genetically correlated may be even more interesting. Acoustic startle response was correlated with prepulse inhibition of the acoustic startle response, which may not be too surprising. In addition, acoustic startle was correlated with open field activity and y-maze activity. Interestingly, cued fear conditioning was not genetically correlated with contextual fear conditioning. This may be because contextual fear conditioning is critically dependent on the hippocampus, but cued fear conditioning is not, and thus, the engagement of different brain regions may also engage different groups of genes. Perhaps even more surprisingly, contextual fear conditioning and Morris water maze performance (both of which require the hippocampus) were not genetically correlated. Thus, it cannot be assumed that just because tasks share a common neural area, they are dependent on common molecular and genetic substrates.

Issues with inbred mice

These studies not only highlight the power of behavioral genetics but also bring forward issues that must be

considered when conducting behavioral genetic studies. First and foremost is that background strain matters. ‘A mouse is a mouse is a mouse’ is simply not true; all mice are not created equal. Thus, statements such as ‘mice are poor learners’ are too general and should be questioned. For example, LP/J mice are poor learners in the Morris water maze but are good at contextual fear conditioning, whereas 129/Svev TacfBr mice are good at both fear conditioning and the Morris water maze. Second, parental strain performance does not always predict performance in F1 crosses. For instance, mice from the 129B6F1 cross outperform both parental lines (i.e., 129/Svev TacfBr mice and C57BL/6 mice) in contextual fear conditioning. This demonstrates heterosis, also known as hybrid vigor, which may result from: (1) the reversal of inbreeding depression associated with the expression of harmful genes and/or (2) overdominance resulting from the heterozygote F1 generation having a greater variety of beneficial gene products.

Whereas studies of inbred mice are a powerful research tool for numerous disciplines such as behavioral, genetic, and neuroscience research, there are some important caveats to remember. First, inbred mice can differ in copy number of a gene and this could influence gene expression. Second, genetic drift can alter behavior between substrains of an inbred mouse strain that are bred by separate sources. Thus, a behavioral phenotype of interest seen in an inbred strain from one vendor may differ from the behavior of the same strain from another vendor. Third, care must be taken in deciphering the cause of the phenotypic variation between inbred strains. For example, an inbred strain could show poor Morris water maze learning, but the behavior could reflect a genetic defect that alters visual acuity and not learning processes. Another example would be the case where one strain shows greater conditioned place preference for a drug of abuse than another strain. One could conclude that the first strain finds the drug more rewarding and attribute the behavioral differences to genes involved in reward processes. However, it is also possible that the strains differ in genes involved in the metabolism of the drug or in learning. Finally, whereas the behavior of mice from an inbred strain should be consistent across laboratories when environmental factors are controlled, this is not always the case. In an eloquent series of experiments, Crabbe, Wahlsten, and Dudek compared behavioral profiles of several strains of inbred mice tested at different laboratories while environment and methodologies were controlled to produce the highest degree of consistency across laboratories as possible. Surprisingly, there was variation in behavior within strains across laboratories. Thus, even small changes in environment may influence gene expression and related behavior. These findings reinforce the strong relationship between genes and the

environment, and the fact that it is not nature or nurture but both that influence behavior.

Natural genetic variance in mice

An example of the power of using genetics to understand behavior comes from a series of experiments by Stitzel and colleagues examining the genetics of sensitivity to nicotine. Studies in both human and animal subjects indicate that genetics contributes to the effects of nicotine. Multiple polymorphisms could produce changes in sensitivity to nicotine. It was found that sensitivity to nicotine covaried with a single nucleotide polymorphism in the gene coding for the $\alpha 4$ nAChR subunit, Chrna4, that results in either alanine (A) or threonine (T) at position 529 on the $\alpha 4$ protein. This polymorphism alters $\alpha 4$ nAChR function and sensitivity to the behavioral effects of nicotine across strains of mice that differ in expression of the A529 versus T529 variant. These results are consistent with human data that suggest altered sequence of CHRNA4 influences smoking behavior, and thus could influence development and persistence of nicotine dependence.

Mouse QTL studies

While QTL studies have been successful in linking behavioral phenotypes to chromosomal regions, there are two behavioral QTL studies that stand out as excellent examples of how a QTL study can link behavior all the way down to a specific gene. Flint, Mott, and colleagues successfully used QTL analysis to link the gene *Rgs2* on chromosome 1 to changes in anxiety-related behavior. They used outbred MF1 mice to identify a QTL related to changes in anxiety as measured in the open-field arena. They next used quantitative complementation (the process in which mice with the identified QTL are crossed to mutants that contain candidate genes of interest) to demonstrate that *Rgs2* modulates anxiety-related phenotypes. *Rgs2* codes for a protein that regulates G-protein signaling and it may be through altering such signaling that mutations in *Rgs2* increase anxiety.

QTL analysis has also been used to identify a gene linked to withdrawal-related seizures. Buck and colleagues used congenic mice that possessed small chromosomal segments from C57BL/6J mice combined with greater than 99% DBA/2J background. DBA/2J mice show severe withdrawal symptoms compared to mild symptoms shown in C57BL/6J mice. Thus, depending on which segment of C57BL/6J DNA segregates, congenics could show C57BL/6J or DBA/2J withdrawal phenotypes. Sixteen candidate genes for alcohol and pentobarbital withdrawal sensitivity were identified in a 1.8 Mb QTL interval on chromosome 4. Of the 16, five emerged with high confidence and only one of the five, *Mpdz*, was significantly associated with withdrawal severity. Buck and colleagues proposed that *Mpdz* expression

may mediate drug effects through actions on targets such as γ -aminobutyric acid-B (GABA-B) receptors and 5-HT₂ receptors.

Mouse mutagenesis

Just as QTL analysis in mice can be used to link genes to behavior, so can mutagenesis studies. One of the classic mutagenesis studies comes from the laboratory of Takahashi. This study examined genes related to circadian rhythms. Male C57BL/6J mice were treated with ENU and, after recovery, were mated with untreated female C57BL/6 mice. Changes in wheel running behavior in offspring during normal light/dark cycle and during constant darkness were used to assess changes in circadian rhythm. One mouse out of 304 showed a change in circadian rhythm, a lengthening of the circadian rhythm during testing in constant darkness. The phenotype was shown to be heritable and offspring were backcrossed to BALB/cJ and C3H/HeJ mice. The mutation, *Clock*, segregated as a single gene and a (BALB X B6)F1 X B6 backcross was used to generate 100 mice for linkage analysis with simple sequence length polymorphisms as DNA markers. The *Clock* mutation was linked to chromosome 5. A follow-up study used positional cloning to identify the candidate *Clock* gene.

Mouse knock-out studies

It is beyond the scope of this article to discuss the generation of knock-out and transgenic mice and all of the important studies conducted with such mice. Instead, one example is provided of how knock-out mice can be used to address issues not resolved through other techniques. Nicotine withdrawal refers to a host of symptoms that include somatic changes, insomnia, increased appetite, anxiety, decreased affect, difficulty in concentrating, and learning and working memory deficits. A major issue in research on nicotine addiction is which nicotinic acetylcholinergic receptors are involved in nicotine-withdrawal symptoms and whether all symptoms are mediated by common receptors. The problem confronting this research is that there are numerous nicotinic acetylcholinergic receptor subtypes composed of α 2– α 10 and β 2– β 4 subunits, but nicotinic receptor antagonists do not have the selectivity to distinguish between the subunits involved in the behavior change.

Genetic knock-out studies have resolved the issue of what subtypes are involved in various withdrawal symptoms. β 2 Knock-out mice showed somatic symptoms during withdrawal. In contrast, β 4 knock-out mice exhibited significantly reduced somatic withdrawal symptoms, suggesting that somatic symptoms of nicotine withdrawal involve β 4-containing but not β 2-containing nicotinic acetylcholinergic receptors; further studies in knock-out mice suggest that the somatic signs of withdrawal may

also involve α 7 nicotinic acetylcholinergic receptors and α 5-containing nAChRs. β 2-Containing nicotinic acetylcholinergic receptors are, however, involved in the affective and cognitive nicotine-withdrawal symptoms. By demonstrating that different nicotinic receptor subunits are involved in different nicotine-withdrawal symptoms and by suggesting how genetic variation in nicotinic receptor function could alter withdrawal symptoms and potentially nicotine addiction, these studies show how genetic manipulations such as knock-out techniques can be used to answer questions that would not be answerable with other techniques.

Summary

Animal models continue to help us understand the intricate relationship between environment and genetics, which has many benefits including advancements in medicine. The links between behavior and genes have led to identification of related gene products that serve as targets for therapeutic interventions. The field of behavioral genetics, however, is only beginning. As we learn more about the genome and epigenetics, behavioral genetics studies will continue to play a critical role in understanding the relationship between genes and behavior.

See also: Analysis of Learning in Invertebrates; Animal Models of Learning and Memory; Genetics of Memory in *Drosophila*; Knock-Outs: Learning and Memory; Mouse Genetic Approaches to Psychiatric Disorders; Nicotine; Role of Gene Transcription in Long-Term Memory Storage; Transgenic Technologies and Their Application to the Study of Senile Dementia; Value of Animal Models for Predicting CNS Therapeutic Action.

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Relevant Websites

<http://www.flyname.com> – A database of *Drosophila* nomenclature.

Knock-Outs: Learning and Memory

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Glossary

Fear conditioning – A Pavlovian learning task in which rodents learn the association between a conditioned stimulus (CS; e.g., tone or context) and an unconditioned stimulus (US; e.g., mild electric footshock). Memory is assessed by measuring freezing (unconditioned response; UR) during the presence of CS. Freezing is the cessation of all movement, except respiration, and is thought to reflect rodent predator-escape responses.

Inducible transgenic mice – To avoid the potential drawbacks of conventional knock-out and transgenic mice, such as expression during development and disruptions in multiple tissues and cell types, methods capable of improved spatial specificity and temporal control were developed. For example, expression of a transgene under the tet promoter can be temporally regulated by doxycycline, and fusions with mutant versions of the ligand-binding domain of the estrogen receptor allow for temporal control of proteins of interest.

Long-term potentiation (LTP) – A stable and long-lasting change in synaptic strength, which is induced by repeated synaptic stimulation. This form of synaptic plasticity is found in many types of synapses in different brain areas both *in vivo* and *in vitro*. Importantly, many studies have indicated that LTP occurs during memory formation, and the results reviewed here demonstrate a role for this synaptic mechanism in learning and memory.

Morris water maze – A behavioral task classically used to study spatial learning and memory. Rodents are trained to learn the position of a submerged platform by using spatial cues in the testing room. Following several training sessions, memory is assessed by a probe trial where the platform is removed from the pool and the mice are allowed to search for its location (target quadrant) for a short amount of time (e.g., 60 s).

Object recognition task – A nonaversive, nonspatial hippocampal learning task. Mice are allowed to interact with two objects for a certain amount of time (e.g., 10 min) in a training session. Memory is assessed in a test session in which one of the objects is replaced by a new object. The stronger the memory of the initial objects, the more time the mice spend with the novel object.

Introduction

In the past 20 years, the development of powerful molecular genetic approaches has dramatically changed neuroscience, including studies of learning and memory (L&M). Knock-outs (gene deletions), knock-ins (point mutations), and traditional transgenics (gene additions) allowed exquisitely specific molecular manipulations that had the additional virtue of being highly reproducible, a considerable departure from previous drug manipulation studies that often fell short on both accounts. With these novel molecular genetic approaches, it was possible to target any gene/protein/molecular process of interest. Most importantly, once the mutant mice were engineered, they could be easily shared with other investigators, thus allowing for collaborative multidisciplinary studies of behavior. This integrative approach forged a new brand of multilevel analyses that has changed the scope of behavioral studies and led to the emergence of a new field in *Neuroscience: Molecular and Cellular Cognition*. Molecular and cellular cognition has evolved into a large field with its own international society with branches and meetings in America, Europe, and Asia and with nearly 3000 members. It is no longer sufficient to address neuroscience problems at one level of analysis; the flexibility and efficiency of molecular genetic tools have raised the standards, and now it is both commonplace and expected that the best neuroscience studies should include mechanisms and explanations that cut across levels, from molecules to behavior. But how do we make compelling connections between levels of analyses? For example, how do we connect gene function with behavior?

In making a compelling argument for the function of a specific gene/protein in behavior, there are four different convergent strategies that are often used in molecular and cellular cognition studies. These strategies include: (1) negative alteration (delete, mutate, etc., the gene and determine whether the behavior is affected), (2) positive alteration (upregulate the function of the gene with transgenic, viral manipulations, etc., and determine if the behavior of interest is altered in a manner opposite to negative alteration), (3) nonintervention (determine whether gene expression/protein activation precedes the behavior of interest, and (4) integration (find out how the gene of interest fits within known molecular mechanisms of the behavior of interest, so as to make predictions of its mechanistic role).

Molecular and cellular cognition studies started with a couple of articles published in 1992 reporting the effects of the deletion of the alpha calcium calmodulin kinase II (α CaMKII) gene in hippocampal long-term potentiation and spatial L&M. Fifteen years later, there are now over 1000 papers published on more than 200 genes included in studies of hippocampal-dependent L&M. Majority of these manipulations resulted in deficits in L&M, but, unexpectedly, more than 30 different genetic manipulations actually resulted in enhancements of L&M. Because of the large scope of molecular and cellular cognition studies and the numerous reviews available of studies focused on how mutations disrupt L&M, this review is focused specifically on the genes whose bidirectional manipulations cause bidirectional changes in L&M: when overexpressed, enhance performance, but when deleted or inhibited, impair L&M, or vice versa.

In addition, since molecular and cellular cognition studies have focused on the connections between molecular and cellular mechanisms of behavior, we also discuss how genes regulate physiological processes and their implications to L&M. We classify the studies reviewed into several groups according to the signal transduction pathways affected and briefly summarize how each of the mutations is thought to affect synaptic plasticity and L&M.

NMDA Receptors

Negative Alterations of NMDAR and Impaired L&M

There is considerable evidence that lesions of various types of NMDA receptors (NMDARs), as well as drugs that interfere with this receptor's function, disrupt L&M. NMDARs are composed of a core subunit (NR1) and other modulatory subunits, including NR2 (with A, B, C, and D subtypes) and NR3 (with A and B subtypes). Deletion of NR1 impaired long-term potentiation (LTP) and spatial memory without confounding nonspatial learning ability. In addition, hippocampal subregion-specific deletion of NR1 in CA3 and dentate gyrus also impaired different aspects of cognition, including pattern completion and separation, respectively. Deleting NR2A, which is the dominantly expressed NR2 subunit in the adult brain, also resulted in deficits in hippocampal LTP and hippocampus-dependent learning tasks. The behavioral role of NR2B could not be examined with knock-out (KO) mice since these mutants died shortly after birth.

Positive Alterations of NMDAR and Enhanced L&M

To investigate the role of NR2B in memory and synaptic plasticity, NR2B was overexpressed in the mouse forebrain (the *doogie* mouse). The longer NMDAR currents,

resulting from overexpression of NR2B, led to enhancements of hippocampal CA1 LTP in this mutant. Importantly, *doogie* showed enhanced performance in several hippocampal L&M tasks, including novel-object recognition, spatial memory tested in the Morris water maze, and contextual conditioning. Aged mutants were also found to outperform controls of similar ages. These results were interesting, but alone they would not have made a strong case for a role for NMDARs in LTP and L&M. Below, we review a number of other molecular genetic manipulations that affected NMDAR levels, LTP, and L&M.

A mutant mouse in which the $\beta 3$ subunit of the voltage-dependent Ca^{2+} channel $\text{Ca}_v\beta 3$ was knocked out also showed enhanced LTP and memory. The $\text{Ca}_v\beta 3$ KO mice outperformed controls in several hippocampal L&M tasks, including contextual fear conditioning, novel object recognition, and social transmission of food preferences. Interestingly, NMDA-mediated currents and NMDAR-dependent LTP were increased in the hippocampus of these mice. Although the underlying molecular mechanism is unclear, NR2B expression was slightly, but significantly, increased in the KO mice. Similarly, NR2B mRNA and protein levels were also higher in transgenic mice overexpressing KIF17, a protein that is thought to transport NR2B along microtubules. These transgenics also showed superior spatial learning and working memory than controls. It is possible that enhanced transport of NR2B, and subsequent higher levels of this subunit in synaptic NMDARs, could be the cause for better learning in mice overexpressing KIF17.

Cyclin-dependent kinase 5 (Cdk5) may regulate calpain-dependent proteolysis of NR2B. Calpain is activated by Ca^{2+} entry through NMDARs, and it rapidly cleaves NMDAR subunits, resulting in a decrease in the number of functional NMDA receptors in the postsynaptic density. Accordingly, deletion of Cdk5 in adult mouse forebrain reduced NR2B degradation, consequently increasing NMDA-mediated currents, which resulted in larger LTP and enhanced contextual fear conditioning, faster fear extinction, and more flexible learning in the reversal water maze task. Transient expression of p25, an activator of Cdk5, in mice forebrain increased NR2A phosphorylation and NMDAR-mediated currents, enhanced synaptic plasticity and hippocampus-dependent memory, including contextual fear conditioning and the Morris water maze. However, prolonged activation of p25 impairs synaptic plasticity and L&M.

Mice lacking nociceptin receptor (ORL1) showed enhanced L&M in the Morris water maze and passive avoidance tasks. This mutation resulted in enhanced NMDA receptor function, more rapid activation of its key downstream effector α CaMKII, and, consequently, larger LTP. Interestingly, application of the nociceptin peptide and the ORL1 agonist Ro 64-6198 had opposite

molecular, electrophysiological, and behavioral effects to the nociceptin receptor KO. Thus, these results suggest that nociceptin-mediated signaling regulates NMDA-receptor-dependent activation of α CaMKII and functions as a key constraint of plasticity and L&M. The findings described in this section demonstrate that upregulation of NMDAR function can result in enhancements of L&M, while deletions of this receptor cause deficits.

Kinases and Phosphatases

There is growing evidence that opposing kinases and phosphatases downstream of NMDARs determine whether incoming signals enhance or suppress synaptic plasticity and therefore facilitate or dampen L&M processes. CaMKII, PKA, MAPK, and PKM ζ are well-known positive regulators, while the phosphatase calcineurin (PP2B) and protein phosphatase 1 (PP1) serve as negative regulators of both synaptic plasticity and L&M. Moreover, it has been shown that shifting the balance between kinases and phosphatases can enhance memory.

Negative Alterations of Kinases and Impaired L&M

The critical roles of α CaMKII in synaptic plasticity and L&M have been addressed by using multiple mutant lines in addition to the original null mutant mentioned above. For example, transgenic expression of a constitutively active form of α CaMKII (α CaMKII T286D) in the adult brain showed impaired synaptic plasticity and L&M. One of the interesting features of this kinase is that it undergoes autophosphorylation (e.g., Thr286 and Thr305/306). The importance of these autophosphorylation events was investigated by using knock-in mice. Mice with a point mutation that prevents autophosphorylation at Thr286 (T286A) were severely impaired in both LTP and spatial L&M. While the phosphorylation at Thr286 is facilitatory, the autophosphorylation at Thr305/306 is inhibitory. Mutant mice expressing α CaMKII with a T305D mutation, which mimics the inhibitory autophosphorylation, were impaired in both LTP induction and hippocampal-dependent L&M, such as Morris water maze and contextual fear conditioning. Blocking inhibitory autophosphorylation by substituting α -CaMKII $^{Thr305/306}$ by nonphosphorylatable amino acids (α CaMKII $^{TT305/6VA}$ mice) reduced the threshold for LTP induction, but still impaired L&M. These results suggest that lowering the thresholds for synaptic plasticity does not always lead to an enhancement in L&M.

The Ras–Raf–MAPK signaling cascade plays critical roles in various functions in the nervous system, including in synaptic plasticity and L&M. Transgenic mice expressing a dominant-negative MEK1, the upstream activator

of ERK1/2, showed impaired fear conditioning, implicating this signaling pathway in L&M. However, ERK1 KO mice did not show deficits in behavior, raising the possibility that ERK2 compensates for the deficiency. Alternatively, ERK2 is the major MAPK involved in L&M, and ERK1 has other roles in the central nervous syndrome (CNS). Indeed, either deletion or reduction of ERK2 resulted in impairments in L&M, including fear conditioning and Morris water maze, showing that ERK2 is the isoform responsible for the L&M.

Adenylyl cyclases (ACs) couple NMDAR Ca^{2+} signaling to downstream cAMP-dependent pathways, including the PKA-dependent phosphorylation of I-1 and therefore the inactivation of PP1. Of all the ACs identified, AC1 and AC8 are the only Ca^{2+} -sensitive ACs in brain. Deletion of AC1 resulted in slight impairment in the initial slope of LTP, but L-LTP was comparable with that of wild-type controls. AC1 KO mice were impaired in Morris water maze, but showed normal performance in contextual fear conditioning and passive avoidance tests. However, mice lacking both AC1 and AC8 showed severe deficits in L-LTP and long-term memory in contextual fear conditioning, passive avoidance, and object recognition, suggesting that cAMP–PKA signaling has a critical role in long-term synaptic plasticity and memory.

Positive Alterations of Kinases and Enhanced L&M

Genetic manipulations using viral vectors provide an alternative approach to mutant mice, and generally have better regional specificity since the viral vectors are delivered to specific areas of interest. Viral overexpression of α CaMKII in the hippocampus was shown to enhance spatial L&M in the Morris water maze. Although LTP in α CaMKII-overexpressing hippocampus remains to be examined, this result suggests that bidirectional manipulations of α CaMKII expression produce bidirectional changes in L&M. Previous transgenic studies indicated that in some cases overexpression of CaMKII could lead to deficits in L&M. This kinase is in abundance in the CNS, and it can phosphorylate many different substrates. It is possible that inappropriate expression of the kinase can lead to phosphorylation of proteins that are not usually modulated by this kinase, and thus to physiological pathologies that leads to L&M deficits.

AC1 transgenic mice showed an enhancement of PKA-dependent LTP. Moreover, this mutant showed enhancements of both memory in an object-recognition task and remote memory for contextual conditioning. The activities of MAPK and CREB, which are downstream molecules of cAMP/PKA pathway and also critically involved in long-term memory (see below), were significantly increased in the hippocampus of AC1 transgenic mice.

A positive alteration of MAPK signaling also enhances synaptic plasticity and memory. Studies of mice expressing a constitutively active form of H-ras (H-ras^{G12V}) in the axons of pyramidal neurons of the postnatal hippocampus revealed a role for Ras/MAPK signaling in presynaptic neurotransmitter release during high-frequency stimulation in LTP and L&M. H-ras^{G12V} presynaptic expression resulted in increases in the activation of MAPK and in the phosphorylation of its presynaptic substrate, synapsin I. In addition, these mutants showed a number of other convergent presynaptic phenotypes, including higher number of docked vesicles, increased frequency of mEPSCs, and altered paired-pulse facilitation. Accordingly, LTP induced under physiological parameters was increased in this mutant. Behavioral studies demonstrated dramatic hippocampal-dependent learning enhancements in several tasks tested. Importantly, a synapsin I mutation, which alone had no measurable effect in LTP and learning, reversed the physiological and behavioral enhancements of the H-ras^{G12V} mice, indicating that H-Ras/MAPK-dependent phosphorylation of synapsin I played a key role in the learning enhancements of these mutants. These results provide strong evidence that the learning enhancements described were caused by presynaptic mechanisms involving Ras/MAPK upregulation and subsequent phosphorylation of synapsin I at its MAPK site.

Positive Alterations of Phosphatase and Impaired L&M

Calcineurin is a Ca²⁺-activated phosphatase, which is composed of catalytic and regulatory subunits. Calcineurin can affect synaptic plasticity in many different ways, including modulation of NMDAR-mediated currents and regulation of another phosphatase PP1. Mice expressing an active form of calcineurin showed increased phosphatase activity and exhibited deficits in hippocampal LTP. Moreover, these transgenic mice have defective long-term memory whereas short-term memory is normal in spatial and object-recognition tasks, supporting the idea that this phosphatase can act as an inhibitory gate for LTP and learning.

Negative Alterations of Phosphatase and Enhanced L&M

A calcineurin inhibitor was expressed in an inducible manner using the doxycycline system. The phosphatase activity in the hippocampus and cortex of the adult mutant mice was significantly reduced, leading to enhancements in LTP both *in vitro* and *in vivo*. Importantly, memory was found to be enhanced in various behavioral tasks, including object recognition, which had been found impaired in transgenic mice

overexpressing calcineurin. These and other studies have indicated that decreasing calcineurin activity facilitates LTP and L&M. However, not all published studies reported findings consistent with this simple idea. For example, deletion of the regulatory subunit of calcineurin (CNB1) in excitatory forebrain neurons of adult mice led to the suppression of hippocampal LTD and only to slight enhancements of LTP. This mutant failed to show enhancements and even showed impairments in L&M tasks. Another study in rats also reported ambiguous results; blocking calcineurin expression by infusing anti-sense oligonucleotides (into brain ventricles) enhanced hippocampal LTP induction and contextual fear conditioning, but not spatial learning in the Morris water maze. The problem with being certain that the results reported truly reflect the L&M capacity of the animals is that not all publications demonstrate that the tests were carried out under conditions that avoid both floor and ceiling effects. Considering the complexity of calcineurin signaling in neurons, it is not entirely surprising that two different manipulations with different degrees of calcineurin inhibition resulted in inconsistent cellular and behavioral phenotypes.

Calcineurin can regulate the activity of another serine/threonine phosphatase (PP1) by dephosphorylating Inhibitor-1 (I-1). Phosphorylated I-1 is a potent inhibitor of PP1, and thus dephosphorylation of I-1 by calcineurin results in the activation of PP1. PP1 has an important role in LTD, and like calcineurin, PP1 is thought to be a negative regulator of memory. To suppress PP1, a constitutively active form of I-1 (I-1*) was inducibly and reversibly expressed in the adult brain. The induction of I-1* expression suppressed PP1 activity by ~70% in the hippocampus. Mice expressing I-1* showed improved object-recognition memory when they were trained in short intervals (massed training). It is well known that longer intervals (e.g., 10 min) between training trials generally trigger better L&M than shorter intervals (e.g., 1 min). Interestingly, the mutant mice were not different from wild-type controls when they were trained with longer intervals, suggesting that these longer intervals occluded the advantage conferred by the expression of the I-1* transgene. The mutants also showed enhanced L&M in the spatial version of the Morris water maze. Strikingly, inhibition of PP1 after training strengthened memory, suggesting that PP1 functions as a negative regulator of memory stability. Whether PP1 is involved in memory erasure or retrieval remains to be resolved. CaMKII, the AMPA receptor GluR1, and CREB-dependent gene expression are modulated by PP1, and changes in any of one of these components could modulate the stability of memory.

The studies reviewed in this section demonstrate that the balance between kinases and phosphatases is crucial for plasticity and learning and that shifting this balance

toward kinases either by activating kinases or by suppressing phosphatases can enhance L&M.

CREB-Mediated Transcription

In addition to the covalent modification of preexisting molecules, the transcription and then translation of new proteins can lead to molecular changes required for L&M. For example, the cAMP response-element-binding protein (CREB) is a transcription factor known to be required for the stability of synaptic plasticity and memory. The role of CREB in plasticity and memory is evolutionarily conserved from invertebrates to mammals. Recently, CREB has also been suggested to affect the probability that a given neuron in a circuit is recruited into a specific memory trace (memory allocation).

Negative Alterations of CREB and Impaired L&M

The targeted deletion of the CREB α and δ isoforms in mice lead to deficits in both LTP and long-term memory tested in a wide range of tasks, including Morris water maze, fear conditioning, social recognition, and conditioned taste aversion. In addition, conditional activation of a dominant negative form of CREB (CREB^{S133A}), with the ligand-binding domain (LBD) of the estrogen receptor transgenic system, impairs the consolidation of fear memory and conditioned taste aversion, confirming the critical role of CREB in long-term memory.

The activity of CREB can be regulated by CaMKIV-dependent phosphorylation of Ser 133. The CaMKIV null mutant mice showed lower CREB activation and, subsequently, deficits in both hippocampal LTP and long-term memory assessed by fear conditioning (1 and 7 days after training). Another CaMKIV mutant mouse expressing a dominant negative form of CaMKIV are impaired in the Morris water maze and contextual fear conditioning (not 1 day, but 7 days after training). Accordingly, this mutant showed deficits in the late-phase of LTP. These results indicate that downregulation of CREB activity by reducing CaMKIV impairs LTP and memory.

Positive Alterations of CREB and Enhancements L&M

Transgenic overexpression of a constitutively active form of CREB (VP16-CREB) lowered the threshold for L-LTP. Gene expression analysis of this transgenic showed BDNF as a key molecule for the maintenance of LTP. However, excessive activation of CREB resulted in abnormalities, such as hippocampal neuronal loss and epileptic seizure, and thus L&M could not be assessed in this transgenic. On the other hand, viral overexpression

of wild-type CREB was shown to enhance memory: viral expression in amygdala enhanced fear conditioning while expression in the hippocampus enhanced spatial memory.

The activity of CREB can be regulated by molecular interactions with other transcription regulators in the nucleus. In *Aplysia*, inhibiting a negative regulator of CREB, ApCREB2 was shown to lower the threshold for long-term synaptic plasticity. Recently, three different mice models show that manipulating CREB suppressors can also enhance synaptic plasticity and memory in mammalian brain. First, expression of a broad dominant-negative inhibitor (EGFP-AZIP) of the C/EBP family of transcription factors in mouse forebrain was found to suppress the repressor isoform of C/EBP β and decrease the expression of the activating transcription factor 4 (ATF4). ATF4 is a negative regulator of CREB. Thus, EGFP-AZIP, by suppressing two different transcriptional repressors, shifts the balance toward transcriptional activators, in both the CREB and C/EBP families. Importantly, expression of EGFP-AZIP lowered the threshold for LTP and memory formation: a tetanus that induces only early stages of LTP (E-LTP) in controls can induce transcription-dependent later forms of LTP (L-LTP) in EGFP-AZIP mice. Behavioral analysis revealed enhanced learning in mutant mice trained with a weak protocol in the Morris water maze. Second, mice heterozygous for a point mutation that prevented phosphorylation of the eIF2 α at serine 51 (eIF2 $\alpha^{+/S51A}$) showed decreased levels of ATF4 protein. Stimulation that induced E-LTP in controls was capable of inducing L-LTP in the eIF2 $\alpha^{+/S51A}$ mutants. Importantly, these mice showed improved L&M in a variety of behavioral tasks, including contextual and cued fear conditioning, conditioned taste aversion, and latent inhibition. Finally, deletion of GCN2, a conserved eIF2 α kinase, reduced phosphorylation of eIF2 α , subsequently suppressed the translation of ATF4 mRNA. Similar to the eIF2 $\alpha^{+/S51A}$ knock-in mutant, the threshold for L-LTP was lowered and spatial learning was enhanced in the GCN2 KO mice. These studies support the hypothesis that the transcriptional repressor ATF4 is an important negative regulator of synaptic plasticity and memory and suggest that translation may be an important regulatory node in plasticity and memory.

Overexpression of CaMKIV in mouse forebrain led to an increase in the levels of CREB activity and to larger LTP in both hippocampus and anterior cingulate cortex. Moreover, these transgenics showed enhanced contextual fear and social recognition memory. Interestingly, CaMKIV overexpression rescued age-related decline in fear conditioning. Taken together, these studies showed that enhancing CREB activity either by suppressing its negative regulators or by activating its positive regulators can enhance synaptic plasticity and memory.

Extracellular Structure

Another important feature of long-term memory and synaptic plasticity is structural changes such as the growth of new synapses. For example, studies in both invertebrates and vertebrates showed that abnormal regulation of cell adhesion impairs long-term synaptic plasticity and synaptic growth.

Negative Alterations of Structural Proteins and Impaired L&M

Downstream from CREB, tissue-type plasminogen activator (tPA) was identified as an immediate early gene whose mRNA is rapidly induced by neuronal activity in the hippocampus. The tPA expression is also induced by LTP. A tPA is an extracellular serine protease that converts plasminogen into plasmin. Genetic deletion of tPA in mice resulted in deficits in L-LTP, supporting the idea that the downstream products of CREB-mediated gene expression are crucial for L-LTP. This mutant is also impaired in several forms of memory, such as contextual fear conditioning, object exploration, and active avoidance tasks.

The brain-growth-associated protein, GAP-43, has been shown to be involved in neural development, structural plasticity, and presynaptic neurotransmitter release. The homozygous GAP-43 KO is lethal, but the heterozygous KO mice ($\text{GAP-43}^{+/-}$) could be studied for behavioral phenotypes. $\text{GAP-43}^{+/-}$ mice showed deficits in contextual fear conditioning but normal cued fear conditioning, suggesting that hippocampus-dependent L&M is impaired. GAP-43 is a presynaptic substrate of PKC. Interestingly, mutant mice overexpressing a non-phosphorylatable form of GAP-43 (S42A) were also impaired in spatial learning.

Heparin-binding growth-associated molecule (HB-GAM), an extracellular matrix-associated protein, is known to regulate neurite outgrowth, axon guidance, and synaptogenesis. Mice lacking HB-GAM were impaired in hippocampus-dependent memory tasks, such as the Morris water maze and contextual fear conditioning. However, anxiety-like behavior is altered in HB-GAM KO mice, and this might confound the interpretation of cognitive phenotypes in these mice.

Positive Alterations of Structural Proteins and Enhanced L&M

Transgenic neuronal overexpression of tPA enhanced both LTP and hippocampus-dependent spatial memory. Recent findings indicated that plasmin converts brain-derived neurotrophic factor precursor (proBDNF) to mature BDNF (mBDNF) and that this conversion is

critical for L-LTP in mouse hippocampus. There is a variety of findings that indicate that BDNF has a key role in synaptic plasticity and learning. It is possible that the increased conversion of proBDNF to mBDNF in the tPA mutants contributes to enhanced LTP and memory.

Overexpression of GAP-43 and HB-GAM also enhanced spatial memory. GAP-43 transgenics showed enhanced *in vivo* LTP. Interestingly, moderate overexpression of GAP-43 was shown to enhance memory in the Morris water maze, whereas higher expression impairs learning in the water maze. Taken together, these studies show that beyond CREB-mediated transcription, structural proteins, which orchestrate or mediate synaptic structural changes, play critical roles in synaptic plasticity and memory.

Glial Regulation

Although studies of L&M have largely focused on neuronal cells, there is increasing physiological and behavioral evidence that glial cells play an active role in L&M. For example, mice lacking glial fibrillary acidic protein (GFAP) showed altered synaptic plasticity in hippocampus and cerebellum, which is accompanied by impaired eye-blink conditioning.

Positive Alterations of S100B and Impaired L&M

S100B is a Ca^{2+} -binding protein, which is secreted from astrocytes. Overexpression of S100B in astrocytes impaired hippocampal synaptic plasticity and spatial learning in the Morris water maze. Interestingly, elevated S100B has been observed in pathologic brains like Down's syndrome and Alzheimer's disease.

Negative Alterations of S100B and Enhanced L&M

Targeted disruption of S100B enhanced LTP and exogenous S100B treatment reversed this enhancement. Null mutant mice showed better performance in the spatial version of the Morris water maze. In addition, contextual fear memory was also enhanced in KO mice. However, it is still unclear how memory was enhanced in this mutant. S100B might bind its receptor in the neuron and trigger signaling involved in synaptic plasticity. These and other results suggest the involvement of glial processes in L&M.

Conclusions

KOs and other transgenic manipulations have had a considerable impact in neuroscience, including in the study of L&M. Although in the early days of molecular and

Table 1 List of mutant mice with enhanced/impaired learning and memory (L&M)

<i>Signaling pathway</i>	<i>Mutants with enhanced L&M</i>	<i>Mutants with impaired L&M</i>
NMDA receptors	NR2B Tg, $\text{Ca}_v\beta 3$ KO, KIF17 Tg, Cdk5 KO, p25 Tg (transient), ORL1 KO	NR1 KO, NR2A KO
Kinases	α CaMKII (virus), AC1 Tg, H-ras ^{G12V} Tg (in presynaptic neuron)	α CaMKII KO, α CaMKII ^{T286D} Tg, α CaMKII ^{T286A} KO, α CaMKII ^{TT305/6VA} KO, AC1/AC8 KO, ERK2 KO
Phosphatases	Calcineurin (inhibitor) Tg, Inhibitor-1 (active) Tg	Calcineurin (active) Tg
CREB-mediated transcription	CREB (virus), EGFP-AZIP Tg, eIF2 $\alpha^{+/S51A}$ KO, GCN2 KO, CaMKIV Tg	CREB $\alpha\delta$ KO, LBD-CREB ^{S133A} Tg, CaMKIV KO
Structural proteins	tPA Tg, GAP-43 Tg, HB-GAM Tg	tPA KO, GAP-43 KO, HB-GAM KO
Glial protein	S100B KO	S100B TG

Tg, transgenic; KO, knock-out; Ki, knock-in

cellular cognition most studies focused on the phenotypes of specific genetic manipulations, current studies in the field are focused instead on connections between molecular, cellular systems and behavioral function. These connections cannot be made with single mutant mice, but require instead a number of convergent manipulations, including transgenic and KO mutations that overexpress and delete the gene of interest. The emerging gold standard in the field is to determine whether opposite results are obtained with pairs of bidirectional genetic manipulations. To illustrate this process, we reviewed a set of mutations of genes that have been manipulated in this manner (**Table 1**). These studies identify NMDAR signaling and CREB-dependent transcription as important mechanisms of L&M and indicate several other signaling pathways involved in processing and storing spatial and contextual information in hippocampal networks.

Remarkably, bidirectional manipulations of L&M phenotypes were highly correlated with similar changes in LTP in the vast majority of mutant mice reviewed, a result that demonstrates that this form of synaptic plasticity has a key role in L&M. It is important to note, however, that not all increases in LTP lead to L&M enhancements, since often there are other parallel changes that affect behavior, including L&M. The remarkable consistency of the reviewed findings provides considerable evidence for a role of LTP mechanisms in L&M. Furthermore, there is compelling evidence that, during learning, synaptic transmission is strengthened in a manner consistent with LTP. Perhaps, the most surprising finding is that the vast majority of these results converge on a signaling pathway that starts with NMDAR activation and eventually involves CREB-dependent transcription. The consistency and convergence of these findings establish the signaling mechanisms underlying LTP as a cornerstone of the biology of memory.

Nevertheless, it is also important to note that there are some caveats that need to be considered when interpreting

each of the findings reviewed: most of the studies mentioned included only a limited behavioral analysis and did not explore in detail the behavioral nature of the L&M enhancements reported. It might be possible that some of the L&M enhancements reviewed have unknown behavioral costs that escaped the incomplete behavioral characterization of these mutants. In addition, tests that are more extensive might reveal other behavioral deficits that were not detected yet. Nevertheless, the extent and consistency of the findings reviewed demonstrate that LTP-like mechanisms have a role in L&M, and that there are at the core of rate-limiting mechanisms that could be used to enhance L&M. Targeting these core mechanisms may contribute to the development of general therapies for cognitive disorders.

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See also: Analysis of Learning in Invertebrates; Animal Models of Learning and Memory; Cerebellum: Associative Learning; Cognition: Attention and Impulsivity; Cognition: Learning and Memory: Pavlovian; Cognition: Learning and Memory: Spatial; Cognitive Decline in Laboratory Animals: Models, Measures, and Validity; Declarative Memory; Episodic and Autobiographical Memory: Psychological and Neural Aspects; Eyelid Classical Conditioning; Genes and Behavior: Animal Models; Genetics of Memory in *Drosophila*; Learning and Memory: Computational

Models; Mechanisms of Memory Formation and Storage in *Hermissenda*; Memory and Aging, Neural Basis of; Memory Consolidation; Memory in *Caenorhabditis elegans*; Memory in the Honeybee; Mouse Genetic Approaches to Psychiatric Disorders; Neural Basis of Classical Conditioning; Neural Plasticity of Spinal Reflexes; Neurogenesis and Memory; Neuron Excitability and Learning; Plasticity in the Primary Auditory Cortex: Substrate of Specific Long-Term Memory Traces; Role of Gene Transcription in Long-Term Memory Storage; Synapse Formation and Memory; Synaptic Mechanisms for Encoding Memory; Transgenic Technologies and Their Application to the Study of Senile Dementia; Value of Animal Models for Predicting CNS Therapeutic Action.

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- <http://www.molcellcog.org> – Molecular and Cellular Cognition Society.
- <http://www.informatics.jax.org> – Mouse genome informatics.

Maternal Deprivation

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Glossary

- Adrenocorticotropin hormone (ACTH)** – A polypeptide hormone, which is secreted by the anterior pituitary gland in response to stress. It mediates synthesis and release of glucocorticoids from the adrenal cortex.
- Corticotrophin releasing factor (CRF)** – A 41-amino-acid polypeptide involved in coordination of behavioral, endocrine, and autonomic responses to stress. It acts via two main systems: (1) CRF neurons in the paraventricular nucleus of the hypothalamus and (2) the CRF neurons in the central nucleus of the amygdala and the bed nucleus of the stria terminalis.
- Central benzodiazepine (CBZ)** – The CBZ binding site is a component of the GABA_A receptor complex. Binding of an agonist to the CBZ site increases affinity of the GABA_A receptor to GABA and enhances GABAergic neurotransmission.
- γ-Aminobutyric acid (GABA)** – A main inhibitory neurotransmitter in the mammalian CNS.
- Long-term potentiation (LTP)** – A long-lasting improvement in communication between two neurons as a result of their simultaneous stimulation. It is considered as a form of synaptic plasticity and a cellular mechanism of learning and memory.
- Neuropeptide Y** – A 36-amino-acid polypeptide which acts as a neurotransmitter in the brain and the autonomic nervous system. It is involved in several functions, including regulation of food intake, learning/memory, and epilepsy.
- Oxytocin** – A peptide of nine amino acids that can act as a hormone and as a neurotransmitter. It is synthesized in magnocellular nuclei of the supraoptic and paraventricular nuclei of the hypothalamus and released from the posterior pituitary into the blood stream. Oxytocin is involved in a number of reproductive functions, pair-bonding, maternal behavior, and social recognition.
- Arginine vasopressin (VPA)** – A peptide which is synthesized in the hypothalamus, stored in the posterior pituitary, and can act as a hormone and as a neurotransmitter. VPA has been involved in a number of functions, including circadian rhythms, pair-bonding, and aggression.

Early neonatal environment has profound impact on the development of the mammalian central nervous system (CNS). Environmental stimuli interact with the genetic blueprint in shaping individual phenotypes and their subsequent perception and reactivity to various challenges. Increasing basic, clinical, and epidemiological evidence support the notion that exposure to an adverse early environment may underlie vulnerability to, and later expression of medical illness and psychopathology. A number of laboratories employed the procedure of maternal deprivation to investigate early adverse experience as a developmental risk factor for psychopathology.

The importance of mother-child interaction and its enduring effect on human behavior was first postulated by Freud in the beginning of the twentieth century. However, the critical importance of maternal environment in normal behavioral development of the offspring was clearly shown by Harlow in his seminal studies of maternally deprived rhesus monkeys. Animals that were raised with peers in the absence of the mother developed abnormal behaviors and altered reactivity to stress. In the early 1960s in a series of experiments, Ader, Denenberg, and Levine showed that in rodents, early environmental stimulation in the form of neonatal handling can have enduring effect on behavioral and neuroendocrine reactivity to stress. The last several decades have seen heightened interest in the long-term effects of early environment. Numerous procedures, including prenatal stress, maternal deprivation, handling, and isolation rearing have been developed by many laboratories to study the effects of early adverse environment on behavioral reactivity and the CNS. The key feature of virtually all of these manipulations has been long-term alteration of the hypothalamic–pituitary–adrenal (HPA) axis and neural circuits that modulate or are modulated by this system.

This article is primarily focusing on the consequences of maternal deprivation in the rat. A brief introduction into the regulation of the HPA axis, mother–infant interaction, and CNS plasticity of the neonate is presented before introducing maternal deprivation models.

Introduction

Regulation of the HPA Axis

Stimuli associated with perceived or actual challenges, external or internal threat, pain, infection, or metabolic perturbations are communicated to the hypothalamus

through stressor-specific pathways where this information is integrated in the paraventricular nucleus (PVN) of the hypothalamus. Parvocellular neurons of the PVN secrete corticotrophin-releasing factor (CRF) and arginine vasopressin (VPA) which are transported to median eminence nerve terminals and released into circulation. At the level of anterior pituitary they stimulate synthesis and release of adrenocorticotrophic hormone (ACTH). When released into the systemic circulation, ACTH in turn stimulates synthesis and release of glucocorticoids (GRs) (corticosterone in rodents and cortisol in primates) from adrenal cortex; they are essential for energy mobilization. Concentrations of glucocorticoids are regulated by glucocorticoid-mediated negative feedback inhibition of further HPA activity at pituitary and hypothalamic sites via two types of corticosteroid receptors, mineralocorticoid (MR) or type I and glucocorticoid or type II. MR receptors have a high affinity for glucocorticoids and show high degree of saturation even under basal HPA activity. MR is thought to be involved in modulation of the circadian rhythm of glucocorticoid secretion, in particular its diurnal trough. GR receptors, in contrast, have a low affinity for glucocorticoids and are thought to modulate negative feedback during the circadian peak and following an acute stressor. The central distribution of these receptors varies in a species-typical and brain-region-specific manner. In rodents MR is distributed primarily in the hippocampus and septum, whereas GR is present in the PVN, hippocampus, cortex, and cerebellum. The central distribution of MR and GR in primates is broader than in rodents with high levels of expression in the neocortex. This may reflect greater role of glucocorticoids in modulation of primate cognition, mood, and social behavior.

In addition to its major role as a regulator of the HPA axis, CRF acts as a neurotransmitter in the widespread circuitry throughout the CNS, integrating information and mediating endocrine, autonomic, and behavioral responses relevant to signs of stress, depression, and anxiety. Acting via neuronal projections that connects amygdala and hypothalamus with the locus ceruleus (LC) CRF stimulates noradrenergic neurotransmission forming a feed-forward cascade of the circuit, which regulates vigilance, anxiety, and fear-like reactivity. In contrast, gamma-aminobutyric acid (GABA), neuropeptide Y (NPY), and oxytocin appear to offset the effects of CRF in stress response.

CNS Developmental Plasticity and Mother-Infant Interaction

While the basic CNS structure and connectivity are programmed by the genetic blueprint, environmental stimuli play a key role in shaping the patterns and connectivity that, in part, are responsible for individual differences in

reactivity to the environment. Developmental processes that occur during the neonatal period include dendritic development, synaptogenesis, granule cell neurogenesis, and apoptosis. The CNS in neonatal rodents is protected from pronounced fluctuations of the HPA axis that can be detrimental to the development of the immature brain. Specifically, there is reduced sensitivity of the HPA axis to activation during the neonatal period (postnatal day (PND) 3–16), which is also known as stress hyporesponsive period (SHRP). This period appears to be essential for the normal brain development in rodents. However, the reduced HPA axis responsiveness during this period is not absolute and can be circumvented by sufficiently strong physical or psychological stressors, including maternal deprivation.

For most newborn mammals, the mother is the most salient feature of early environment and the primary source of environmental stimulation. The newborn rat pups are almost entirely dependent upon the mother for thermoregulation, nutrition, stimulations of micturition, and protection for at least the first 2 weeks of life. Maternal behaviors exhibited by the lactating female rat, include licking, grooming, and arched-back nursing.

Normal mother–infant interactions are believed to be essential for the proper development of the CNS. It has been suggested that the mother acts as a hidden regulator of the infant physiological system. For example, stimulation associated with feeding stimulates heart rate in the infant rat, while licking (stroking) facilitates growth via stimulating secretion of growth hormone and expression of ornithine decarboxilase, a rate-limiting enzyme involved in growth. Furthermore, maternal behaviors play an essential role in suppressing adult-like stress response, in maintenance of the SHRP. In particular, feeding causes desensitization of the adrenal glands to circulating ACTH, whereas licking/stroking inhibits the release of ACTH from the anterior pituitary. Therefore, it is not surprising that separation from the mother is potent enough stimulus that can induce a substantial HPA response in the neonatal rat during the SHRP.

Rodent Models of Maternal Deprivation

There are numerous procedures of maternal deprivation developed to study the long-term effects of early adverse experience. These procedures vary greatly among various research groups, ranging from a single 24-h separation to repeated episodes of separation lasting 12, 6, or 3 h and administered on PND 1–2 through PND 14–21. Also, while in some of these procedures newborns are kept together as a litter, in others they are kept isolated from each other during the separation episode.

In rodents, the best-characterized procedure on long-term effects of maternal separation involves Long Evans hooded rats separated daily from the dam on PND 2–14.

In particular, maternally separated (MS) animals are removed from the dam as a litter and are kept in a warm incubator for 180 min daily. The MS group is compared to animals with the experience of daily separations from the dam for 15 min (handled, H) or animal facility reared (AFR) controls. Some studies also include animals that were undisturbed (or nonhandled, NH) during the same period. These manipulations are performed between 08:00 and 12:00 a.m. each morning. Pups are reared normally beginning PND 15. They are weaned on PND 21 and tested beginning after PND 60. This model has been developed based on naturalistic and seminaturalistic observations of mother-litter interaction in the rat. Under normal conditions, the lactating female rat leaves the maternal nest regularly. However, the duration of these, naturally occurring mother-litter separation episodes appears to be linked to her social status. Dominant female rats build their nests in close proximity to food and water resources and thus can keep regular separations from the offspring relatively brief (15–30 min). However, subordinate females are forced to build their nests in the periphery, resulting in markedly longer (2–3 h) foraging periods and longer periods of separation from the litter.

Consequences of Maternal Deprivation

Changes in Maternal Behavior

Behavior of the mother toward the offspring appears to undergo significant change as the consequence of the maternal deprivation protocol. These changes include increases in latency to retrieve pups to the nest and to initiate licking and nursing. Also, normally coordinated pattern of maternal behavior is disrupted and appears disorganized in the mother as the result of the maternal deprivation protocol. MS pups exhibit elevations in circulating ACTH and corticosterone within 5 min of reunion with the mother. At weaning MS animals weigh less than H or AFR animals, which is another sign of a deficit in maternal care. This difference in body weight can persist well into adulthood. Also, according to recent evidence maternal deprivation protocol results in profound and long-lasting changes in emotional reactivity of the mother and her sensitivity to opioid drugs.

These observations have been the basis for the maternal mediation hypothesis according to which deficits in maternal care upon reunion of the mother with her litter account for a large portion of the neurobehavioral differences observed in the adult offspring. This hypothesis is supported by the observation that many effects of the separation procedure observed in adult offspring can be eliminated by cross-fostering procedure. Also, naturally occurring variations in the frequency of pup licking and grooming (LG) provided by the mother is associated with individual differences in stress responsiveness,

emotional reactivity, and cognitive functioning in adult offspring. As adults, offspring of low compared to high LG mothers show features that are similar to those of MS animals. These include enhanced HPA response to stress and elevation of fear- and anxiety-like behaviors. Furthermore, offspring of low LG mothers exhibited impaired performance in tests of spatial learning and object recognition that was paralleled with shorted dendritic branch length and lower spine density in CA1 cells as well as impaired long-term potentiation (LTP).

Behavioral Adaptations

Using multiple tests for emotional reactivity (novel arena, novelty-induced suppression of appetitive behavior, open field, elevated plus maze, defensive withdrawal, acoustic startle, and startle-induced ultrasonic vocalization) many research groups found that MS animals exhibit signs of neophobia and increases in anxiety, fear, and depression-like reactivity in comparison to H or AFR animals.

MS animals are markedly more hesitant than H or AFR animals to explore a novel arena, which is interpreted as a sign of neophobia. Similar results are found in a test of novelty-induced suppression of appetitive behavior. In this test, food-deprived animals are presented with food in the center of a novel open arena, thus assessing internal conflict between voluntary approach and withdrawal tendencies. In accord with notion of increased neophobic responses, MS animals exhibit longer latencies to approach the food and to feed, also spending less time feeding than H animals.

Furthermore, in the open field test MS animals spend markedly less time than H animals exploring the inner area of the arena. In the elevated plus maze test, a classical test of anxiety and fear-like reactivity, MS animals spend less time on open arms of the maze than either H or AFR animals, often freezing or jumping off the apparatus. Signs of elevated anxiety and fear are also seen in defensive withdrawal test in which MS animals are slower to leave a safe compartment, re-enter to this compartment more often, and spend more time in it than either H or AFR animals. While in the tests for emotionality discussed above, an animal is tested for voluntary exploration of an aversive environment, in the acoustic startle response test an animal is challenged by an acute, uncontrollable stress (loud noise). MS animals exhibit exaggerated startle responses (higher amplitudes) and are quicker to respond than H animals. Similar to other types of aversive stimuli, such as attacks from conspecifics, or electric foot-shock, startle-inducing acoustic stimuli induce rats to emit ultrasonic distress vocalizations. In accord with the data obtained from other tests, MS animals are more likely to vocalize in response to startle than H controls.

In an animal model of anhedonia, one of the key signs of depression, MS animals consume markedly less sucrose solution than either H or AFR animals.

HPA Axis Adaptations

HPA axis adaptations can be considered as a key feature of the maternal deprivation procedure. MS animals exhibit exaggerated HPA responses to stress compared to either H or AFR animals, as indicated in **Table 1**. MS, H, and AFR animals do not differ in basal concentrations of ACTH and corticosterone. However, in response to psychological stressor (air puff, novel environment, and brief handling) MS animals exhibit augmented ACTH and corticosterone response. Furthermore, exogenously administered steroid drug dexamethasone fails to restrain diurnal rise in plasma ACTH and corticosterone in MS animals. Thus, both endogenous and exogenous negative feedback appears to be reduced in MS animals as a result of maternal deprivation procedure.

Table 1 HPA effects of neonatal maternal deprivation as compared to either handling or animal facility rearing

Variable	Effect
<i>HPA axis function</i>	
Basal ACTH	↔
Basal corticosterone	↔
ACTH stress response	↑
Corticosterone stress response	↑
Dexamethasone resistance	↑
<i>CRF receptor binding</i>	
PVN	↑
Pituitary	↓
<i>CRF content</i>	
PVN	↑
Median eminence	↑
<i>CRF mRNA</i>	
PVN	↑
<i>MR mRNA</i>	
Overall hippocampus	↑
CA1	↑
CA2	↔
CA3	↑
DG	↑
Septum	↔
<i>GR mRNA</i>	
Overall hippocampus	↓
CA1	↔
CA2	↔
DG	↔
PVN	↔
FC	↓
CG	↓
<i>MR/GR mRNA ratio</i>	
CA1	↑
CA2	↑
DG	↑

This reduction in negative feedback can be explained by opposing changes in central MR and GR densities in MS versus H groups. MS animals have increased densities of MR mRNA in the hippocampal regions of CA1, CA3, and the dentate gyrus (DG), and have decreased GR mRNA densities in overall hippocampus as well as in the frontal cortex (FC) and the cingulate gyrus (CG). The increased hippocampal MR/GR mRNA ratio in MS animals may contribute to the increased fear-like reactivity and impaired cognitive functions.

Exaggerated responses to stress found in MS animals can also be explained by increased expression of CRF mRNA, especially in the PVN. Furthermore, MS animals exhibit increased CRF content in the medial eminence and increased CRF receptor binding in the PVN. Therefore, the CNS of maternally deprived animals is characterized by increased CRF content and increased sensitivity to CRF.

Extrahypothalamic Effects

There are numerous adaptations in the CNS of MS animals especially in those neurocircuits that modulate or are modulated by the HPA axis, as shown in **Table 2**. The involvement of the extrahypothalamic CRF neurocircuits is particularly important since these circuits mediate neuroendocrine, behavioral, autonomic, and cognitive responses to stressors.

CRF mRNA in the central nucleus of the amygdala and bed nucleus of the stria terminalis (BNST) paralleled by CRF content in those regions is increased in MS animals in comparison to either H or AFR animals.

Table 2 Extrahypothalamic effects of maternal separation in comparison to handling or animal facility rearing

Variable	Effect
CSF CRF	↓
CRF receptor binding	
LC	↑
Raphe	↑
<i>CRF content</i>	
BNST	↑
Central nucleus of the amygdala	↑
Hippocampus	↔
LC	↔
<i>CRF mRNA</i>	
BNST	↑
Central nucleus of the amygdala	↑
Hippocampus	↔
<i>α2 adrenergic receptor binding</i>	
LC	↓
Nucleus of the solitary tract	↓
<i>GABA_A receptor binding</i>	
Amygdala	↔
LC	↓
Nucleus of the solitary tract	↓

These CRF pathways project directly to brain stem nuclei, including the LC where CRF directly activates noradrenergic neurons. The LC has been well established as a site that mediates vigilance and anxiety-like states in rodents and primates. It sends wide projections to multiple cortical and subcortical areas, including prefrontal cortex, hippocampus, amygdale, and hypothalamus. Thus, activation of the LC can further drive the HPA axis. MS animals exhibit reduced α 2-adrenergic receptor binding in the LC and nucleus of the solitary tract, suggesting that once neurons of the LC are active it will be difficult to reduce their firing rate. Also, in addition to the LC, MS animals exhibit increased CRF receptor binding to the raphe nuclei. Thus, changes in the CRF system as the result of maternal deprivation may lead to cascade of adaptations, including both the noradrenergic and serotonergic systems. MS animals are also characterized by reduced central benzodiazepine (CBZ) receptor binding in the LC and the nucleus tractus solitarius when compared to H animals. Therefore, increased CRF, reduced CBZ, GABA_A, and α 2-adrenergic receptor binding all contribute to increased responsiveness to psychological stressors as the result of maternal deprivation.

Summary

In summary, studies involving the procedure of maternal deprivation in the rat provide direct evidence that exposure to adverse events early in development results in exaggerated stress reactivity and alterations in several neural circuits that persist into adulthood. Many of the neurobiological alterations seen in maternally-separated animals parallel those described in patients with depression and anxiety disorders that have a history of various childhood traumas, such as physical or sexual abuse, neglect, psychological maltreatment, or chronic illness. Taking into account the contribution of early adverse events to mental illness can result in improved understanding of the biology of the disorder, its classification, and treatment.

See also: Animal Tests for Anxiety; Depression; Fear, Anxiety, and Defensive Behavior in Animals; Infant Bonding and Attachment; Neural Substrates of Unconditioned Fear, Defense, and Anxiety; Parental Behavior.

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Motivation

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Glossary

- Addiction** – A behavioral pattern of drug use characterized by compulsive drug taking, compulsive drug seeking, and a high tendency to relapse after withdrawal.
- Conditioning** – A phenomenon by which responses are acquired by virtue of pairing with (classical or Pavlovian conditioning), or allowing one to obtain or avoid (operant or instrumental conditioning), a biologically motivating stimulus.
- Dependence** – A physiological state whereby after prolonged use of drugs, a marked physiological withdrawal syndrome occurs upon cessation of drug use.
- Drive** – An internal motivating state (e.g., hunger or thirst) that energizes behavior when homeostasis is unbalanced. Drives are assumed to be aversive in nature and the subject seeks to reduce them.
- Hedonism** – A view of motivation in which people are motivated by obtaining pleasure and avoiding punishment.
- Incentive** – An external motivating stimulus that elicits an appetitive response.
- Instinct** – Heritable dispositions or patterns of behavior.
- Motivation** – An internal state (need, desire, or want) that serves to activate behavior and give it direction.
- Reinforcement** – The strengthening of a response by means of application of reward (positive reinforcement), or removal of an aversive stimulus (negative reinforcement); not synonymous with reward. In the context of fear conditioning, an aversive stimulus could also positively motivate defensive responding.
- Reward pathway** – A hypothetical common mechanism of reward in the brain, argued to be the mesolimbic dopamine system.
- Sensitization** – A progressive increase in response to the same dose of the drug with repeated administration.
- Tolerance** – A progressive decrease in response to the same dose of the drug with repeated administration, or, the need to take a higher dose of the drug to achieve the same drug effect as before.

Motivation may be described as an internal state (need, desire, or want) that serves to activate behavior and give it direction. What we do is largely determined by the

expected consequences of our actions. Anytime reward or punishment is considered as a cause of behavior; motivation is an essential principle of explanation. However, our understanding of exactly what motivates us has been the subject of debate since at least the time of the ancient Greeks. Here we review some basic approaches to motivation – with special emphasis on two dominant viewpoints: drive and incentive theories.

Historical Views on Motivation

Since the time of the ancient Greeks, motivational views could be divided into two categories: those that emphasize intrinsic causes of motivation, and those that emphasize environmental causes. The fourth-century Greek philosopher Thrasymachus (*c.* 459–400 BCE) theorized that people are motivated by self-interest. Any appearance of societal interest or greater good will ultimately be reducible to some sort of calculated long-term self-interest. This form of motivation, in which people and animals are motivated by obtaining pleasure and avoiding punishment, has come to be known as ‘hedonism.’ However, Socrates rejected this view, arguing that people use moral judgment, regardless of their self-interest, to guide their behavior. He posited that individuals can generally agree on what is right or wrong, and are motivated to do what is right. The dichotomy between environmental and intrinsic determinants of actions closely models contemporary incentive and drive theories of motivation.

The first scientific approaches to motivation are typically attributed to René Descartes (1596–1650), who originated the concept of the reflex. Descartes noted that an environmental input, which he called a stimulus (e.g., fire), could sometimes elicit a direct response (e.g., withdrawal). He suggested that nervous pressure was transmitted to the brain by nerves and then reflected back to the muscle, causing it to contract. This causal analysis suggested that behaviors could be studied mechanically, similar to examining the workings of a machine. Although this ingenious theory was poorly received at the time, it is the basic reductionist principle of functional neuroscience today. Over time, biologists consistently found that the machinery of animals was well suited for the environment in which they lived. That is, animals were highly successful at finding food, mates, mothering, and other skills necessary for living in

their home environment. Charles Darwin (1809–82), in the *Origin of Species* (1859), argued that species that were successful in surviving and breeding possessed biological and behavioral traits selected for by nature – traits adaptive for the particular environment. The machinery that controlled behavior generated instincts, or heritable dispositions and action patterns of behavior. Originally, instincts were used to explain behavior only in animals, but over time the concept became accepted as an explanation of some human behavior as well.

Perhaps no other person had more influence on motivational theories than Sigmund Freud (1856–1939). Freud's psychoanalytic theory emphasized unconscious motivation, in which individuals are strongly motivated by thoughts of which they are not fully aware. Freud referred to unconscious motivation as the Id, which contains basic drives, and is compelled to seek pleasure and reduce pain. These basic drives include instinctive needs for food, water, and sex. Freud emphasized the persistence of these urges, in that they arise in childhood, can only be suppressed, and become manifest in complex and sophisticated ways, reflecting the Id's hedonic essence. In contrast, the SuperEgo is our conscience and strives for moral perfection. Finally, the Ego utilizes reason and common sense, navigating external stimuli to mediate the conflict between the Id and SuperEgo. In essence, Freud's theory of behavior was the beginning of drive theory, because the goal of behavior was to reduce the conflict between the Id and SuperEgo. Freud's theories made common sense, and have become everyday explanations of behavior among educated people, even today. However, Freud was a neurologist by training, who emphasized case studies, and his lack of scientific approach and literary license meant that most of his ideas would not be directly integrated into psychology as it emerged as a science in the early twentieth century.

The beginning of the academic discipline of psychology includes John B. Watson (1878–1958) and Ivan Pavlov (1849–1936), both influential behaviorists. Watson and Pavlov redefined psychology as the study of behavior, emphasizing the circumstances surrounding behavior, and specifying the relationship between stimulus and response. The behaviorists endeavored to eliminate in scientific discourse the discussion of all unobservable mental events – including consciousness, which Watson believed was superfluous. They posited that behavior could be explained in terms of reflex stimuli (termed unconditioned stimuli, or USs) which generate automatic, instinctive responses (termed unconditioned responses, or URs), becoming associated with neutral stimuli (termed conditioned stimuli, or CSs). By virtue of being paired with a US, a CS would come to produce a conditioned response (CR) functionally related to the UR, a phenomenon known as Pavlovian or classical conditioning. Likewise, the behaviorist Edward L. Thorndike

(1874–1949) extended learning to include voluntary action in his famous law of effect: "Of several responses made to the same situation, those which are accompanied... by satisfaction... will... be more firmly connected with the situation, so that, when it recurs, they will be more likely to recur... those... followed by discomfort... have their connections... weakened" (Thorndike, 1911, quoted in (Bolles, 1978)). This form of learning came to be known as instrumental, or operant, conditioning. Together, behaviorists believed that Pavlovian and instrumental conditioning could explain the causation of most behavior. While Darwin undoubtedly influenced behaviorist thinking, the behaviorists stressed extrinsic factors as sole causes of behavior.

Around the same time, zoologists and biologists studying behavior in the natural setting were emphasizing instinctive patterns of intrinsically generated behavior. This science came to be known as 'ethology.' Ethologists described the presence of a number of instinctive behaviors, including those that behaviorists had categorized as USs. Eventually, ethologists and behaviorists found a middle ground, and depicted a complex interplay between instinctive and learned patterns of behavior. However, modern motivational theories tend to favor either intrinsic or extrinsic factors.

Drive Theory

Whereas behaviorist psychology emphasizes observable behavior, drives are thought of as organizational motivational states evoked by internal physiological regulatory processes. Drive theorists, such as Clark Hull and Kenneth Spence, argued that drives were a major cause of behavior. Two fundamental concepts are important for understanding drives: homeostasis and the negative-feedback loop. Homeostasis is the required regulation of physiological and psychological variables within biologically defined limits. In some early conceptions of drive (such as those of Hull), drives did not generate specific behaviors but rather generally energized behavior. This became quickly untenable and other theorists, such as Guthrie, added mechanisms to make the behavior specific to the need. For example, if blood sugar becomes too low, our body generates a hunger drive, which energizes behavior to obtain and consume foods with sugar in them. Alternatively, if sugar rises too high, a sense of satiety terminates eating. Thus, feeding behavior is viewed as part of a homeostatic system that keeps sugar levels at an optimal equilibrium. Other drives play a similar role: thirst leads us to drink water; and sexual arousal leads us to pursue sexual relief. In the concept of relief, we can understand the negative-feedback component of homeostatic regulation. Each drive accumulates energy, pressuring the individual to reduce it. In turn, when the action is performed, the drive is reduced (the energy

released) and the subject experiences relief. Thus, drives can be viewed as aversively motivating through the mechanism of negative reinforcement. During negative reinforcement, we produce behavior to terminate an ongoing aversive stimulus. Models involving drive theory therefore are also known as drive-reduction or tension-reduction models.

In an influential series of experiments supporting drive reduction as a substrate of behavior, the physiologist Walter B. Cannon examined hunger, which he believed was a sensation arising from the stomach. Cannon had subjects swallow a pneumatic balloon that measured stomach muscle contractions, and found that when the empty stomach contracted (hunger pangs), subjects reported being hungry. Others extended this work and showed that low blood sugar caused the stomach contractions. Cannon concluded that hunger originated from a signal in the body, and a similar view was adopted for thirst. In these cases, it is presumed that hunger, thirst, and other drives are inherently aversive, and that subsequent behavior arises from a desire to reduce them.

Theorists, such as Hull, argued that drives were also a major cause of learning, although their role is less defined. Primary reinforcers (e.g., food) are those that can reduce drives directly. However, secondary reinforcers (those, such as money, which have been paired with primary reinforcers) can also come to motivate behavior. Drive theory cannot adequately explain how secondary reinforcers reduce drives. Likewise, with the increasing discovery of physiological processes that stimulate behavior, such as the influence of androgens on male sexual behavior, it became unclear why the concept of a sexual drive (or energy) would be required. However, drive states are still used to explain some behaviors, especially in cases where the circuitry is not adequately understood, such as sleep. When we are sleep deprived, an aversive sleep pressure and sleep debt are believed to build up, which can only be relieved with proper sleep.

Today, the emphasis on drive theory has decreased, and research has focused more on the role of secondary reinforcers, and how they come to influence behavior.

Acquired Motivation

Most modern views on motivation hold that biologically based drives and incentives account for only a tiny fraction of behavior. Behavior can occur when we are not hungry, sexually aroused, thirsty, etc.; learned motives are therefore critical to understanding behavior. Two kinds are typically considered, ‘acquired drives’ and acquired ‘incentives.’ An example of an acquired drive is the sense of doom (fear) elicited by the sight of a tornado. This drive would motivate one to seek shelter or hide, which

offers a goal or incentive – the relief from fear and avoidance of harm. One learns that the sight of a tornado is something to be feared. Acquired incentives can include foods that are normally unpalatable, but over time, acquire incentive value. Consider liquor, which is commonly considered foul tasting the first time it is consumed. Access to liquor is limited, requires us to spend considerable money, and can put some of us at risk with the law. Yet, many people work hard to obtain liquor. There is no clear inborn motivational state that generates this action. We may not be thirsty at all, and consumption of liquor might actually increase thirst. Thus, the desire to consume liquor is a learned phenomenon.

Whereas acquired drives generate an internal pressure for action that has been learned, acquired incentives offer an external pull for action. By understanding the mechanisms of learning, we can understand how these factors come to control behavior. Conditioned fear provides an excellent example of acquired drive. Pain is a powerful motivator, and the threat of pain generally elicits both conditioned fear and avoidance. Conditioned fear reflects Pavlovian conditioning, whereby stimuli associated with pain come to elicit the fear response. Avoidance, on the other hand, reflects instrumental conditioning where the responses of escape come to be reinforcing because expected pain and fear are averted. The buildup of conditioned fear exerts pressure on the animal or person much in the same way as instinctive fear. Thus, conditioned drives can be viewed as enhancing fitness by expanding the effective repertoire of instinctive drive.

A second type of acquired motivation is conditioned reward (conditioned incentive). This reflects a process by which a stimulus that has no reinforcing properties on its own (such as money) comes to acquire the power of natural rewards, through learning. Conditioned reinforcement occurs when a stimulus is consistently paired with a natural reward through Pavlovian or instrumental conditioning. For example, animals can be trained to work for tokens that can be traded for food. However, subjects will also come to value cues that have no value. For example, if a clicking noise is paired with the delivery of food consistently, subjects will, over time, work for the click alone. These stimuli will eventually lose their effectiveness as reinforcers if the food is never delivered, but it is puzzling that they alone can act as reinforcers, supporting the acquisition of new behaviors. Conditioned incentives, such as that elicited by the click, may arise because they predict the occurrence of a natural reinforcer. Alternatively, it may simply be that pairing with the natural reinforcer confers some of its characteristics to the conditioned reinforcer. For example, Ludwig and colleagues gave detoxified alcoholics a small amount of alcohol when they pressed a button. Subjects rated their subjective craving. The subjects were then exposed to

cues that had been associated with alcohol in the past – namely the sight, and a small taste of, their favorite bottle of liquor. After exposure, subjects worked harder at pressing the button, and reported greater craving. The stimuli had thus been imbued with qualities of the incentive, and subsequently motivated behavior that gained access to alcohol. Conditioned reinforcers can serve a number of functions, such as providing information on how to obtain natural reinforcers, or simply becoming pleasant in their own right. This phenomenon can be understood in the context of classical conditioning and is typically referred to as Pavlovian incentive learning.

Acquired drives are thus internal states that motivate behavior. Similar to biological drives, they generate negative feelings or internal states (such as fear) that can be reduced when the appropriate behavior is performed (such as avoidance). Conditioned incentives are likewise learned, but reflect an external draw on behavior by virtue of their association with natural reinforcers.

Opponent-Process Theory

Opponent-process theory is a more complex theory that attempts to integrate both aversive and appetitive reinforcement. Richard Solomon and John Corbit developed this theory based on the time course of positive and negative feelings that emerge over time. The general concept is that over time any emotional state (A process) will convert into the opposite (or opponent, B process) state. After the experience of a happiness-invoking situation, a swing toward negative feelings will emerge over time. This is, in essence, a homeostatic theory of mood. Opponent-process theory brings emotion into the same general conception of equilibrium that is appreciated throughout biology. In Solomon and Corbit's original conception, the B process could change over time and was subject to conditioning. The theory has been especially useful in explanations of dependence and addiction. Thus, as an exercise in understanding motivation, the rest of this article focuses on theories of dependence and addiction.

Drug Dependence and Addiction

Most scientists agree that certain drugs may initially be taken to bring about an end to some unpleasant state (e.g., pain or depression), while others are taken in order to bring about some pleasant state (e.g., euphoria). Thus, initial drug use can involve both negative and positive reinforcement. However, as drug use becomes problematic and habitual, two competing views on addiction emerge.

Negative Reinforcement Models

Negative reinforcement theories of addiction emphasize aversive outcomes of terminating drug use, especially withdrawal and depression. These theories explain escalating drug use as being a result of tolerance to drug effects. Thus, these theories can be directly mapped to drive-reduction theory. Positive reinforcement theories of addiction, on the other hand, emphasize the ability of drugs to induce positive hedonic states, or reinforcement, by activating reward-related circuitry in the brain. Positive reinforcement theories rely on sensitization (described below) to explain escalating drug use. Finally, to explain relapse, both theories rely on conditioning – either conditioned withdrawal, or craving elicited by cues associated with drug use.

Physical dependence is generally defined as the presence of an aversive withdrawal syndrome upon cessation of the drug. Withdrawal is typically defined as a marked physiological disturbance that occurs upon cessation of the drug. Withdrawal symptoms are usually the opposite of drug-induced effects, and can be viewed as opponent, or drive, processes. In this view, opponent processes (known as compensatory adaptations) increase over time, forming the basis for tolerance, and increased drug use. The physical dependence theory of addiction has become a less tenable explanation for drug addiction for several reasons: First, physical dependence and withdrawal fade fairly quickly after drug cessation, and can be effectively managed in rehab; however, high relapse rates persist for years. That is, withdrawal is an acute condition, but addiction appears to be a chronic disease. Second, many drugs that produce addiction do not produce a marked physical withdrawal syndrome, such as the stimulant drugs amphetamine and cocaine. Moreover, the intensity of the withdrawal syndrome is not necessarily predictive of a drug's addictive potential. For example, the withdrawal syndrome produced by barbiturates and alcohol is life threatening, and far more intense, than that produced by heroin or cocaine, and yet the latter are viewed as more addictive. Third, persistent high doses of drugs are required to produce physical dependence, yet habitual drug taking can occur at much lower doses. Modern use of hydrocodone, a fairly weak but popular narcotic, would usually fall into this category. Finally, addictive potential depends heavily on how quickly the drug acts in the brain, and requires the use of a rapid route of administration (e.g., injected heroin is much more addictive than morphine tablets); however, equivalent dependence can be produced by slow and fast methods of administration. Given these findings, the definition of addiction (below) has come to exclude physical dependence as a necessary or sufficient component of addiction.

Nonetheless, several modern negative reinforcement theories can explain addiction and still resemble drive

theory. Prominent among these are psychological dependence models and the allostatic theory of addiction. Allostasis proposes that under chronic instability outside of the homeostatic range (such as chronic activation of the reward circuit), physiological systems become mobilized to find an equilibrium around the altered allostatic set point, including a new set point for mood. Opponent-process compensatory adaptations over time come to mask the initial hedonic effects of the drug. Aside from physiological adaptations that manifest as physical withdrawal, the allostatic (and psychological dependence) model proposes that there exists also a reward-related adaptation in mood that manifests as persistent depression, irritability, anxiety, and generalized emotional distress. This distress syndrome is posited to outlast physical withdrawal. In this sense, when the addict relapses and begins taking drugs again, the reinforcing effects of the drugs can be viewed in terms of drive reduction – rather than producing pleasure or positive reinforcement, they merely attenuate aversive drive states of the addict (such as depression).

A third negative reinforcement view of addiction that closely mirrors drive theory is the self-medication hypothesis. In this view, addicts begin, and continue, to take drugs in order to correct innate homeostatic imbalances in mood or other affective states. The drug of choice is not random, but rather matches some psychological need; opiates might be taken because of their ability to mute psychological pain, while cocaine might be taken to correct inherent behavioral depression.

Overall, a number of negative reinforcement models of addiction have been proposed which closely mirror drive (or acquired drive) theory. In all of these models, drug taking reduces intrinsic negative physical or emotional states.

Positive Reinforcement Models

In positive reinforcement models of addiction, addicts take drugs because of their rewarding or reinforcing properties. Some recent definitions of addiction stress incentive properties of drugs, and seek to exclude dependence as a necessary or sufficient condition for addiction. Jaffe describes addiction as a behavioral pattern of drug use characterized by: (1) overwhelming involvement with the use of a drug (compulsive drug taking), (2) the securing of its supply (compulsive drug seeking), and (3) a high tendency to relapse after withdrawal. Positive reinforcement has been the central emphasis of addiction research for many years, with the mesolimbic dopamine system viewed as the central reward pathway. This system innervates areas involved in action, planning, and learned behavior (e.g., nucleus accumbens prefrontal cortex and amygdala) with dopamine from the ventral tegmental area, and is well positioned to control behavior. In

particular, the nucleus accumbens has been argued to be an interface between motivation and subsequent action. Indeed, natural reinforcers and all drugs of addiction activate this pathway, and by one argument, addictive drugs are essentially hijacking this natural reinforcement pathway.

Positive reinforcement models of addiction gained popularity when animal models of reinforcement (drug place preference and self-administration, in particular) emerged in the twentieth century. These behavioral models of drug reward predict addictive potential better than the strength of withdrawal symptoms. In most reward models, drugs function as biological incentives, directly activating reward circuitry. However, in this simplest form, the reward theory of drug addiction inadequately explains escalating drug use and the emergence of compulsive over-involvement with the use and seeking of the drug.

Robinson and Berridge have proposed that sensitization, a process by which repeated intermittent administration of the same dose of drug leads to an increase in response, can help us understand the transition to addiction. In particular, the psychomotor response, mesolimbic dopamine activity, and reinforcing properties of many drugs, especially psychostimulants, undergo sensitization, and this is accompanied by synaptic remodeling in reward-related circuitry in the basal ganglia and prefrontal cortex. Robinson and Berridge propose that the incentive qualities, or psychological wanting, of the drug also undergo sensitization, which leads to escalating drug seeking, drug use, and loss of executive control. In this conception, the hedonic properties of the drug (psychological liking) are separate from incentive salience, because addicts report decreased pleasure with repeated drug use (i.e., tolerance). However, the ability of drugs to reinforce responses and increase approach undergoes sensitization, consistent with a view of increased incentive. Finally, CSs that are present at the time of administration (e.g., the sight of the drug, or even contextual stimuli) become imbued with conditioned incentive value which escalates, and can elicit craving and energize behavior toward drug taking. As an alternative explanation, which does not necessarily require sensitization, Di Chiara, in 1999, proposed that one key difference is dopamine response to rewards: the response to natural rewards habituates, whereas the response to drug rewards does not diminish. This allows drugs to exert an ever greater influence on learning as compared to other reinforcers. Conceptually, this is similar to an addict who pays more and more attention to drugs, and less and less attention to other matters in his life, such as home, family, and work.

Positive reinforcement models of addiction are frequently viewed as the mirror opposite of negative reinforcement models. This conception can create an unnecessarily narrow perspective, in which positive or negative reinforcement is exclusively viewed as the basis of drug addiction. However, these processes are not mutually exclusive, and could actually work together to produce addiction. That is, people may initially take drugs for pleasure, and subsequently transition to compulsive behavior as the incentive value of the drug sensitizes. In turn, withdrawal syndrome (or reward allostatic) could make the termination of drug use very difficult. Finally, conditioned cues may trigger relapse by eliciting craving. In this sense, both drive-like and incentive states contribute to the phenomena of addiction and dependence. Nonetheless, several addiction theorists have focused on what is necessary and sufficient for addiction, in which case reward-related processes are more central to addiction than physical dependence. Even Koob's allostatic theory focuses heavily on reward homeostasis, and discredits physical withdrawal.

Contemporary theories of addiction, incentive sensitization and reward allostatic, differ in terms of emphasis on positive or negative reinforcement, with origins ultimately tracing back to the earliest drive and incentive views of motivation, as reviewed at the beginning of this article.

See also: Acute Dependence; Animal Models of Behavior; Alcohol Addiction; Animal Models of Learning and Memory; Brain Stimulation and Addiction; Cognition: Learning and Memory: Pavlovian; Drug Addiction; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Drug Sensitization and Drug Abuse; Emotions; Incentive Motivation and Incentive Salience; Motor Function and Motivation; Neural Systems of Motivation; Psychostimulants; Rewarding Brain Stimulation; Sexual Motivation; Sleep Genetics; Transition to Addiction.

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Mouse Genetic Approaches to Psychiatric Disorders

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Glossary

Epistasis – The contribution of multiple genes to one phenotype.

Genotype – The genetic makeup of an organism, described as a combination of alleles for a gene.

Phenotype – A measurable observable trait that is a product of the genotype and environment.

Pleiotropy – The multiple consequences of one gene in different systems.

Polymorphism – A variation in gene sequence.

trait related to a psychiatric disorder, a bottom-up approach. This article does not seek to describe genetic models of human psychiatric disorders but describe how mouse genetic studies contribute to our knowledge of disorders and how different gene manipulations can be used to study behavioral outcomes. As a companion to disease-specific articles this should allow the power, complexities, and caveats of the different approaches to be understood.

Top-Down Approaches

Mouse Breeding and Behavioral Traits

As with domesticated animals, mice have been bred for specific traits for hundreds of years. For example, a popular breed among mouse fanciers was the Japanese Waltzing Mouse, selectively bred because they dance in circles. It has since been established that this trait is due to a genetic mutation causing inner ear dysfunction. More recently, researchers have taken advantage of advances in genetics to map specific genetic components of heritable behavioral traits. This has been greatly facilitated by the sequencing of the mouse genome (completed in 2002), which has allowed mapping of single base pair changes within the mouse genome. Two main strategies have been taken to pinpoint genes involved in behavioral traits. First, through comparing behavior of inbred strains of mice and identifying naturally occurring polymorphisms and second, by inducing genetic mutations, and selecting mice with abnormal behavioral traits.

Inbred Strains

The foundation work for much of the mouse behavioral genetic research occurring today stems from work in the early 1900s developing inbred strains of mice where all offspring carry identical DNA. Screening inbred strains of mice, and congenic lines derived from crosses of the parental strains, allows different behavioral traits to be ascribed to alterations in specific areas of the chromosome, termed quantitative trait loci. Genetic polymorphisms within these loci can be identified and then the role of the affected genes further elucidated. The use of congenic lines has enabled an efficient mapping of genetic variants and since the publication of the genomic sequence of one host strain of mouse (C57BL6/J), the identification of changes in DNA sequence has become

Introduction

Heritability studies have shown that almost every psychiatric disorder has a genetic component, for example, if a person suffers a disorder there is an increased likelihood that their parent or sibling will suffer from it too, and this is more likely the more genes they have in common. For example, it is more likely a monozygotic twin (identical DNA), than a dizygotic twin (sharing 50% DNA), of a schizophrenic will also suffer. However, no single gene variant has been discovered to explain a psychiatric disorder. Rather, the psychiatric disorders are products of a complex blend of variations in multiple genes and contributing environmental factors – physical, psychological and social. A specific gene polymorphism may confer susceptibility to a disorder but for the disorder to manifest it may need to be present with particular variations of other genes and the occurrence of additional environmental factors or stressors, such as drug exposure or an adverse life event.

For many years mice have provided a valuable tool for studying the genetic contribution to behavior. Mice share 99% of the approximately 30 000 genes expressed in man. This conservation across species and the ability to genetically manipulate mice makes them a valuable model organism with which to study the role of specific genes in disorders. Mouse genetics has been used to approach the identification and study of disease-related genes from two directions: First, genomic studies seek to find novel genes that might be implicated in a disorder, a top-down approach, from the behavior to the gene; and second, candidate gene studies, where specific genes are manipulated, and their role determined in a behavior or complex

more straightforward. A collation of genetic and behavioral measures collected from many research groups working on the same strains of mice is making it possible for an *in silico* approach to be taken where researchers can correlate their behavioral data with the current genetic information (WebQTL).

Random Mutagenesis

Rather than looking for naturally occurring polymorphisms, which may have relatively subtle effects on behavior, another strategy has been to induce changes in the genome by random mutagenesis, and then search for heritable abnormal behavioral traits in the mutagenized lines. Random mutations are introduced into the genome by means of a chemical mutagen or radiation. Historically, induced mutagenesis has been a successful strategy for finding novel genes that have a role in disease. However, it is a costly process requiring vast colonies of mice and resources. There are several sites around the world that have embarked on large-scale mutagenesis studies, screening for genes relating to a number of traits, including neurological and behavioral changes. They rely on an initial high-throughput screening to identify mice performing abnormally on standard behavioral tests. These mice are then bred and the heritability of the behavioral trait tested. At this point the genetic location of the mutation is identified and the gene function and altered phenotype further explored and characterized. To date this strategy has proved successful in identifying genes involved in a number of neurological functions and the maintenance of circadian rhythmicity.

Utility of Genetic Association

These strategies begin with no formal hypothesis as to what gene may be involved in a behavioral trait. Looking for naturally occurring polymorphisms that influence behavior by comparing across strains of mice most closely mirrors heritable behavioral traits in humans as they are likely to be evolutionarily stable. As a result, the effects of these genetic variations are subtle (not life threatening to the animal) and to elicit a large effect probably requires variation in a number of genes (polygenic). Thus through this strategy genes with relatively small effects may be missed or their actions only become apparent on a specific genetic background (in one strain). On the other hand, searching for mutations that cause a change in behavior, in mutagenized mice, allows us to identify novel genes, but not necessarily genes whose differential expression predisposes a person to a psychiatric disorder as genes whose mutations cause large (possibly deleterious) effects on behavior are less likely to have evolutionary stability and would often be bred out of populations. This kind of study does however provide a powerful tool for

identifying novel targets for further investigating the underlying neurobiology of behavior.

Bottom-Up Approaches

Traditionally, lesion studies or pharmacological approaches have been used to understand how the brain works. While these remain important in the last two decades, with the advent of genetic engineering and the utility of mice as a genetically tractable species, there has been a rapid increase in studies determining the contribution of specific genes and their products to behavior. These techniques, developed to study different physiological systems, have been exploited by neuroscientists to study the function of the wealth of genes expressed in the brain. They have enabled the identification of pharmacological targets of drugs and allow a dissection of the molecular mechanisms by which behavioral changes occur.

Genetic Manipulation of the Mouse

The methodologies used to mutate genes *in vivo* to ascribe function to their products are becoming ever more elegant and precise. The first transgenic mice (in which a gene is introduced to a mouse) were made in the early 1980s followed by the first knockout mice, in 1989, where mutations were introduced to endogenous genes in embryonic stem cells that could be used to generate mice bearing the gene, often functional. Current techniques now allow us to investigate the function of genes with both regional and temporal specificity. We can test the precise role of a protein in a specific cell population at a specific time in development. Studying the behavioral consequences of manipulating genes has allowed us to investigate both the intrinsic mechanisms of behavior and the actions of drugs in mediating behavioral change. Targeting specific genes known to have a role in psychiatric disorders enables us both to research the molecular events and pathologies leading to the disorder, and perform a behavioral characterization of the genetic model, that might point to new endophenotypes of the disorder.

Utility of Genetic Manipulations

Directed manipulation of genes allows hypothesis-driven research by exploiting how the expression of individual genes contribute to behavior and by extrapolation to the underlying neuropathologies of different psychiatric disorders. Candidate genes may be chosen for study due to: their high expression in specific brain circuits; their regulation in response to a perturbation; a prediction based on pharmacological or neurochemical knowledge; or identifications of disease associated polymorphisms in the human genome. For example, studies regarding the

properties of drugs of abuse, that lead to dependence and addiction, have benefited greatly from genetic manipulations. It has been possible to ascribe specific proteins the site of action of drugs of abuse. For example, it became evident when studying knockouts of the monoamine transporter genes that the rewarding actions of cocaine were not solely through action at the dopamine transporter (DAT) but also at the norepinephrine and serotonin transporters (NET and SERT). It was not until all three transporters were knocked out that cocaine had no pharmacological activity. As with the genetic association studies described above, studying the behavioral profiles of different strains of mice, it is often found that a phenotype may be visible in one strain but not another. For this reason most studies are carried out on mutant mice and sibling control mice, and ideally on mice where the parents have been backcrossed to a specific inbred strain. A mixed genetic background may increase the variability meaning a subtle effect might be lost in the noise or alternatively modifier genes may be present in one of other strain that are needed for the phenotype to be visible and thus in one strain, or in a proportion of siblings on a mixed background, the phenotype will not predominate.

Transgenic Mice

Transgenic mice have new DNA introduced to the genome at a random location. A gene or genetic element is injected into the nucleus of a newly fertilized egg cell and may randomly insert into the genome. It is not targeted, and its expression is dependent on its insertion site. By incorporating specific promoter elements into the transgene, it is possible to direct expression to specific populations of cells. Once inserted into the genome, the transgenic DNA is stable and can be passed from generation to generation in a heritable fashion. Because the random insertion of DNA into the genome may itself disrupt function of a gene at the point of insertion, or it may be differentially expressed due to regulatory elements in the surrounding genome, studies using transgenic mice often require the characterization of more than one founder line of mice. As tools for manipulating gene expression are developed, there are many different ways transgenes can be used to study the function of genes. Gene overexpression, for example, can be driven by simply introducing a gene under the control of its own or other promoter elements. On the background of a knockout mouse (where the endogenous gene has been mutated (see below)), transgene expression can enable behavioral deficits ascribed to the absence of the gene to be attributed to it specifically, presuming that they are restored with transgenic expression. Transgenes with genes encoding dominant negative forms of a protein or micro-RNAs can be used to establish the role of proteins

by blocking their function directly or by reducing their expression in specific neuronal populations respectively. Using the promoters and regulatory regions of genes as vectors for carrying the transgene to be expressed allows targeting of the construct to specific neuronal populations. Bacterial artificial chromosomes (BACs), which can carry large chunks of genomic sequence up and downstream of a gene of interest, have proved especially valuable in increasing the size of transgenes and providing the necessary regulatory components to drive expression in specific neuronal populations. This strategy has been used to generate a library of transgenic mice with green fluorescent protein expressed in the place of thousands of different genes. These BACs are also being used to express Cre-recombinase, an enzyme used to selectively knock-down genes (see below).

Constitutive Knockouts

Rather than random insertion, making knockout mice requires the endogenous gene itself to be mutated. A specific gene is mutated and reintroduced into the mouse genome by homologous recombination (e.g., it is switched for the normal gene) in mouse embryonic stem cells. The embryonic stem cells carrying the mutation are then injected into a blastocyst (mouse embryo at an early developmental stage, a few cell divisions) and divide and differentiate. A chimeric mouse, made up of cells derived from both the host blastocyst and the embryonic stem cells, is born and crossed to a normal mouse. Presuming the gametes were derived from the embryonic stem cells, the mutation becomes heritable and will be present as a heterozygote (one copy of mutant, one copy of wild-type (normal) gene). Crossing these heterozygotes should then produce, by mendelian genetics, 50% heterozygotes, 25% knockouts, and 25% wild-type mice. These offspring can then be phenotyped to determine the consequences of loss of that gene in behavioral tests. This methodology is now commonly used and it is relatively easy to generate knockouts for almost any gene. However, there are issues of this technique both in interpretation and logistics of mutation of certain gene products.

As highlighted at the beginning of this article over 80% of genes in the genome are expressed in the brain. This is not to say that only 20% are expressed elsewhere but that genes may be ubiquitously expressed in all cells or they have multiple roles serving functions during development of tissue and in the mature brain, or be necessary for the functioning of other organs (pleiotropy). Thus, it might be that a gene not only has a specific role in a behavioral trait in the adult but has an essential role in early development as well. In this case the mutant mouse may not survive through development making it impossible to test in adulthood. Genes may have other pleiotropic functions that preclude behavioral profiling,

for example, mice with a neurological impairment might be unsuitable for testing in a complex cognitive task. For this reason, it is also important that new mutants are put through standard neurological tests, to ensure that behavioral impairments reflect a change in the targeted behavior and not an underlying deficit. For example, an impairment in a visual learning task (such as the water maze) may be due to a visual rather than cognitive deficit.

Knock-In Mice

Knock-in mice are generated in much the same way as knockout mice; however, instead of generating a null mutation where the gene cannot be expressed, the mutation introduces a change to the gene that alters its function or introduces new regulatory elements. An example of a way this strategy is used to study human disorders is to swap the endogenous mouse gene for a humanized version of the gene where a genetic variant, that may cause an increased susceptibility to a psychiatric disorder is introduced into the mouse so behavioral changes attributed to the specific polymorphisms of the gene can be characterized. Another manipulation that can be made to genes is to make alterations to the structure of the gene, to alter its function. Examples such as this can be used to make a dominant negative or functionally inactive protein, one with an alteration that changes the function. For example, a key mechanism of action of nicotinic receptors includes both its activation and a subsequent desensitization. A mutation (relating to a naturally occurring polymorphism associated with human epilepsy) that renders the receptor unable to desensitize has been instrumental in elucidating the role of these receptors. Behavioral effects such as locomotor sensitization are seen with much lower doses of the drugs showing the importance of the desensitization in the receptor's normal activity. Drugs with the site of binding of ligands modified have also enabled the study of drug actions. For example, gamma aminobutyric acid (GABA)-A receptor alpha subunits confer binding to benzodiazepine at a site different to binding of their natural ligand, GABA. A mutation at this site altering one amino acid is sufficient to make these receptors benzodiazepine insensitive, but not alter the actions of GABA. These have been used to tease out the specific receptor subtypes mediating the anxiolytic actions of benzodiazepines as opposed to side effects such as sedation and muscle relaxation.

Inducible Expression

Inducible transgenic systems have also been developed whereby the gene can be switched on or off, and its function tested within the same mouse. These systems generally utilize transgenic technologies where regulatable sequences are incorporated into the promoter region

of the transgene. The most common strategy is adapted from a bacterial antibiotic resistance system where the presence of an antibiotic, tetracycline, activates a transcription factor (transactivator) that drives expression of an enzyme that confers resistance to the antibiotic. The transactivator and resistance gene promoter have been adapted and introduced into mammalian systems to enable the switching on or off of a specific gene (behind the tet-promoter) in the presence of antibiotic (delivered to the mouse in drinking water). Using this system, behavioral changes attributed to a neuropathological change in gene expression can be directly tested. By expressing these transgenes on a knockout background, the role of the gene can be tested acutely. Interestingly, on occasion when selective gene expression has been shown to restore wild-type behavior, it has been shown that switching off the gene does not re-instigate the knockout phenotype – pointing toward a developmental role of the gene product in mediating the behavior, not the presence of the gene at the time of testing. The localized expression shows us the importance of genes at particular periods of development. These studies provide important controls for constitutive knockout studies.

Inducible Knock-Downs

To circumvent the issues of epistatic effects of a gene knockout during development (e.g., embryonic lethality), or compensatory changes that may occur during the long-term absence of the gene, several methods have been developed to create inducible knockouts where the gene can be knocked out in specific regions or cell populations at specific timepoints (e.g., adulthood). The most successful strategy has adapted the cre-recombinase system used by bacteriophage viruses to integrate their viral genome into and out of the host bacterial genome. Lox-P sites (specific DNA sequences) are introduced into the gene to be mutated, and the new sequence recombines into the endogenous mouse gene. These two lox-P sites if introduced into noncoding regions should have no effect on their own but, in the presence of cre-recombinase enzyme (not expressed intrinsically in mammalian cells), will join together and the DNA sequence between them will be excised. Thus if the internal sequence contains part of the coding region of the gene, its function will be knocked out. The inducibility of this system comes from the expression of the cre-recombinase, which can be driven either through a transgene where its expression can be regulated by specific promoter elements or by viral injection into a specific brain region. This strategy has been especially useful in looking at the role of genes in specific circuits, where results in a constitutive knockout may be confounded by brain-wide alteration in function. For example, glutamate receptors have been inducibly knocked out in specific hippocampal populations and

striatal medium spiny neuron populations to study their function in specific networks or circuits related to learning and memory, addiction, and schizophrenia.

RNAi

A more recent strategy to downregulate gene expression is the use of small interfering RNAs (RNAi). It has been found that an endogenous method of regulating gene expression is the production of small pieces of RNA that interact with the messenger RNA (transcribed to make proteins) and cause its degradation. Understanding the sequence structure of these RNAs has enabled the design of RNAs to target specific genes. Expression of these driven by transgene or through viral vector expression allows the localized (and potentially reversible) downregulation of expression.

Viruses

A cheaper and faster way to manipulate gene expression *in vivo* has been the use of viruses. Viruses are manipulated so they can no longer reproduce and transgenic DNA introduced. Intracerebral injection of the viral particles to the brain allows a local infection of surrounding brain cells. In this way localized overexpression or knockdown (by shRNA) can be achieved. Common viruses that have been adapted for this purpose include: the Herpes Simplex virus, however, this only confers a short period of gene expression. Alternatively Lentiviruses and Adeno-associated viruses (AAV) which confer long-term expression of the gene of interest, can be used. A caveat of using viruses is that it is difficult to direct the infection or expression to one population of neurons. It is likely all neurons in the vicinity of the injection site will be infected (rather than say just excitatory or inhibitory neurons, for example). Currently, researchers are investigating the selectivity of specific serotypes of AAV vector for specific cell types and this may prove useful for targeting specific cell populations.

Considerations for Interpreting Phenotypic Data

As the targeting of genetic manipulations becomes more precise elucidating the specific contribution of genes to behavior and psychiatric disorders, becomes a more feasible task. Refinements to targeting strategies and the precise localization of genetic mutations to individual cell populations should allow us to construct a picture of what individual pathways and molecules in the brain do in relation to behavior.

The polygenic basis of most psychiatric disorders does not point to simple associations between genes and behavior. Single genes are active in the presence of other genes and it is the combined activity of all these genes that is

measured in behavioral output. A polymorphism in one gene may only show an apparent phenotype in the presence of certain polymorphisms of other genes. The effects of multiple genes (epistasis) on one behavior can confound establishing the role of single genes. Future genetic studies may begin to take this into account, manipulating multiple genes simultaneously.

A factor when considering the pleiotropic effects of genetic mutations is how they might interact with maternal behavior. It might be possible that a knockout mother may show differences in maternal care of her offspring. For this reason it is important that (and often required for publication) that mice of different genotypes are generated from the same matings e.g. heterozygous matings used to make both wild-type and knockout mice. More general environmental factors also need to be considered. In some cases a phenotype may only become visible in combination with manipulations of the environment, for example after introduction of a stressor. This can also account for why the described phenotype of a mutant mouse can vary between laboratories. A phenotypic change in locomotor activity, for example, may be visible in a novel environment but absent in a familiar environment, or in differential lighting conditions.

By recognizing the strengths and limitations of different manipulations of the mouse genome we can interpret behavioral data and begin to understand the complexities of how genes may play a role in psychiatric disorders. In making links from the behavioral phenotyping of mutant mice to the role of the mutated gene in behavior and a psychiatric disorder requires putting together a cohesive picture with parallel pieces of evidence: Is a mutant phenotype carried across more than one behavior associated with the psychiatric disorder? Is it preserved in related mutations? Does it resemble the effect of a pharmacological antagonist? Does the gene appear in human (or mouse) genetic association studies?

Conclusions

There is a place for both top-down and bottom-up approaches in the use of mice to study psychiatric disorders. Gene association studies in inbred strains of mice provide a simpler genetic system to study behavioral variation, than the equivalent human studies where there is more genetic variability and less control of the environment. The tools available to probe the roles of candidate genes (either hypothesis driven or from the association studies) are becoming ever more sophisticated, and provide a powerful way to link gene function to behavior. Though we need to be aware of limitations and pitfalls in analysis of genetically manipulated mice, as new technologies are developed these can be addressed. In combination with lesion and pharmacological studies

which themselves are beset by caveats and limitations, we have a great number of tools that will prove highly valuable in the understanding of how the molecules and circuits of our brains control our behavior, and what the neuropathological changes are that mediate the symptoms of psychiatric disorders.

See also: Animal Tests for Anxiety; Cognition: Learning and Memory: Spatial; Depression; Feeding; Schizophrenia; Value of Animal Models for Predicting CNS Therapeutic Action.

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Neural Bases of Defensive Aggression

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Glossary

Cytokines – Pleiotropic cell-to-cell-signaling proteins or glycoproteins that are produced by different cell types in the periphery and the brain.

Defensive rage behavior – A naturally occurring behavior that takes place in the presence of another animal of the same or of a different species, usually within the domain of its territory; this animal can be of the same or of another species. Basic features of this form of aggression include: flattening of the ears, lowering of the body, drawing in of the head, piloerection, hissing, pupillary dilatation, and stiffening of the tail that becomes motionless.

Enkephalin – A peptide neurotransmitter synthesized in the central amygdala and whose inhibitory actions are mediated upon the periaqueductal gray (PAG) to suppress defensive rage behavior in the cat.

γ-Aminobutyric acid (GABA) – A major inhibitory neurotransmitter in the central nervous system. In the medial hypothalamus and the PAG, GABA inhibits defensive rage behavior by acting through GABA_A receptors.

Glutamate – A major excitatory neurotransmitter of the central nervous system; a principal neurotransmitter of the pathway linking the medial hypothalamus with the midbrain PAG whose functions are mediated through N-methyl-D-aspartic acid (NMDA) receptors.

Hypothalamus – Together with the thalamus, it forms the diencephalon and serves as the primary structure for the integration of defensive rage behavior as well as a variety of other visceral processes.

Interleukin 1 (IL-1) – A cytokine whose actions in the brain serve to facilitate defensive rage behavior by acting through IL-1 β receptors and serotonergic (5-HT₂) receptors both in the medial hypothalamus and PAG.

Interleukin 2 (IL-2) – A cytokine whose actions in the brain serve to suppress defensive rage behavior in the medial hypothalamus by acting through IL-2 and GABA_A receptors and to facilitate defensive rage behavior in the PAG by acting through IL-2 and neurokinin-1 (NK₁) receptors.

Kluver-Bucy syndrome – A disorder associated with lesions of the amygdala or adjoining piriform cortex that is characterized by abnormal docility, hypersexuality, and marked oral tendencies.

Limbic structures – A region of the forebrain consisting of the hippocampal formation, amygdala,

septal area, prefrontal cortex, and cingulate gyrus.

These structures are linked by the fact that they receive cortical and monoaminergic inputs and modulate hypothalamic functions.

Midbrain periaqueductal gray (PAG) – A key structure of the midbrain that receives major output of the medial hypothalamus for the integration and expression of defensive rage behavior.

Monoamine neurotransmitters – These include norepinephrine, dopamine, and serotonin. Both norepinephrine and dopamine facilitate defensive rage behavior by acting through α_2 and dopamine-2 (D₂) receptors, respectively; serotonin facilitates defensive rage through 5-HT₂ receptors and suppresses defensive rage through 5-HT_{1A} receptors.

Septal rage – Marked increase in aggressive behavior following large lesions of the septal area in which the intensity of the aggressive reactions in these animals is so intense that the animals are extremely difficult to handle.

Substance P – A peptide neurotransmitter that powerfully facilitates defensive rage behavior by acting upon NK₁ receptors both in the medial hypothalamus and PAG.

Vasopressin – It acts both as a hormone and as a neurotransmitter. When acting at V₁ receptors.

Overview of the Model of Defensive Aggression

Defensive (aggressive) behavior (referred to interchangeably as ‘defensive rage’ or ‘affective defense’) has been studied most intensely in felines, although varieties of studies have been conducted in other species, including the rodent, dog, and monkey. In addition, there is a close similarity between the characteristics of defensive rage seen in animals and those seen in the human. In the human literature, this form of behavior has been referred to as ‘reactive’ aggression which contains marked impulsivity. It is seen, for example, in certain classes of patients with schizophrenia and borderline personality disorder. An example of this form of aggression in nonpatient populations includes ‘road rage.’ The following discussion concerns research derived from studies conducted in felines.

In the cat, defensive rage is a naturally occurring behavior that takes place in the presence of another animal, usually within the domain of its territory, and involves both defensive and offensive components. This animal can be of the same or of another species. Concerning defensive components, the basic features of this form of aggression include: flattening of the ears, lowering of the body, drawing in of the head, piloerection, hissing, pupillary dilatation, and stiffening of the tail that becomes motionless. Frequently, this pattern of responses serves to cause the opponent (i.e., the threat object) to withdraw from the immediate environment, thus achieving the objective of defensive rage behavior. If the cat decides to attack, its initial response is typically by paw strike, which is a primary means by which it defends itself. However, if the opponent responds to the attack with an aggressive act of its own, the cat will frequently roll over on its back with its anterior surface facing the opponent. With respect to offensive components, the fighting behavior may then result in a change in strategy on the part of the cat attempting to defend itself by exhibiting responses such as scratching and biting of the opponent, which are viewed as offensive. Other elements of offensive behavior that may be present include stretching or extension of the limbs, a hooked-shaped bending of the tail that may change in position to appear erect. Thus, in defensive rage behavior, while the overriding behavior is predominately defensive in nature, this response pattern may be superimposed by components of offense as described above.

Neural Substrates of Defensive Rage

Historical Basis

The first recorded studies of defensive aggression in animals made use of cortical ablations in cats and dogs and took place over the last decade of the nineteenth century and first two decades of the twentieth century. These studies typically demonstrated that such ablations were associated with the induction of marked vocalization, and other indices of rage behavior in response to innocuous tactile stimulation.

While these studies suggested forebrain involvement in aggressive behavior, they provide little information with regard to localization of function. In the 1930s and 1940s, several other approaches were employed to obtain a better estimate of the regions of the brain associated with rage behavior. Three general regions of the brain were the general focus of study. These included the hypothalamus, midbrain PAG matter, and limbic structures.

Hypothalamus

In one approach, decorticate preparations in cats in which the thalami were destroyed but the hypothalamus

remained intact were nevertheless capable of still displaying rage reactions, which suggested the importance of the hypothalamus in the rage process. Other studies attempted to more precisely specify the regions of the hypothalamus involved in rage behavior. Selective lesions of the posterior hypothalamus in the cat or the monkey were shown to reduce activity and eliminate signs of aggression, especially when provoked by tactile stimulation, which appears to underscore the relative importance of the posterior aspect of hypothalamus. Consistent findings were observed following selective lesions in the human where lesions of the posterior hypothalamus had a calming effect upon patients subjected to this procedure. In contrast, selective lesions of the medial preoptico-hypothalamus were shown to block aggressive reactions in mice. These findings may suggest that different parts of the hypothalamus may be species specific. Not all reports of lesions placed in the hypothalamus were reported to block aggressive behavior. In fact, several investigators reported that lesions placed in the ventromedial nucleus of the cat induced rage behavior. Such an effect is similar to reports in the human literature where tumors of the ventromedial hypothalamus were associated with the presence of rage behavior. It is possible that the type of lesions placed in the ventromedial hypothalamus, similar to the presence of the tumors, may have generated an irritative focus and thus served to stimulate the neighboring regions (see discussion of the effects of electrical stimulation below). Alternatively, such lesions may have resulted in the development of a supersensitivity of the descending neurons from hypothalamus that remained intact, which mediated this form of aggression.

Midbrain PAG

The role of the PAG was initially identified by reports that lesions of this region of the midbrain could successfully block the basic features of the rage response, including the vocalization component. Such observations were essential in identifying the PAG as a critical link in the downstream circuit from the hypothalamus for the expression of defensive rage that is described in detail below.

Limbic structures

The limbic system consists of the hippocampal formation, amygdala, septal area, prefrontal cortex, and cingulate gyrus. An extensive literature has emerged from studies of all of these regions, the most notable ones involving lesion and ablation studies of the amygdala, septal area, and prefrontal cortex in various species of animals. In addition, considerable information with regard to the role of limbic structures has been gained through clinical

studies involving the behavioral effects of tumors and seizure activity involving these structures.

One of the earliest and well-known studies involving lesions of the amygdala and adjoining regions of piriform cortex of monkeys described the resulting behavior later known as the Kluver–Bucy syndrome. It is characterized by abnormal docility, hypersexuality, and marked oral tendencies. Other studies involving perhaps regions of amygdala not involved in the induction of the Kluver–Bucy syndrome have produced dramatically different results in which the animals have become hyperaggressive. Later studies involving electrical-stimulation procedures provided new information which clarified the conflict in the literature with regard to the effects of amygdaloid lesions upon rage behavior. In brief, activation of the medial amygdaloid nucleus and adjoining regions potentiates defensive rage behavior, while activation of more lateral regions of amygdala, such as the lateral, central, and lateral aspects of the basal complex, suppresses this form of aggression.

Concerning other areas of the limbic system, studies involving the septal area conducted in rodents have revealed a marked increase in aggressive behavior following large lesions of this region. The intensity of the aggressive reactions in these animals became so intense that they became extremely difficult to handle. The phenomenon was frequently referred to as ‘septal rage.’ With respect to the prefrontal cortex, the first recorded evidence that this region regulated emotional behavior involved the case of Phineas Gage, a railroad worker in Vermont during the early part of the nineteenth century. He was struck by an iron bar that penetrated his brain and damaged parts of his frontal lobe, effectively destroying the connections between the prefrontal cortex and other cortical and subcortical structures. Afterwards, he displayed marked personality changes, such as childlike behavior and the passions of a strong man. Small lesions placed in the frontal lobe of monkeys were reported to result in a more placid animal, while lesions placed in the prefrontal region of rodents resulted in heightened aggressive behavior. Other studies involving electrical stimulation of the prefrontal region of the cat induced marked suppression of aggression and rage behavior.

Neural Circuits Underlying Defensive Rage Behavior

Definition and Properties of Stimulation-Induced Defensive Rage Behavior

Prior to an analysis of the neural circuitry governing defensive rage behavior, it is useful to first briefly describe and characterize defensive rage as induced by electrical brain stimulation in the cat. In this species, brain-

stimulation-induced defensive rage behavior (or affective defense) was originally defined operationally primarily in terms of the defensive elements inherent in defensive behavior by earlier investigators. These properties of the behavioral response are highly similar if not identical to the characteristics of defensive rage which were described earlier in this article with respect to feline defensive behavior occurring in nature. They include flattening of the ears, lowering of the body, drawing in of the head, piloerection, hissing, and pupillary dilatation. With the exception of paw striking, which is present following electrical stimulation, other elements of offensive behavior seen in nature are clearly absent in this model. Thus, defensive rage behavior, as applied in the present context as well as by investigators over the past five decades, reflects a behavioral response that is limited primarily to the defensive components of the overall behavioral repertoire. In spite of the absence of the offensive components, this model has been utilized effectively to provide an understanding of the neural mechanisms governing defensive rage which is the principal focus of this article.

In attempting to understand the nature of the neuroanatomical circuits governing defensive aggression, it is useful to distinguish between two classes of neural structures. The first class refers to structures associated with the elicitation of defensive rage and includes, principally, the medial hypothalamus and midbrain PAG. The second class refers to structures from which defensive rage is not elicited; however, these structures produce powerful modulation (i.e., suppression or potentiation) of this form of aggression, and these regions include the limbic system.

Hypothalamus

Efferent connections

Defensive rage behavior is elicited by electrical stimulation along the rostrocaudal extent of the medial preoptico-hypothalamus. The regional sites within the medial hypothalamus which give rise to the long-descending pathways that project to the brainstem are localized within the anterior third of the medial hypothalamus. Other aspects of the medial hypothalamus such as the region of the ventromedial nucleus associated with defensive rage behavior do not project directly to the brainstem but, instead, project rostrally to the anterior hypothalamus. Thus, neurons within the ventromedial nucleus associated with defensive rage behavior can affect other neurons in the brainstem related to defensive rage through a disynaptic pathway, whose initial synapse lies in the anterior medial hypothalamus. The transmitter released onto anterior medial hypothalamic neurons from the ventromedial nucleus is not known but, since the pathway is excitatory, it is likely that glutamate is a good candidate. The primary target of neurons in the

anterior medial hypothalamus that mediates defensive rage behavior is the dorsal aspect of the midbrain PAG. The neurotransmitter released onto dorsal PAG neurons from the anterior medial hypothalamus mediating defensive rage has been shown to be glutamate which acts through *N*-methyl-D-aspartic acid (NMDA) receptors.

Other projections from the medial hypothalamus project back to limbic structures, such as the amygdala, hippocampal formation, septal area, and even prefrontal cortex, and serve as feedback pathways to regions that significantly modulate hypothalamic processes. An additional important connection links the medial and lateral hypothalamus. Both regions are reciprocally connected. In contrast to the defensive rage response elicited from the medial hypothalamus, the lateral hypothalamus is associated with the expression of predatory attack behavior – a response virtually identical to the predatory (hunting) response seen in nature by felines and related species. Both of these neurons are inhibitory and each of the functions is mediated by γ -aminobutyric acid (GABA) acting through GABA_A receptors. One possible and perhaps likely function of this reciprocal connection is suggested here: namely, when one of these responses such as defensive rage is active, it is essential that other (competing) responses such as predatory attack be suppressed in order for the defensive rage response to be most effective. Therefore, the GABAergic projection from the medial to lateral hypothalamus serves this function.

Afferent connections

The primary inputs to the medial hypothalamus originate from the limbic system. Limbic structures possess the unique properties of receiving inputs from secondary or tertiary sensory sources, inputs from reticular formation monoamine neurons, and, as noted above, they project directly or indirectly to the medial hypothalamus. In this manner, activation of any one or more of these limbic structures by their afferent sources could provide output signals that could powerfully modulate processes associated with the medial hypothalamus, which includes defensive rage behavior. Two of the limbic structures whose effects upon defensive rage behavior have been systematically examined include the amygdala and septal area. Analysis of these regions is considered below along with a brief discussion of the anatomical substrates and role of the prefrontal cortex.

Amygdala. The amygdala – situated in the rostral aspect of the temporal lobe – is composed of a complex of nuclei. Several different projections arise from amygdaloid nuclei that project to the hypothalamus or PAG and provide the substrates for modulation of defensive rage.

One pathway – the stria terminalis – arises principally from the medial nucleus of the amygdala and adjoining regions of the basomedial nucleus. The stria terminalis

projects extensively to wide regions of the anterior-half of the medial hypothalamus, including the ventromedial nucleus. This pathway has a potent excitatory effect upon the medial hypothalamus, in particular, with respect to defensive rage. The potency of this pathway is reflected by the observation that electrical stimulation applied directly to this pathway is sufficient to induce defensive rage behavior. This response is presumed to be the result of massive excitation of the ventromedial hypothalamus by the stria terminalis not unlike the effects seen following direct electrical stimulation of this region of hypothalamus. The facilitating effects upon defensive rage behavior are initiated from substance P neurons of the medial amygdala and are mediated through neurokinin-1 (NK₁) receptors in the medial hypothalamus.

A second pathway arises from the central nucleus of amygdala and adjoining portions of the lateral nucleus and principally supplies the dorsal aspect of the midbrain PAG. These neurons are enkephalinergic, act through opioid μ -receptors in the PAG and powerfully suppress defensive rage behavior. In summary, the opposing effects of lesions and ablations of the amygdala, described above, can be understood in terms of the differential modulatory properties of the amygdala upon defensive rage behavior. Two mechanisms are involved, one facilitatory and the other inhibitory. The effects are mediated from two different regions of amygdala; they utilize two different pathways and the neurotransmitters are clearly different and opposite in function.

Septal area and prefrontal cortex. Parts of the medial septal nucleus and adjoining aspects of the lateral septal nucleus project directly to the medial hypothalamus. This pathway also mediates the potentiating effects upon defensive rage behavior of the medial aspect of the septal area. The far lateral aspect of the septal area appears to suppress defensive rage behavior, and the pathway mediating such effects appears to be disynaptic. The first limb of this pathway projects to the lateral hypothalamus and facilitates predatory attack, which is elicited from this region and is thus presumed to be excitatory to neurons in this region. The second limb represents a short, GABAergic (inhibitory) neuron projecting from the lateral to medial hypothalamus, thereby providing the functional anatomical basis for suppression of defensive rage which is elicited from the medial hypothalamus. The neurotransmitter(s) associated with the pathways arising from the septal area which mediate these effects remain unknown.

The prefrontal cortex produces potent suppression of several different forms of aggressive behavior, which include both predation and defensive rage. There are several possible routes by which such suppression may be manifest. One involves a multisynaptic pathway from the prefrontal cortex whose initial link targets the mediodorsal thalamic nucleus; the additional connections involve several short interneurons projecting from the

mediodorsal thalamic nucleus rostrally to the anterior hypothalamus. A second projection involves a monosynaptic pathway from the prefrontal cortex to the hypothalamus involving smaller quantities of axons arising from the prefrontal cortex. A third pathway is also a monosynaptic projection to the dorsal aspect of the PAG typically associated with the expression of defensive rage behavior. In this manner, activation of these routes to the hypothalamus and PAG endows the prefrontal cortex with significant capacity to modulate aggression and rage behavior. Since all major outputs of the cerebral cortex are believed to utilize glutamate as their neurotransmitter, it is presumed that the outputs of the prefrontal cortex likewise utilize this neurotransmitter, which is excitatory upon its target neurons. The neurotransmitters associated with interneurons passing from the mediodorsal thalamic nucleus remain unknown.

Midbrain PAG

Efferent connections

The dorsal PAG constitutes the most caudal region of the neuraxis of the brain where integration of the defensive rage response occurs. The integrated response involves at least two components: somatomotor and autonomic. The somatomotor components reflect descending pathways from the PAG directly to motor nuclei of cranial nerves V and VII, which govern jaw opening and the vocalization component of the defensive rage response. Other downstream projections reach, directly or indirectly, the spinal cord motor nuclei that control movements of the upper limbs essential for striking at a threatening object in the environment. The autonomic component is subserved through descending fibers that supply either, directly, the preganglionic sympathetic neurons situated in the intermediolateral cell column of the thoracic and lumbar spinal cord or, indirectly, through synapses in the solitary nucleus or ventrolateral medulla. In either manner, activation of the dorsal PAG will result in significant increases in blood pressure, heart rate, and pupillary dilatation which are principal autonomic characteristics of this behavior. The outputs from the PAG for the expression of defensive rage are arranged in neuronal pools, especially within the caudal two-thirds of the neuropil, which are neuroanatomically distinct from other pools of neurons in more ventrolateral and caudal levels of the PAG which are associated, instead, with withdrawal or avoidance of a threatening stimulus.

Afferent connections

At least three different sources of input to the PAG relate to its activation pattern for the expression of defensive rage behavior. A primary source, which was described above, is the anterior medial hypothalamus. It projects in dense quantities mainly to the dorsal and lateral aspects

of the PAG. The largest quantities of fibers terminate in the rostral half of the PAG, while some extend further caudally within the neuropil of the PAG. In this context, the PAG may be viewed as a relay nucleus of the descending pathway of the medial hypothalamus for the expression of defensive rage behavior. Consistent with this view is that, as indicated previously, the excitatory effects of descending fibers from the medial hypothalamus upon the PAG are mediated through NMDA receptors and the transmitter is likely glutamate. The arrangement of this circuit would suggest that the initial integration for the expression of defensive rage takes place in the medial hypothalamus rather than the PAG, where afferent sources of the medial hypothalamus, and, in particular, limbic structures, provide the essential inputs for induction of defensive rage behavior, whose expression is then mediated through the PAG.

A second afferent source includes limbic inputs. These include fibers projecting from the central and lateral nuclei of amygdala as well as from the prefrontal cortex. These afferent sources serve as powerful modulators of defensive rage at the level of the PAG. As noted above, a major enkephalinergic input to the PAG is provided from these nuclei of amygdala and provide significant suppression of defensive rage behavior. While the functions of the prefrontal cortex upon the PAG with respect to defensive rage have not been clearly elucidated, it is likely that this pathway is also inhibitory because the prefrontal cortex, itself, suppresses defensive rage behavior. Thus, the contributions of the limbic system in modulating defensive rage behavior are manifest both at the levels of the hypothalamus as well as the PAG. The principal connections involving the hypothalamus, PAG, and limbic structures are depicted in **Figure 1**.

The third significant afferent source includes fibers such as those contained in the spinothalamic tracts that mediate both tactile and noxious (pain) sensory information. It is well known that collaterals of these tracts supply the PAG. These stimuli may activate neurons in the PAG subserving defensive rage and, accordingly, thus serve to evoke defensive patterns. Such a phenomenon would be particularly true with respect to noxious stimulation, which, when applied to a cat, will evoke a marked defensive reaction that typically includes hissing.

Neurochemical Mechanisms within the Hypothalamus and PAG

Neurotransmitters, Neuromodulators, and Receptors

Both excitatory and inhibitory neurotransmitters in the hypothalamus and PAG play important roles in the regulation of defensive rage behavior. Some of these neurotransmitters have been identified with respect to

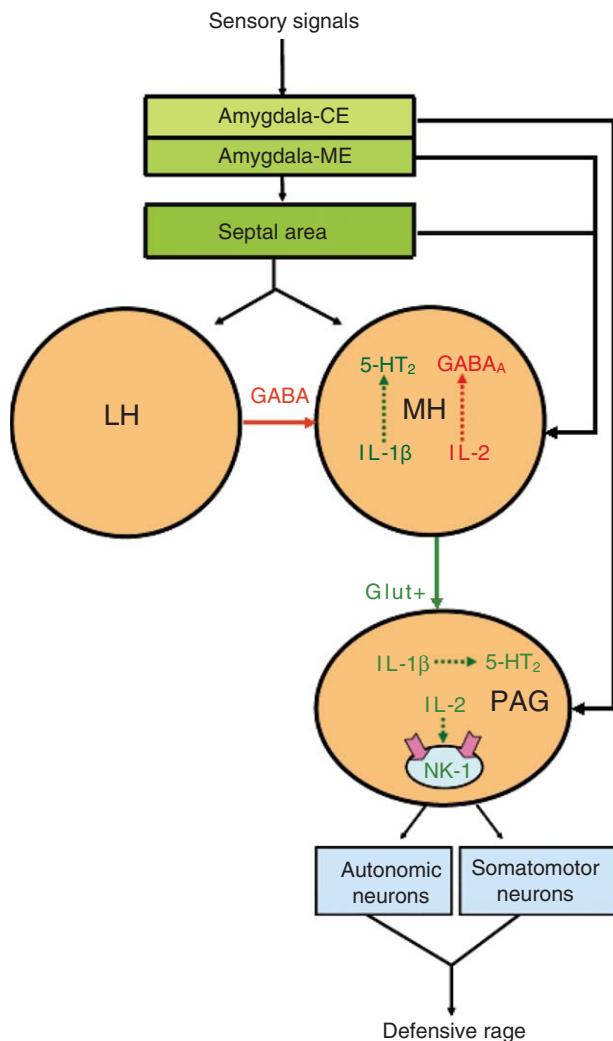


Figure 1 Schematic diagram depicting critical neural relationships and mechanisms involving several limbic structures, the hypothalamus, and the midbrain periaqueductal gray (PAG). Changes in levels of excitability within the septal area and amygdala affect excitability levels and functions of the medial hypothalamus and PAG, which include defensive rage behavior. The expression of defensive rage behavior is mediated through neurons in the medial hypothalamus and a descending glutamatergic pathway to the midbrain PAG. The PAG represents the lowest region of the neuraxis of the central nervous system at which integration of the autonomic and somatomotor components of defensive rage behavior occurs. GABAergic neurons from the lateral to medial hypothalamus provide a major source of inhibition upon defensive rage from a neighboring region of hypothalamus. Also shown in this diagram is the role of several proinflammatory cytokines, IL-1 and IL-2. IL-1 in the medial hypothalamus and PAG facilitate defensive rage and act through 5-HT₂ receptors in both regions. IL-2 in the medial hypothalamus suppresses defensive rage and its functions are mediated through GABA_A receptors. In contrast, IL-2 in the PAG facilitates defensive rage behavior by acting through substance P-NK₁ receptors in the PAG. 5-HT = serotonin; CE = central nucleus of amygdala; GABA = γ -aminobutyric acid; IL = interleukin; LH = lateral hypothalamus; ME = medial amygdala; MH = medial hypothalamus; NK₁ = neurokinin-1.

specific neuronal pathways while others have not. The discussion below (and **Table 1**) summarizes the modulating effects of these neurotransmitters and their receptors upon defensive rage behavior.

Excitatory neurotransmitters and their receptors

Neurotransmitters that have been shown to facilitate defensive rage behavior include acetylcholine, dopamine, norepinephrine, serotonin (acting through 5-HT₂) receptors, glutamate, and the neuropeptides, vasopressin, cholecystokinin, and substance P.

Cholinergic agents, administered systemically, facilitate aggressive responses acting through muscarinic receptors. Moreover, when microinjected directly into the medial hypothalamus, cholinergic agents can induce rage behavior. Norepinephrine and dopamine have similar effects upon defensive rage. The cholinergic pathways over which facilitation is mediated have not been identified. It is quite possible that the pathways involved include local interneurons within the hypothalamus.

Noradrenergic neurons, situated mainly in the locus ceruleus, project widely to the forebrain, including the

Table 1 Effects of neurotransmitters, receptors and cytokines upon defensive rage

<i>Compound</i>	<i>Receptor + anatomical locus</i>	<i>Effect</i>
<i>Monoamines</i>		
Dopamine	D-2, medial hypothalamus	↑
Norepinephrine	Alpha-2, medial hypothalamus	↑
Serotonin	5-HT _{1A} , medial hypothalamus	↓
Serotonin	5-HT ₂ , medial hypothalamus	↑
<i>Amino acids</i>		
Glutamate	NMDA, dorsal PAG	↑
GABA	GABA _A , medial hypothalamus, PAG	↓
<i>Peptides and other classes</i>		
Substance P	NK-1, medial hypothalamus, PAG	↑
Cholecystokinin	CCK-B, PAG	↑
Enkephalin	Opioid- μ , dorsal PAG	↓
Vasopressin	Vasopressin-1A, medial hypothalamus	↑
Acetylcholine	Muscarinic, medial hypothalamus	↑
<i>Pro-inflammatory cytokines</i>		
Interleukin 1	IL-1 + 5-HT ₂ , medial hypothalamus, dorsal PAG	↑
Interleukin 2	IL-2 + GABA _A , medial hypothalamus	↓
Interleukin 2	IL-2 + NK-1, dorsal PAG	↑

Key: ↑, facilitation; ↓, suppression.

hypothalamus. Norepinephrine, acting through α_2 -receptors in the anterior medial hypothalamus, potentiates defensive rage behavior. Likewise, similar functions are mediated by dopamine, arising from the ventral tegmental area, and acting through dopamine D₂ receptors in the same region of the anterior medial hypothalamus. Thus, the catecholaminergic pathways supplying the medial hypothalamus serve a general activating or potentiating function with respect to aggressive behavior. The effects of serotonin differ from catecholamines in that their functions differ in opposite ways, depending upon the receptors through which they act. Serotonin neurons arise from raphe neurons, mainly of the pons and midbrain, and project widely throughout the brain, including the hypothalamus and midbrain PAG. Serotonin, acting through 5-HT₂ receptors in both the hypothalamus and PAG, has been shown to facilitate defensive rage behavior.

Several excitatory neurotransmitters acting at the level of the anterior medial hypothalamus include substance P, which arises from the medial amygdala, (as discussed earlier in this article) and vasopressin, acting through V₁

receptors. While the pathway mediating the excitatory effects of substance P reaches the medial hypothalamus through the stria terminalis, the pathway mediating vasopressin remains unknown.

As noted earlier in this article, the principal descending pathway mediating defensive rage behavior from the hypothalamus arises from the anteriomedial hypothalamus and targets the dorsal PAG. The neurotransmitter released from these neurons is glutamate and their excitatory functions of this pathway are mediated through NMDA receptors in the dorsal PAG. Several peptides, which could either function as neurotransmitters or neuromodulators, also facilitate defensive rage behavior within the dorsal PAG. These include substance P, and cholecystokinin (acting through CCK_B receptors). The pathways associated with these peptides have not been identified.

Inhibitory neurotransmitters and receptors

There are at least three different neurotransmitters that suppress defensive rage behavior. Serotonin, acting through 5-HT_{1A} receptors both in the medial hypothalamus and dorsal PAG, produces powerful suppression of defensive rage behavior. Likewise, enkephalin, whose origin is the central and lateral amygdaloid nuclei, project directly to the dorsal PAG and its powerful suppressive effects are mediated through μ -opioid receptors in the PAG. GABA neurons are found throughout much of the central nervous system (CNS) and, within both the medial hypothalamus and PAG, act through GABA_A postsynaptic receptors to suppress defensive rage behavior. While the GABAergic pathway within the hypothalamus has been identified as a short neuron projecting from the lateral to medial hypothalamus, the origin of the GABAergic pathway within the PAG remains unknown. However, it is suggested that GABA is mediated through a local interneuron within the PAG.

Role of Cytokines

Cytokines may be defined as pleiotropic cell-to-cell-signaling proteins or glycoproteins that are produced by different cell types in the periphery and the brain. While cytokines have typically been studied for the effects on immunity, their relationship to other processes such as development, feeding, and sleep has been described. Cytokines exert their effects by binding to receptors, which may then be followed by the activation of signaling pathways. Cytokines can affect functions of the brain by several routes. Cytokines formed in the periphery may enter the brain through several routes. One involves the blood-brain barrier; and a second may involve entrance to the brain through circumventricular organs or sensory afferents. Cytokine release can also be stimulated by brain endothelial cells and produced by glial and neuronal cells. The discussion below describes

the results of recent studies indicating how cytokines within regions associated with the expression of defensive rage behavior can act as neuromodulators to regulate this form of aggressive behavior.

Evidence of a possible relationship between changes in cytokine levels and aggression has been suggested from studies conducted in the human. A population of normal, healthy adults who were subjected to an enhancement of proinflammatory cytokines displayed elevated hostility scores and, consistent with these findings, a positive correlation was observed between the presence of hostile marital conflict and increased production of plasma proinflammatory cytokines. Moreover, patients with hepatitis C who have been treated with cytokine immunotherapy displayed higher than normal levels of aggression as determined by measures of hostility, anger, and hostility.

Recently, a series of studies have revealed that cytokine-receptor activation within the medial hypothalamus or PAG can powerfully modulate defensive rage behavior in the cat. Activation of interleukin (IL)-1 β receptors in either the medial hypothalamus or dorsal PAG at sites from which defensive rage could be elicited potentiates defensive rage behavior. Further studies revealed that the potentiating effects of IL-1 β are mediated through 5-HT₂ receptors. In contrast, activation of IL-2 receptors in the medial hypothalamus suppresses defensive rage behavior. However, activation of the IL-2 receptors in the dorsal PAG, instead, facilitates defensive rage behavior. The suppressive effects of IL-2 are mediated through GABA_A postsynaptic receptors in the medial hypothalamus, while the facilitating effects of IL-2 in the PAG are mediated through substance P-NK₁ receptors. It is suggested that the difference in effects seen with IL-2 in these two regions can be accounted for in the following manner: in the medial hypothalamus, the epsilon (ϵ) subunit of the GABA_A receptor is absent, which is not the case with respect to the GABA_A receptor in the PAG. In order for GABA to be effective, the ϵ subunit of the GABA_A receptor must be lacking. These findings indicate the following: (1) that activation of cytokine receptors within the CNS significantly modulates defensive rage behavior; (2) that the modulating effects of cytokines are mediated through classical neurotransmitter systems; and (3) the effects of cytokines may possess regional specificity. The effects of cytokines upon

defensive rage within the hypothalamus and PAG are shown in **Figure 1**.

See also: Antisocial Substance Dependence; Communication of Emotions in Animals; Emotions; Fear, Anxiety, and Defensive Behaviors in Animals; Human Fear and Anxiety; Intermittent Explosive Disorder; Motivation; Mouse Genetic Approaches to Psychiatric Disorders; Neural and Pharmacological Substrates of Aggression; Neurobiology of Offensive Aggression; Neural Substrates of Conditioned Fear and Anxiety; Neural Substrates of Unconditioned Fear, Defense, and Anxiety; Neural Systems of Motivation; Offensive and Defensive Aggression; Psychoneuroendocrinology of Stress.

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Physical Cognition and Reasoning

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Glossary

Associative learning – A form of learning where an individual learns by temporal or spatial contiguity that two events are related. The association may be further reinforced and enhances future learning experiences by generalization to categories of events that share relevant characteristics with the initial one.

Causal agent – Any entity that produces an effect or is responsible for events or results.

Causal knowledge – A weak causal knowledge refers to a knowledge acquired from associative learning after an individual has been repeatedly exposed to a spatial or temporal contiguity between two events. A strong causal knowledge refers to an immediate or rapid interpretation of how events may relate to each other.

Causal reasoning – Reasoning is the cognitive process by which an individual draws conclusions, makes decisions, or forms judgments based on experiences, events, and observations. When strong causal knowledge emerges from understanding and allows flexible responses to novel contingencies, it becomes causal reasoning.

Inferential reasoning – The process of deriving the logical consequences of assumed premises or the process of arriving at some conclusion that – though it is not logically derivable from the assumed premises – possesses some degree of probability relative to premises.

challenging. When an animal solves a problem, the question is to know whether the animal's response is the outcome of a blind associative learning process, or if the animal recognized certain properties of the relationships between a stimulus and an event, and consequently formulated abstract categories and representations from these associations. In the first case, the animal relies on repeated associations between two events to direct its behavior, which may lead to weak causal knowledge. In the second case, the animal understands how events are related to each other, builds mental representations, and therefore may reason about elements and make inferences on the forces involved. This would be considered as a stronger causal understanding and does not need repeated exposures to the association.

To detect whether animals can reason beyond associative learning, several approaches have been proposed: animals may infer – from observable or unobservable elements – the solution of a problem, they may rely on reasoning to foresee what is to come and prepare their action accordingly, they may build their own hypotheses on what physical forces caused an effect, and they may reason about other individuals from their social world.

Inferential Reasoning

Transitive Inference

Many species are known to be able to match a stimulus with a given reaction. Temporal or spatial proximity between events act as powerful re-enforcers in associative learning. There are reports to support the view that most animals may only solve a task through associative learning and fail to show strong causal understanding of their environment. An approach to explore the reasoning skills of animals has consisted of testing responses of animals during 'transitive inference tasks.' In these tasks, animals first learn the association between a pair of elements. They are then asked to reason about this knowledge and to infer the relative position of pairs compared to each other. In logic, if A is superior to B and B is superior to C, then A is superior to C. Most species – rats, pigeons, crows, New and Old World monkeys, and great apes – have been shown to succeed in transitive inference tasks. In a recent review, a meta-analysis was conducted on responses of animals in inference tasks and different models were created to understand performances of different species. The most robust models – accounting for the

Introduction

In the eighteenth century, the Scottish philosopher Hume concluded that it was vain to search for proof of causality in the universe. Reflecting on causal knowledge, Hume suggested that we can only perceive conjunctions of events. Whatever comprehension we may have of the mechanisms that govern our life, we will never know for certain that our understanding is nothing more than assumptions. Despite this, human beings seek observable or nonobservable explanations for effects, even though they may never prove causal relationships. In nonhuman animals – where the lack of spoken language prevents direct exploring of causal reasoning – assessing what animals understand from their environment remains

performances of most species – were not the cognitive models (inferences via logical reasoning) but were instead reinforcement-based models where associative-learning rules were implemented. It would seem that – by weighing the relative strengths of the learned association against each other – animals could solve transitive inference tasks without calling upon stronger causal knowledge.

Inference by Exclusion

The capacity of animals to infer one element from the presence of another has limits since it may be strongly biased by associative learning. Inferring one solution by using the absence of an element is a more cognitively demanding task, as it requires reasoning about a missing stimulus or event. Vervet monkeys do not infer from a carcass in a tree that a leopard may be close by, and traces left by a snake do not elicit fear responses such as alarm calls in them. One could argue that animals failed to link an observed consequence (the trace) to its causal agent (the snake), but another interpretation would be to consider predatory traces as a poor signal for predatory activity: such traces may be abundant in the wild, preventing potential preys from building strong, accurate, temporal, or even spatial associations between these traces and predators. To explore the extent to which animals might reason about invisible or missing elements, researchers have used approaches based on ‘inference by exclusion’ tasks. In these tasks, animals are given only partial knowledge of where a food reward may be hidden. It is the lack of expected information that cues the animals into choosing an alternative correct option. One version of the task is to detect whether subjects can retrieve the location of a hidden food among two by using the absence of visual cues. Between two boxes A and B, an experimenter displays the content of box A, – the empty one. To solve the task, the subject needs to infer that if A is empty, then B is the box that contains the reward. Another variant is to infer from the lack of auditory cues the position of the reward (**Figure 1(a)**). An experimenter shakes the empty box B, which produces no sound. Subjects need to infer from the lack of sound in box B, that box A contains the reward. These investigations have shown that some animals were inferring the location of the food by exclusion: if not B then A – a process that cannot be solved by associative learning is set up where animals are exposed to a limited number of trials. This line of research has been initially investigated in great apes. Most species of great apes successfully solve these tasks, thereby reasoning about missing information. Other primate species like brown capuchin monkeys show similar responses as well as dogs and to some extent pigeons. This may not be so surprising since some researchers have suggested that animals need to mentally represent food-items hidden inside shells, tree trunks, or piles of leaves,

as they search for them. In fact, this was specifically demonstrated in an experiment with capuchin monkeys, where the monkeys were able to discriminate between an empty nut and a full one based on their weights and the sound produced when shaken. These types of experiments support the idea that animals, in general, can reason about something they do not visually or acoustically perceive.

Inference from the Integration of Multiple Factors

Inferring a correct response by reasoning upon unobserved cues is a capacity that may prove valuable in a species whose access to resources depends on conditions that render the distribution of feeding resources difficult to predict. Foraging behavior has long been assumed to be a random process – based mainly on the shortest route between trees known to potentially bear fruits. Recent studies suggest far more elaborate foraging strategies, for example, primates memorize a network of routes within their home range using landmarks and possibly mental maps of their environment. They are able to modify their foraging route when it becomes inaccessible and use shortcuts. They remember trees they exploited during previous seasons and are capable of generalizing the search to other trees of the same species, which are sometimes not visible from the initial discovery position. In a typical foraging activity, they may have to decide between traveling to a nearby feeding site that may be reduced in size, or, traveling to a distant one that may provide a larger amount of resources. Several decision-making processes may be at stake when foraging and primates might choose their next course of action by reasoning about multiple elements that are not directly visible. When fruits reach maturity, they provide abundant supply but for a limited amount of time. The maturity of fruits may be ruled by seasonal variations but also by less predictable factors, such as local weather conditions. Making accurate predictions about when food will be ready to eat is therefore challenging. Mangabeys from the Kibale forest (Uganda) have been shown to choose the trees they would exploit before even seeing or smelling them. They anticipated the maturational state of the fruits on the trees they visited – a process that could be based on their experience from earlier visits. In addition, they visited and returned more frequently to the trees they knew to be matured, when the sun had been shining (favoring additional maturity of fruits) compared to the days where weather had been fresh or covered. A complete understanding on how weather affects fruits’ maturity is probably not what drove the mangabeys toward the most interesting trees. They may have learned instead that warm-weather periods are associated with faster renewing of food at the feeding sites. In this regard

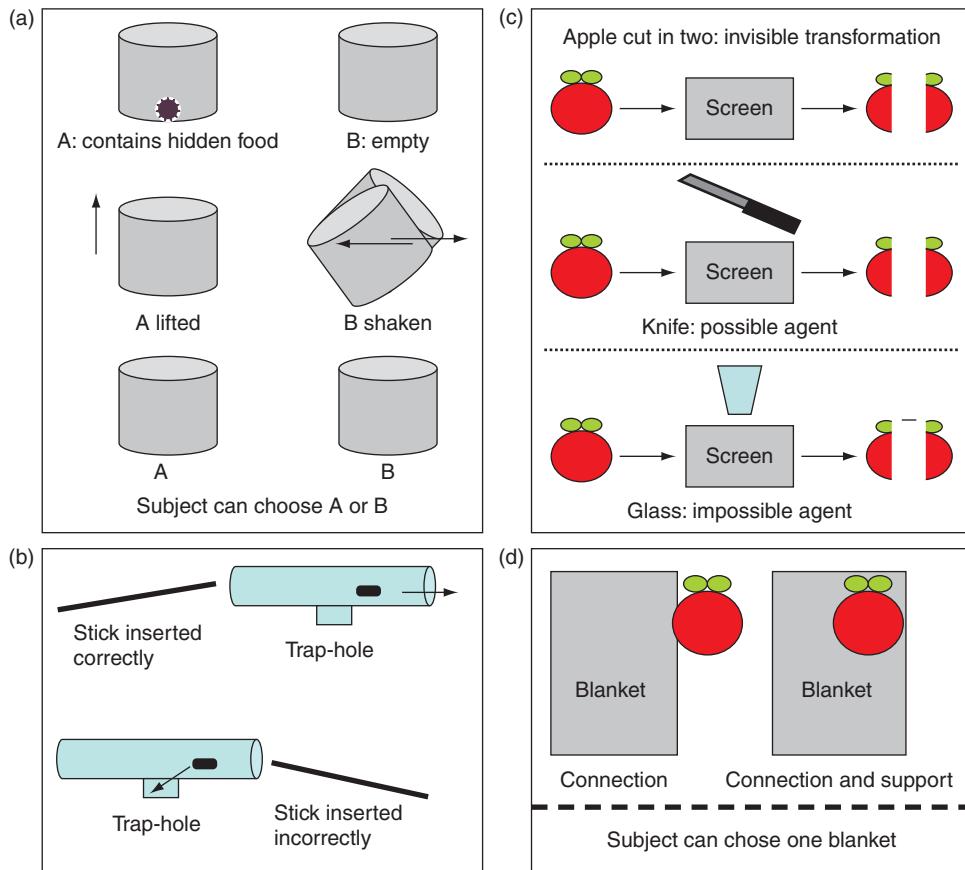


Figure 1 (a) Inference by exclusion. First, the experimenter hides a reward in box A, out of sight. In front of the subject, the experimenter shakes box B (no noise) and lifts box A without shaking it (no noise). The subject can then choose either box A or B. To succeed, the subject needs to infer that the reward is in box A, since no noise was produced when box B was shaken. (b) Trap-tube task. A reward is available if pushed out of the tube with a stick (correct action). However, it can fall into the trap-hole if pushed from the wrong side (incorrect action). Subjects have to foresee the result of the action of the stick on the reward to obtain it. (c) Recognizing causal agents. The experimenter shows an apple before hiding it behind a screen. Then, two halves of an apple are revealed from behind the screen. Objects are then shown to subjects, also from behind the screen: a possible agent of the transformation (knife) or an impossible agent (glass of water). The looking duration toward both possible and impossible agents is recorded. (d) Understanding the role of unobservable forces: Subjects can choose to retrieve one blanket among two. In one case, an apple is left on the blanket and can be retrieved by pulling the blanket. In the other case, an apple is left in contact with the blanket but cannot be retrieved by pulling the blanket. Subjects fail to differentiate between both options and choose equally often the option where the blanket provides connection and support, and the option where it provides only connection.

reasoning is still necessary, since decision making was achieved by weighing different possible options and inferring the best-fruit location, a process that could only rely on mental representations encompassing flexible and complex properties of the environment.

Anticipating Results or Consequences

Reasoning in Relation to Time

Solving problems by relying on anticipatory skills is one of the most recently studied topics in animal reasoning. Individuals may need to reason about missing elements in relation to time – that is, when the solution to a problem is missing now but may be available later. In one study,

orangutans and bonobos were first trained to use a hook to reach for a bottle of juice placed out of their compartment. On following days, individuals were given temporary access to the hooks, but not to the bottles. They were then invited to leave the room and come back 1 h later on the same day. On their return, the bottles were available, but the hooks had all disappeared. To solve the task, individuals had to first collect the hooks before leaving the room, and bring them back later, once the bottles would be set up. In both species, some individuals, but not all, successfully solved that task. In addition, some succeeded with a time interval of one night between the time of collection and return in the bottle room. Experiments with orangutans and chimpanzees have shown similar results for temporal reasoning applying

tool-use tasks. Such planning capacities require reasoning on the mental representation of a need yet to come. Indeed, in most of those studies, researchers controlled the variables such that at the time where subjects had to collect the tools, nothing in their environment could remind them of what would come later on, preventing associative learning from driving the correct behavior. Reasoning in relation to time is also seen in birds. Under experimental conditions, scrub jays are able to adapt their current behavior to solve future tasks. In a particular study, birds flexibly solved planning tasks, relying on anticipatory skills as opposed to hardwired responses. Solving such a task seems possible only if animals use mental representations of what is about to happen; they have to adapt their behavior despite the fact that there is no immediate reason for it. Great apes along with some bird species (corvids) show some similarity with the human in their ability to project future events and organize their responses with regard to future needs.

Foreseeing the Course of Action in Tool Use

Other studies on causal reasoning have addressed animals' understanding more directly of how a present behavior may have future consequences on performances in a task. To do so, these studies have used tool-use-related tasks where animals need to foresee the result of their action on the tool to obtain a reward. One example is the trap-tube task (**Figure 1(b)**), where an animal needs to use a stick to push or pull a reward out of a tube. The tube has an inaccessible compartment, or 'trap,' in the middle of it into which food can fall. The experiment is designed in a way that if the subject does not foresee the correct extractive technique, the reward is lost as it falls into the inaccessible compartment (trap-hole) during the process. Subjects have to insert the stick in the correct side of the tube in order to successfully push the reward outside. Capuchin monkeys generally perform at chance level in this task. One individual was reported to further learn to insert the stick in the hole that was the farthest possible from the reward, suggesting associative learning on how to solve the task, rather than an understanding of causal properties of the relation among the stick, the reward, and the trap-hole. Chimpanzees tested in a similar task succeeded without using the associative rules observed in capuchins, and seemed to accurately anticipate the results of their action on the tool. This may also indicate that they understood better than other primates the role of the tool that could cause either a success or a failure in retrieving the reward. Tests using variants of the trap-tube task have been designed to allow testing of animals that do not usually use tools like rooks. A variation on the trap-tube task was developed with two traps-holes and an inner-pulling system that does not require the insertion of a stick. The task still requires the subjects to foresee the

course of their action and choose from what side to pull in order to retrieve the reward. In this condition, rooks could solve the problem, but no evidence was found that they did so by understanding the causal properties of the task like great apes did.

Understanding Causes

Recognizing Causal Agents

More work has been conducted to explore whether animals recognize the causal agent of a transformation – the tool. Understanding of the causal role of a tool was tested in rhesus macaques. The study consisted in showing an apple before hiding it behind a screen, out of view of the subject (**Figure 1(c)**). The screen was then removed revealing two half apples. The experimenter then took a knife from behind the screen and showed it to the subjects. This computed into a possible transformation – an apple might be divided into two parts under the action of a knife. In additional trials, the experimenter showed impossible transformations, for example, a glass of water was shown to the subject instead of a knife, or two half apples were first hidden behind a screen, and an entire apple was shown afterwards. A comparison between the duration of observation for possible or impossible transformation indicated that macaques looked longer at impossible transformations. The authors interpreted this response as an illustration of the conflict between what macaques expected and what they discovered. This study replicates comparable results previously obtained in experiments with chimpanzees. Rhesus macaques, unlike chimpanzees, are not known for their tool-use abilities; therefore, it is particularly interesting to find evidence for understanding tool-use tasks in this species. Macaques may have recognized the causal role between the knife and the apple by seeing keepers using them – a knowledge not acquired by experiencing the action of the knife themselves. For some researchers, understanding the source of a noise or that a knife is a causal agent reflects causal understanding. Other researchers contest that view. When rhesus macaques identify a knife as a possible causal agent, they would 'only' predict the result of the association (an apple cut in two); there is no proof that any conceptualization of the action itself occurred. For example, they may not understand that the knife works because of a sharpened edge, or because it is held and controlled by a human. The subject does not need to make assumptions about the forces at work to solve the problem.

Explaining and Making Hypothesis

The controversy remains with regard to the animals' capacity to explain how elements are related to each other or why. There is general agreement that animals

can identify the correct solution by reasoning on observable facts – whether visible or momentarily invisible. Animals seem capable of understanding that elements are related to each other; they may also understand that the relationship can have sequential properties – like the fact that one event always precedes another one, or that if a certain element is observed it must have been preceded by another. Therefore, they produce the correct conclusions and anticipate the course of an action accurately. However, it could be that animals cannot conceive the physical forces and theoretical entities which causal explanations rely on. Not all forces responsible for observational events are visible themselves. One of the limits animals encounter is in the understanding that unobservable forces (e.g., gravity) play a causal role. The previously described ‘inference by exclusion’ task is informative in this respect. In such a task the absence of sound when a box is shaken can be used to infer that the alternative box is baited. Different levels of understanding could underlie reasoning processes. First, the correct solution may be reached by reasoning on a well-known association: sound equals presence of food in a container. If there is no sound in box A, then there is no food in box A, thus choose box B. The subjects here infer from the absence of an otherwise ‘observable’ force: the sound. Second, based on their experience that noise always precedes food, animals could also wrongly consider the noise as a causal agent: noise makes food, if there is no noise, then there is no food, thus choose other box. This would be an incorrect assumption but it would still mean success in the response to the task. Third, animals could consider food as a causal agent for the noise and then infer: a food in a box makes noise when shaken, if the box is shaken and makes no noise, then, there is no food and the alternative box should be chosen. Lastly, animals could consider an unobservable force like solidity as the causal agent: both food and boxes are solid elements so that food makes a noise when it hits the inside of the box. If no noise is produced when the box is shaken, there is no food in that box so choose the alternative one. Whatever their level of understanding, apes found the correct box by reasoning about the clues given and made the correct inference. But whether they did so by using the most accurate explanation, the one calling upon solidity, is still open to debate.

For some researchers, proving causal understanding in animals would ultimately consist in showing that they understand that an intermediary nonobservable force mediates the result of an association. In the human, this is referred to as Folk Physics which is defined by Daniel Povinelli as “the kind of understanding of the physical world that develops naturally and spontaneously during the development of human infants and children and later permeates the adult conception of why the world works the way it does.” Researchers have studied the extent to which chimpanzees understood something about physical

laws such as gravity, solidity, or force transmission. For example, chimpanzees do not distinguish between physical contact and necessary connections to make different parts of a tool functional. If chimpanzees have to pull a blanket toward them to obtain an apple, they do not differentiate between the condition where the apple is indeed on the blanket (**Figure 1(d)**), and the condition where the apple is left in contact, but not directly on the blanket. The fact that animals can reason from missing elements does not necessarily prove that they may have a concept of the physical forces at stake. Conceiving an explanation requires more than simply representing what is missing. These studies emphasize the need to distinguish between what is not observed and what is not observable. As put by Daniel Povinelli, “Humans alone think about such things as God, ghosts, gravity and other minds. Animals do not form concepts that refer to merely hypothetical things.” This means also that despite a capacity to represent events from their observable properties and to extrapolate rules from these representations, animals cannot “posit unobservable properties or processes” as mediating variables to explain or to predict observable events or states. Such limitations would also apply toward the social worlds of animals. Animals seem to have representations about the observable properties of their social world, but may not be capable of reasoning about unobservable forces.

Conclusion

The question of how animals reason about their social world was first addressed in the late 1960s by scientists. When people observed that monkeys form coalitions by recruiting support against a third party, they assumed that animals understand something of the relationship between partners and the way they can be manipulated to produce a desired result. Reports about deception also suggested that animals may solve social problems using reasoning. For example, after a fight a chimpanzee would produce an appeasing gesture known to lead the former opponent to approach with peaceful intentions. The chimpanzee producing the gesture would then renew aggression once the former opponent had come close enough. Applying a friendly behavior in a competitive situation may have been a goal-oriented tactic. This would reflect an understanding of others’ mental states, knowledge, or belief. Nonetheless, this behavior might also arise from a single learning process, that is, each time I hold out my hand, my opponent comes closer, where I can attack again. Experimental studies show that most animals possess the skills needed to anticipate others’ behavior, but failing to solve tasks requiring them to attribute mental states to others. As stated by some researchers, cases supporting causal understanding must provide evidence that animals

used previous knowledge and acted in an apparently unrelated way to produce a desire outcome. In most studies assessing social understanding, however, experiments do not succeed into separating causal understanding and potential use of associative learning when solving a problem. When evidences of reasoning about the social world are found, they suggest that reasoning is based on what partners do – observable element – rather than on what partners think – unobservable element. Even if great apes may attribute intention to others, they may not have our ability to infer multiple psychological dimensions. Beliefs and desires play a causal role in behaviors. They are, to the same extent as physical forces, unobservable forces. If they cannot clearly distinguish between thinking, believing, or wanting, primates may not distinguish the causal succession of unobservable thoughts leading to a specific action.

See also: Social Cognition: From Behavior-Reading to Mind-Reading.

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Relevant Websites

- <http://www.subiaul.com> – Francys Subiaul's Lab.
<http://www.klf.ac.at> – Konrad Lorenz Research Station.
<http://www.psychol.cam.ac.uk/ccl> – University of Cambridge, Nicki Clayton Cognition Lab.

S

Schizophrenia

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Glossary

Environmental model – A simulation model in which one or more environmental challenges are given to the animal that play (or are hypothesized to play) a role in the human disease.

False negative – A drug which acts like the gold standard in the human condition, but not in the animal model.

False positive – A drug which acts like the gold standard in the animal model, but not in the human condition.

Genetic model – A simulation model in which one or more genes that play a role (or are hypothesized to play a role) in human disease are altered.

Gold standard – In animal models, a gold standard is the clinically successful drug with which all other compounds are compared.

Screening model – An animal model in which the efficacy of an unknown compound is compared to a clinically successful drug.

Simulation model – A model in which certain aspects of a human disease are modeled in an animal species.

in all domains of cognition, schizophrenic patients show more severe impairment in attention, verbal and visual learning, working memory, and executive functioning.

Although schizophrenia is usually diagnosed for the first time between the ages of 18 and 30, several signs and symptoms can already be seen at a much earlier age. Several prospective studies in different countries have shown that, as a group, children that later go on to develop schizophrenia are slower in reaching developmental milestones, such as sitting, walking, or talking for the first time. Together with many other pieces of evidence, this has led most researchers to conclude that schizophrenia has a developmental component.

The etiology of schizophrenia or what causes this disturbance in neurodevelopment is still largely unknown. Family, twin, and adoption studies have clearly shown that genetic factors play a major role in the development of schizophrenia, but concordance rates of about 50% in monozygotic twins is clear evidence that nongenetic factors are also involved. Moreover, although many genetic studies, including large-scale genome-wide scans, have been undertaken, no crucial gene has so far emerged. Although, theoretically, such a gene might still be identified in the future, it seems more likely that several genes contribute to the overall illness. In addition, schizophrenia is certainly a heterogeneous disorder with multiple causes. Nonetheless, some candidate genes have been identified in more than one study. These will be discussed later on in this article. In addition to identifying the gene(s) involved in the development of schizophrenia, much research is also devoted to identifying the nongenetic, environmental factors. This is even more difficult to assess, since it usually involves retrospective interviews, and environmental factors are usually less clearly circumscribed. However, although the nature of the environmental factors is less clear, studies have shown that there are at least two critical periods in which environmental factors may have a major influence on the development of schizophrenia, namely, the perinatal and

Schizophrenia is a severe mental disturbance which afflicts approximately 1% of the population. Patients typically develop schizophrenic symptoms around or after puberty and the symptoms are generally classified into three different categories. Positive symptoms refer to signs and symptoms that are not normally seen in healthy individuals but are produced as a result of the disease, and encompass hallucinations (most often auditory) and delusions (such as paranoid ideation or feelings of being controlled). Negative symptoms, on the other hand, are signs and symptoms that are normally present in healthy individuals but are absent or significantly reduced in patients and encompass social isolation, anhedonia, and apathy. Finally, patients with schizophrenia often show cognitive symptoms. Although they are generally impaired

the (pre)pubertal period. Major life events during these periods increase the likelihood of developing schizophrenia, especially in genetically high-risk individuals, underlining the now well-accepted idea that most major (psychiatric) disorders result from an interaction between genetic and environmental factors. This interaction will be discussed in more detail later on in this article.

Patients with schizophrenia are generally treated with antipsychotic drugs often in combination with certain forms of psychotherapy. Although psychotherapy by itself is usually not very effective, the combination of both forms of therapy has been proven to be more effective than either alone. Antipsychotic drugs belong to a large variety of chemical classes and are often subdivided into classical and atypical antipsychotic drugs. All antipsychotic drugs reduce the positive symptoms but most of them do not have a major influence on either the negative or the cognitive symptoms. Classical antipsychotic drugs, with prototypes such as haloperidol and chlorpromazine, induce major neurological (so-called extrapyramidal) side effects including parkinsonism, akathisia, dystonia, and (tardive) dyskinesia. Atypical antipsychotic drugs, including clozapine, olanzapine, and quetiapine, on the other hand, have been thought to induce much less extrapyramidal side effects. However, recent nationwide studies in the UK and the USA have questioned this. Indeed, a large study in the USA (>1400 patients) was unable to show a major therapeutic advantage of any of the tested atypical antipsychotic drugs over a classical antipsychotic. In this respect, it should be emphasized that clozapine was not included in this study. When the nonresponsive patients were subsequently treated with clozapine, a significant improvement was seen in a large proportion. It would be beyond the scope of this article to discuss the possible reasons for the lack of differentiation between classical and atypical antipsychotic drugs. However, it is a well-known fact that the incidence of side effects depends, to a large degree, on the dose used and there is evidence that previous studies used relatively high doses of, especially, the classical antipsychotic drugs.

Animal Models

Introduction

The development of an animal model for any disease usually starts with a detailed analysis of the symptomatology of the illness as well as an investigation into the pathology and/or etiology of the disease. Once the etiology or pathology of the disease is known, one tries to mimic this in animals and looks for similar symptoms. Such an approach has been quite successful for several neurological disorders such as Huntington's chorea and Parkinson's disease, but is much more difficult for

psychiatric disorders, including schizophrenia, for several reasons.

The symptoms of schizophrenia, with the exception of the cognitive deficits, can only be assessed through a psychiatric interview, making the modeling in animals impossible. In addition, none of the symptoms of schizophrenia is pathognomonic, and all of them can occur in other diseases as well. This implies that a good animal model for schizophrenia should always include more than one symptom, preferably as many as possible.

Another important hurdle in the development of animal models for schizophrenia is the lack of knowledge about the pathology and etiology. Although many studies have investigated the pathological changes seen in the brains of patients with schizophrenia, as with the genetic studies, no clear picture has so far emerged. This again may in part be due to the heterogeneous nature of the disorder, but also indicates that schizophrenia does not result from large structural deficits in one or a few neuronal structures. Indeed, most researches now consider schizophrenia as a disorder of connectivity, thus emphasizing a disturbance in communication between neuronal structures, rather than a localized deficit in one specific brain region. Although such deficits are more difficult to assess, they may have much more widespread functional consequences and might account for the plethora of signs and symptoms.

As with the pathology, little is known about the etiological factors, save that genetic and environmental factors interact, as discussed earlier. This implies that most of the animal models focusing on the etiology are based on a hypothesis rather than on facts. This does not necessarily mean that the animal models are not valid; however, it should be kept in mind when evaluating such models.

Given the relative paucity of hard findings about the etiology and pathology, many researchers have tried to develop alternative models for identifying novel therapeutic agents. Many of these are based on the effects of acute or subchronic administration of psychotomimetic drugs such as amphetamine or phencyclidine. Alternatively, models which are more based on the effects of known antipsychotic drugs, and less on the diseases itself are often employed, especially in the search for novel drugs. These models, often referred to as screening models, will be discussed here briefly, though one can argue whether they really belong to the category of animal models for schizophrenia.

Screening Model

Screening models, also sometimes referred to as animal models with pharmacological isomorphism or with predictive validity, have played a major role in the development of antipsychotic drugs. Indeed, virtually all

antipsychotic drugs currently on the market have been identified using such models. This also explains why the current antipsychotic drugs are all very similar in mechanism of action and therapeutic efficacy (see above).

The development of a screening model usually starts with the (behavioral) effects of a drug with a known clinical profile, the so-called gold standard. In the case of screening models for the treatment of schizophrenia, the gold standard is usually chlorpromazine or haloperidol. In light of the already discussed clinical differences between haloperidol and clozapine, some screening models are based on both haloperidol and clozapine. This gold standard is then given to a rat or mouse, usually a normal, healthy animal and a specific behavior is investigated. This can be a spontaneous or a learned behavior. In some cases, the effects of the gold standard are evaluated on drug-induced behavior. In these latter cases, the distinction between screening models and simulation models becomes less obvious. There is evidence that dopamine hyperactivity and/or glutamate hypoactivity, in part, underlie the symptomatology of schizophrenia. Moreover, schizophrenic patients suffer from stereotyped behavior, deficits in prepulse inhibition and there is even some indication that they have an increased motor activity. Thus, it becomes somewhat arbitrary whether such models are simulation or screening models. However, since we discuss simulation models, especially from the point of etiological similarities, we decided to include these drug-induced models in the category of screening models. **Table 1** gives an overview of the most relevant screening models.

Once the effects of the gold standard are established, the screening model has to be validated. This is the most crucial step in the process of building any animal model, though it often does not receive the attention it deserves. The criteria for validating screening models are directly derived from clinical studies and include the occurrence of false positives (i.e., a drug behaves like the gold standard in the model, but not in the clinic) and false negatives (i.e., a

drug behaves like the gold standard in the clinic but not in the model). Although these two criteria seem to be relatively straightforward, they are nonetheless difficult to evaluate, especially the (non-) existence of false positives. First of all, it is impossible to test all the existing drugs, not used for the treatment of schizophrenia, in a screening model. However, it is even more problematic to answer the question whether all the drugs tested positive in a screening model are true or false positives. Indeed, many compounds have been tested positive in, for instance, the conditioned avoidance response (probably the most widely used screening model for antipsychotic drugs), but a considerable proportion of these drugs never made it to the market. Although one might consider such drugs as false positives, it should be realized that there are many reasons why a drug may not make it to the market, including pharmacokinetic problems, or issues with safety pharmacology or toxicology.

In addition to these general validating criteria, many clinical studies have shown that anticholinergic or chronic treatment with an antipsychotic drug reduces the extrapyramidal side effects but not the therapeutic effect. These latter two criteria thus can be useful to distinguish between the therapeutic and side effects of antipsychotic drugs. **Table 1** gives an indication how well the different models are validated. Once the validation has been established and is acceptable, one can then use such models to test novel compounds, in the hope they may behave like the gold standard and ultimately, after extensive clinical trials, prove to be true positives.

Although such models, as mentioned before, have been extensively used in the development of antipsychotic drugs, they have an important disadvantage. Screening models are based on a gold standard and therefore they will inevitably identify drugs with a similar mode of action, which also explains why the current antipsychotic drugs are all very similar in mode of action and therapeutic efficacy. Since the current generation of medication does not significantly improve the negative or cognitive

Table 1 Screening models and their validation for schizophrenia

Screening test	False positives	False negatives	Potency correlation	Anticholinergic	Chronic treatment
Conditioned avoidance response	–	+	+	–	+/-
Intracranial self-stimulation	–	+	+/-	–	+
Paw test	+	+	+	+	+
DA agonist-induced hyperactivity	–	+/-	+	+/-	Ø
DA agonist-induced stereotypy	–	–	–	–	–
DA agonist-induced prepulse inhibition deficit	+/-	+	+	–	+
NMDA antagonist-induced prepulse inhibition deficit	–	+/-	–	Ø	+

+: there is ample evidence that the criterion is fulfilled; -: there is ample evidence that the criterion is not fulfilled; +/-: there are contradictory results with respect to this criterion; Ø: this criterion has not been investigated in any detail.

symptoms of schizophrenia and therapeutic compliance is usually quite low, we are in need of better animal models with an improved chance of detecting novel therapeutic targets.

Simulation Models

When aspects of a disease are modeled in animals, we usually refer to them as simulation models, though sometimes the term animal models with construct validity is used. As schizophrenia is a disorder of the brain, we need to disturb the normal brain of animals in order to recreate some of the aspects of schizophrenia. Although there are several ways of doing this, we will focus predominantly on etiological approaches, that is, on those models in which genetic or early environmental interventions are used. We mentioned above the use of psychotomimetic drugs, which are sometimes used to model aspects of psychosis. However, since schizophrenia is a chronic illness, such models seem to be less relevant for simulating the disorder. Similarly, given the difficulty in identifying the neuropathology of schizophrenia, such approaches offer limited possibilities for mimicking the pathology in animals.

Genetic Models

Schizophrenia has a strong genetic component, and although the genes that are involved in the etiology have not been unequivocally identified, some genetic changes have been found in several clinical studies. **Table 2** lists the most important genes that have been associated with schizophrenia. Without going into too much detail, it is important to note that for most genes, more than one single nucleotide polymorphism (SNP) has been linked to schizophrenia. A good example is the regulator of G-protein signaling 4 (RGS-4) gene of which four SNPs have been linked to schizophrenia (commonly denoted by 1, 4, 7, and 18). However, in one study, the haplotype G–G–G–G was found to be more common in schizophrenic patients, whereas in another study the haplotype A–T–A–A was seen more often in patients. Another problem is that in most cases, the functional consequence of this mutation is not known, and could represent both gain-of-function as well as loss-of-function mutations. In several cases, increases or decreases in expression of the protein or mRNA of the candidate gene have been found, but it is not clear whether this is really due to the SNP.

Since neither the specific nature of the SNPs nor the functional consequences of those associated with schizophrenia is generally known, it has proven difficult to develop animal models based on genetic mutations known to be associated with schizophrenia. Most studies have therefore been carried out with either homozygous

or heterozygous knockout mice. In particular, the homozygous knock-out animals may not adequately represent schizophrenia as none of the genes so far identified in schizophrenia lead to a complete loss of function. This has led to some intriguing though difficult-to-interpret finding. The schizophrenia-associated neuregulin haplotype, for instance, is associated with increased levels of neuregulin. However, the heterozygous neuregulin knock-out mouse shows some schizophrenia-like features (such as increased locomotor activity and decreased prepulse inhibition).

Environmental Models

Although genetic factors play a major role in determining the susceptibility for schizophrenia, it has long been recognized that environmental factors also contribute to the actual development of the disease. The nature of these environmental factors has proven hard to identify for several reasons. First, as discussed above, the most important environmental factors often antedate the onset of the disease by a long period. In fact, the perinatal period is possibly the most crucial period in which these factors impinge on the disease process. The relative paucity of prospective studies from birth onwards implies that most studies have relied on memories of a relatively distant past. Second, environmental factors are less well circumscribed than, for instance, genetic factors. For instance, the offspring of mothers that were pregnant during the Dutch hunger winter in 1944 have an increased risk of developing schizophrenia. This has been taken to indicate that malnutrition is a possible cause of schizophrenia. Although this may be the case, the hunger winter will undoubtedly also have led to severe stress (as hunger was very uncommon in the Netherlands) and to a change in the nature of the food (many were known to eat tulip bulbs, for instance). Such factors may equally have contributed to the development of schizophrenia. Third, there is increasing risk that schizophrenia results from an interaction between genetic and environmental factors (see below). This implies that studying environmental factors in isolation may fail to identify certain factors.

In spite of these difficulties, several environmental factors can be deduced from the literature that affect children more often who later develop schizophrenia as compared with control children. **Table 3** lists the most important ones and gives some examples. From the table, it is clear that most of the environmental factors so far identified are especially related to the pre- and early postnatal period, which is in line with the idea that schizophrenia is a disturbance of (neuro)development. However, later life stressors or excessive cannabis use around the period of puberty have also been reported to increase the risk of developing schizophrenia.

Table 2 Candidate genes for schizophrenia and genetic simulation models

Gene	Location (human)	Functional consequence of SNP	Animal model	DA resp.	PPI	P50	Memory	Soc Isol	Anhedonia
COMT	22q11	Val158 SNP has reduced enzyme activity	COMT ^{-/-} COMT ^{+/-}	— Ø	— Ø	Ø Ø	Ø Ø	Ø Ø	Ø Ø
DAOO	12q24	Increased activity and expression?	None						
DISC-1	1q42	Not clear at present	DISC-L100P DISC-Q31L DISC-KO	Ø Ø +	+	Ø Ø Ø	+	— + +	— + Ø
DTNBP1	6q22	Reduced mRNA and protein expression?	Sdy mutant	—	—	Ø Ø	Ø Ø	+	Ø
G72	13q32-34	Not clear at present	None						
mGluR3	7q21-22	Reduced protein expression?	mGluR3 ^{-/-}	Ø	Ø	Ø	Ø	Ø	Ø
NRG1	8p12-21	Increased mRNA expression?	III-NRG1 ^{+/-} TM-NRG1 ^{+/-} CRD-NRG1 ^{+/-}	Ø Ø Ø	+	Ø Ø Ø	+	Ø Ø Ø	Ø Ø Ø
RGS4	1q21-22	Reduced protein expression?	RSG ^{-/-}	—	—	Ø Ø	— +	Ø Ø	Ø Ø

+: the sign or symptom observed in the patients can also be observed in the animal model; -: the sign and symptom observed in the patients was not observed in the animal model; Ø: the sign or symptoms has not been investigated in the animal model. Abbreviations: DA resp.: hyper-reactive response to dopamine agonists; PPI: deficit in prepulse inhibition; P50: deficit in P50 auditory gating; memory: deficit in memory tests; Soc Isol: social isolation.

Table 3 Environmental etiological factors for schizophrenia and simulation models

Environmental factor	Examples	Animal model	DA resp.	PPI	P50	Memory	Soc Isol	Anhedonia
Seasonality	Increased winter birth	None						
Urbanicity	Increased risk in urban areas	None						
Migration	Increased risk in foreign culture	None						
Prenatal malnutrition	Dutch Hunger winter	Prenatal protein deprivation	Ø	+	Ø	Ø	Ø	Ø
Prenatal stress	Dutch Hunger winter (?) Death of close relative Unwantedness of pregnancy Exposure to war	Prenatal stress	+	+	+	+	+	Ø
Prenatal Infection	Influenza Herpes Simplex Rubella	Prenatal PolyI:C	+	+	Ø	+	Ø	Ø
Obstetric complications	Bleeding during pregnancy Pre-eclampsia Asphyxia	Caesarean section Birth hypoxia Postnatal hypoxia	+	+	Ø	Ø	Ø	Ø
Early postnatal stress	Childhood trauma Maternal deprivation Increased risk in urban areas (?) Increased risk in foreign culture (?)	Maternal deprivation	+	++	+	Ø	+/-	Ø
Peri-pubertal drug use	Excessive cannabis use	Peripubertal cannabinoid agonist	-	+	Ø	+/-	+	+
Peri-pubertal stress	Low socio-economic status Social adversities Severe life events	Isolation rearing None Early ventral hippocampal lesion Prenatal MAM treatment	+	+	+		+/-	+/-

+: the sign or symptom observed in the patients can also be observed in the animal model; -: the sign and symptom observed in the patients was not observed in the animal model; Ø: the sign or symptoms has not been investigated in the animal model. Abbreviations: DA resp.: hyper-reactive response to dopamine agonists; PPI: deficit in prepulse inhibition; P50: deficit in P50 auditory gating; memory: deficit in memory tests; Soc Isol: social isolation.

Based on these environmental factors, such as the prenatal stress, prenatal immune challenge, maternal deprivation, and isolation rearing models, a large number of animal models have been developed. In addition, the early ventral hippocampal lesion model has to be mentioned. In this model, the ventral hippocampus of 7-day-old rats is lesioned with an excitotoxic compound. Although, strictly speaking, this is not an etiological model (i.e., there is no evidence that the ventral hippocampal region is lesioned in children who later develop schizophrenia), it has been a very successful model and the lesioned animals show (in adulthood) a number of similarities to schizophrenic patients.

The Symptomatology of Schizophrenia

In spite of the apparent lack of knowledge about the etiology of schizophrenia, quite a number of genetic and environmental factors have been identified that play a role in its development. As discussed in the previous sections, many of these can be modeled in animals. However, the question remains whether such modeling leads to comparable symptoms in animals. Since most symptoms are difficult to assess, even in humans, much research is devoted to identifying signs and symptoms that are more objectively measurable. In this context, much experimental work has, in recent years, focused around the concept of endophenotypes. In order to be called an 'endophenotypes' a sign or symptom should be heritable, co-segregate with the disease and also occur in healthy siblings. Moreover, it should be state dependent, that is, it should also occur in patients in remission. The idea of endophenotypes is that they are more easily quantifiable (using specific cognitive or electrophysiological tests) and more related to specific brain regions or neurotransmitters, and thus more accessible for studying in animals, thereby increasing their translatability.

Several endophenotypes have so far been identified for schizophrenia, though in some cases there is still some doubt as to whether they fulfill all the requirements. These include smooth pursuit eye tracking, P50 sensory gating, prepulse inhibition, sustained attention, and working memory. Most of these relate to the cognitive symptoms of schizophrenia and several of these can also be assessed in animals. In addition, patients with schizophrenia also suffer negative symptoms such as anhedonia and social isolation. Even though several models for anhedonia have been developed in animals, such as decreased sucrose preference and increased threshold for brain self-stimulation, these have not been studied very often in relation to schizophrenia. Social behavior, and more specifically social isolation, on the other hand, has been studied in many animal models for schizophrenia. Finally, patients with schizophrenia show prominent

positive symptoms, which are generally impossible to assess in animals (see discussion at the beginning of this article). One possible biomarker (though it is still unclear whether this is a true endophenotype) is the hyper-reactive dopaminergic state. Several challenge studies have shown that patients with schizophrenia show an increased sensitivity of the dopaminergic system to psychostimulants such as amphetamine, especially in the striatal complex. Since antipsychotic drugs block the dopaminergic transmission and reduce positive symptoms, this increased dopaminergic state may well represent a biomarker for this group of symptoms.

Tables 2 and 3 summarize the main findings of the various animal models in relation to the signs and symptoms discussed here. It is important to realize that this table can not give full and detailed account of each model, and sometimes studies from different laboratories come to different conclusions. Whether this represents true differences, or are due to slight changes in the methodology, or the use of different mutant animals is still unclear. Nonetheless, the tables clearly show that much more work has been done using environmental manipulations than genetic ones. The tables also show that certain symptoms or endophenotypes (such as prepulse inhibition) are much more often studied than others (such as anhedonia).

Validating Simulation Models

In contrast to the screening models for schizophrenia, much less work has been done in trying to validate the simulation models. Since such models are based on the disease itself, they aspire, in addition to predictive validity, also face and construct validity. Although most models show some face validity, most of the manipulations shown in **Tables 2 and 3** also show behavioral abnormalities not seen in schizophrenia, which decreases their face validity. Further, with respect to the predictive validity, most of the simulation models lack in-depth analysis, including the effects of chronic treatment or the influence of anticholinergic drugs. Moreover, with respect to assessing the predictive validity of these models, we have to realize that much is still unknown about the clinical effects of antipsychotic drugs. For instance, whereas cross-sectional studies have provided some evidence that antipsychotic drugs may reduce prepulse inhibition (PPI) and gating, some longitudinal studies have suggested that the effect of both classical and atypical antipsychotic drugs is rather limited. Similarly, as discussed in the beginning of the article, there are, so far, no drugs effective against the cognitive and negative symptoms of schizophrenia, suggesting that the validity can only be assessed by the lack of effect of antipsychotic drugs.

Summary and Outlook: Gene–Environmental Models

In order to develop novel drugs for schizophrenia, we were restricted, for a long time, to screening models, based on known effective drugs such as haloperidol or chlorpromazine. This has certainly hampered the development of really innovative treatments. Fortunately, our increased knowledge on the role of genetic and environmental factors in the etiology of schizophrenia and the identification of specific biomarkers and endophenotypes has led to a wide range of novel simulation models for this severe psychiatric disorder.

These novel models are capable of capturing a number of aspects of schizophrenia. Although their validity has not been assessed in great detail yet, they already have proven to be of great value. In order to further improve the modeling of schizophrenia, interest should now turn to the integration of the two types of models. It is becoming more and more evident that schizophrenia is due to an interaction between genetic and environmental factors. It is therefore not so surprising that many of the genetic models listed in **Table 2** do not show overt schizophrenia-like abnormalities. As in patients, these genes are susceptibility genes, increasing the risk that, upon an environmental challenge, such abnormalities can occur.

Thus, it would be interesting to investigate the effects of stressors in animals with a different genetic background. Some studies have already been done, and it has been shown, for instance, that in contrast to Wistar or Sprague Dawley rats, Lewis rats are more or less resistant to the effects of early maternal deprivation, isolation rearing, and early hippocampal lesioning. It is to be hoped that within the next decade the various environmental manipulations described in **Table 3** will be applied to several of the genetic models displayed in **Table 2**, in order to further improve the simulation models for schizophrenia. At the same time, a better understanding of the clinical pharmacology of the biomarkers and endophenotypes will certainly be beneficial for improving the validity of the simulation models.

Conclusions

The rational development of etiology-based simulation models for schizophrenia has greatly increased over the last two decades. This is mostly due to an increased knowledge of susceptibility genes and environmental risk factors. Further, the identification of specific

endophenotypes and/or biomarkers has increased the translational power of the current generation of animal models. The next steps should now focus on further improving the models by focusing on the gene–environmental interaction, that is, by combining specific environmental manipulations displayed in **Table 3** with some of the genetic models displayed in **Table 2**. In addition, more knowledge of the clinical pharmacology of the various endophenotypes would be very helpful in assessing the validity of the different models.

See also: Genes and Behavior: Animal Models; Mouse Genetic Approaches to Psychiatric Disorders; Psychostimulants; Stress and Brain Morphology; Value of Animal Models for Predicting CNS Therapeutic Action.

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Relevant Websites

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Transgenic Technologies and their Application to the Study of Senile Dementia

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Glossary

β-Amyloid Protein – A form of amyloid protein which is commonly found in abundance in the brains of Alzheimer's Disease patients.

Amyloid Precursor Protein (APP) – The precursor to the β-amyloid protein. The gene associated with APP has been linked with Alzheimer's disease.

Long-term Potentiation (LTP) – A form of synaptic plasticity that requires conjoint pre- and postsynaptic activity. LTP is often asserted to underlie memory formation.

Presenilins – A family of transmembrane domain proteins widely expressed in the brain. Missense mutations in genes coding for two of these proteins (presenilin-1 and presenilin-2) have been linked to a familial form of Alzheimer's Disease.

Reactive Gliosis – The neuroinflammatory response of glial cells to brain tissue insult.

Senile Plaque – Abnormal extracellular deposits of β-amyloid protein commonly found in the brains of patients diagnosed with Alzheimer's disease.

Tau Protein – A microtubule-associated protein found in neurons throughout the central nervous system. Abnormal, hyperphosphorylated, Tau protein is associated with the formation of the neurofibrillary tangles commonly seen in the brains of Alzheimer's Disease patients.

Transgenic animal – An animal which carries an exogenous gene deliberately inserted into its genome.

strategies to assess the biological basis of memory as well as neurodegenerative diseases that impact cognitive processes across the lifespan. However, the molecular genetics revolution brought with it both great expectations as well as questions that pervade colloquial discussions until this day. The belief that dementia and other devastating disorders of the mind could be explained by simple (or even single) gene mutations became pervasive in popular parlance. Furthermore, the expectation that these genes could be manipulated to modulate cognitive function became common place. As a particularly striking example, in the preface of a text summarizing a three day symposium on the chemistry of learning, Corning and Ratner wrote that one impetus for the symposium was the frequent request by nonscientists that they be provided "memory molecules" ... to help some senile relative." Despite the optimism of the early 1960s, progress in satisfying these goals has been slow. Nevertheless, experiments using transgenic animals, when properly conducted and interpreted have been shown to be a useful technique in behavioral neuroscience. In what follows we highlight some of these techniques in order to illustrate how transgenic animals can be effectively used to further our understanding of dementia and other disorders of the mind. To exemplify this approach, we focus on the usefulness of transgenic animal models in behavioral neuroscience by examining the advances made in the study of senile dementia. We hope to demonstrate both the usefulness of this technique as well as its potential pitfalls.

Introduction

Watson and Crick's description of DNA fostered a torrent of new strategies to address the mechanisms of genetics, and subsequently, ushered in a new interest in the role of genetics in brain disorders. By 1960, the laboratory of Francois Jacob had developed new methods to locate specific genes on chromosomal material, and moreover, a process by which genes could be excised or added into the DNA of single cells. These methods were the precursors to modern transgenic science, an approach that has revolutionized

Transgenic Animal Models: Advantages and Disadvantages

Transgenic mice are generated in a process that begins with the injection of a DNA strand fragment (representing a gene) into the male pronucleus of a newly fertilized mouse embryo, after which the DNA is incorporated into a random site on one of the chromosomes. Since the embryo is at the single-cell stage, the newly incorporated gene is replicated and ultimately is expressed in all of the animal's cells,

including its germline. This heterologous expression of a DNA fragment is commonly referred to as a transgene and the gene's product is made in addition to the endogenous gene expression pattern.

The manipulation of embryonic stem cells may be used to induce more specific genetic modifications. When introduced into a host embryo, stem cells can contribute to all cell types as the embryo develops. By homologous recombination of stem cells maintained in culture, modifications can be introduced into specific genetic loci and introduced into the mouse germline to promote mutations of preexisting proteins. Depending on the nature of the recombination and its location on the DNA strand, these mutations can effect deletions (i.e., knockouts) or modifications of existing genes and the expression of novel proteins. Since the engineered strand of DNA replaces a strand that had been endogenously expressed, the effects of the new gene are pure, that is, they are not the summed product of the endogenous gene and the transgene.

Since their introduction, transgenic strategies have been employed to generate mice that lack, or express in a modified form, genes that contribute to molecular and biochemical processes that have been asserted to contribute in various ways to the induction, storage, and expression of memories in the mammalian brain. This approach may be recognized as a modern variant of more widely used pharmacological strategies that for many decades served as a mainstay in the arsenal of tools employed in attempts to elucidate the biochemical substrates of memory. Despite their wide use, pharmacological manipulations were generally acknowledged to be inadequate, owing to the lack of specificity of most (or all) pharmacological agents, the lack of agents for many targets, and the difficulty associated with specifying or restricting the site of action of a pharmacological manipulation. However, although the transgenic and pharmacological approaches are conceptually related, many of the complications incurred with pharmacological manipulations are circumvented with modern genetic techniques. In particular, for every gene there exists a specific target protein, and any protein of interest is coded for by a specific gene.

While superior in many respects to their pharmacological counterparts, the use of transgenic animals is not itself free from interpretative difficulties. First, genetic alterations depending on a transgene are often expressed throughout an animal's development and can adversely impinge on a cascade of developmental processes. Thus, any unique properties of the adult phenotype (for instance, its capacity to learn) might reflect an acute influence of the genetic manipulation, or possibly, a consequence of an abnormal development of the nervous system and/or compensatory effects promoted by the gene deletion. In this regard, it should

be noted that many gene deletions produce gross abnormalities (e.g., neuroanatomical or morphological) and, in many instances, are lethal. Thus, even transgenic animals which appear to be nominally normal (at a behavioral or neuroanatomical level of analysis) can reasonably be expected to be abnormal at levels that escape detection. Second, a transgene is often expressed in every cell in the organism, thus confounding attempts to interpret the effect of the alteration in any specific brain region. Each of these first two complications has been at least partly overcome with newer techniques. Specifically, recent advances in transgenic models have led to the ability to create conditional transgenic animals that express genetic alterations only in specific brain regions and/or only after certain developmental points. Third, the production of a mutant mouse requires several generations of inbreeding, and the inbreeding may introduce uncontrolled mutations that may themselves impinge on the target behavior. Lastly, the genetic approach suffers from a major interpretative difficulty endemic to traditional pharmacologic manipulations. Specifically, a genetic mutation may impinge on a target behavior for reasons unrelated to that which was intended. To illustrate the analogy, a drug that impairs learning may do so via its disruption of a specific component of the learning mechanism, or may do so as a secondary or unintended effect of the drug, for instance, on the animal's state of alertness or its sensory acuity (as but two of many possibilities). Thus while the transgenic approach has certain definite advantages over more traditional pharmacological interventions, the interpretation of results based on these genetic manipulations is not uncomplicated. Overcoming these interpretive difficulties requires a close attention to the issues of experimental design and behavioral methods (an issue that we will return to below).

Alzheimer's Disease: Example of Advances Made through Use of Transgenic Models

With the exception of some primates, Alzheimer's disease (AD) is not known to spontaneously occur in nonhuman animals, making it an ideal target for transgenic models.

AD is the most common form of senile dementia, affecting an estimated 40% of all people over the age of 80 (although this estimate is complicated by comorbid states and imprecise diagnostic tools). The initial symptoms of AD include poor memory of recent experiences, and consequently, an impairment of new learning and the ability to recall that which had been previously learned. While AD is typically diagnosed with

behavioral and cognitive tests, it can only be confirmed upon autopsy by the presence of senile or neuritic plaque – areas of dead tissue containing among other things large deposits of beta-amyloid ($A\beta$) protein. Senile plaque is most commonly seen in the hippocampus and its afferent cortical structures, and until recently, laboratory studies of AD were largely limited to pharmacological manipulations of these structures and their synaptic integration, both in attempts to produce animal models of the disorder, and to assess the efficacy of treatment strategies. Although fruitful, this approach has had limited success in describing the etiology or pathology of AD, or as a tool for the development of effective treatments. However, recent advances in genetics and the development of transgenic technologies have made it possible to study alterations in brain morphology, synaptic transmission, and learning in strains of mice that carry genes associated with the human dementias characteristic of AD. Specifically, transgenic mice have been created to investigate the role of three of the genes (APP, presenilin-1, and presenilin-2) associated with familial forms of AD.

The amyloid precursor protein (APP) is the precursor to the β -amyloid peptide, the principal constituent of the senile plaques that are prevalent in the brains of human AD patients. To determine the action of APP in presenile animals, Zheng *et al.* produced mice lacking the gene that codes APP. During early adulthood, a marked increase in reactive gliosis was observed in the brains of the transgenic animals. Behaviorally, the animals exhibited reduced locomotor activity and grip strength, as well as deficits in spatial water maze learning and a reduced capacity for hippocampal long-term potentiation (LTP; a form of synaptic plasticity that is often asserted to underlie memory formation). This set of results indicates that under normal conditions, APP plays an essential role in the development of the nervous system and its regulation of behavioral function, suggesting that a disruption of APP function may underlie or contribute to the expression of AD. However, although APP deficient transgenic mice exhibit hippocampal neuron loss and declining synapse density in adulthood, only a subpopulation of these transgenic animals display lethargy or learning deficits, suggesting that APP function or expression may alter the susceptibility to neuronal loss and dementia, but may not itself underlie the behavioral or cognitive deficits that characterize AD. This latter caveat highlights the necessity of attending to the performance of individual experimental subjects.

Other APP transgenics have been generated that express mutations associated with early-onset familial AD, and these strains are characterized by accelerated deposition of β -amyloid protein (the constituent of senile plaques), a reduction in neuronal density in the

hippocampus, and often, an impairment of LTP induction. Similarly, these mice often exhibit severe impairments in the rate at which they acquire escape responses in the spatial version of the water maze as well as impairments in other cognitive tasks. For instance, Kelly *et al.* compared the performance of transgenic mice that expressed a human AD-associated mutant form of APP to wild-type controls in the water maze and in passive avoidance. They demonstrated that APP mutant mice showed age-related decline in performance in both of these tasks that were not related to changes in sensory or motor impairments. Furthermore, they (as well as others) reported that the emergence of these learning impairments correlate with the development of plaque formation. However, in other instances, the development of plaques appears to be unrelated to learning deficits or impairment of *in vitro* indices of learning-related synaptic plasticity, such as LTP. In all, variations in the expression of APP appear to be related to neuroanatomical abnormalities and behavioral deficits associated with AD, but its exact role in promoting these abnormalities is unclear and it does not appear sufficient in itself to underlie the dementia.

The early-onset familial form of AD is more highly correlated with mutations in one of two genes known as presenilins (PS-1 and PS-2) than with the APP gene itself. Proteolysis of presenilin proteins in turn stimulates the proteolysis of the APP, and down regulation of PS-1 is associated with a decrease in β -amyloid production. Moreover, overexpression of the PS-1 gene leads to the overproduction of β -amyloid. Thus variations in PS expression may precede irregularities in APP function and thus may be better suited as the determinant of the emergence of AD.

Targeted ablations of the PS-1 gene are lethal during development and are associated with abnormal development of the nervous system, and thus electrophysiological recordings from mice lacking the PS-1 gene have been rare. In contrast, transgenic mice that overexpress the PS-1 gene have been developed, and these mice are amenable to electrophysiological and behavioral characterization in adulthood. In all such lines, increased levels of β -amyloid have been observed during middle adulthood. To examine whether the overexpression of PS-1 influences electrophysiological properties of the hippocampus implicated in learning and memory storage, Parent *et al.* measured field excitatory postsynaptic potentials (EPSPs) at the Schaffer collateral-CA1 synapse in hippocampal slices. Although basal indices of synaptic efficacy (e.g., EPSP slope and amplitude) were unaltered in the transgenic animals, input-specific LTP was more easily induced and was more persistent in the transgenic animals than in the respective wild-type control subjects. Thus if LTP is a substrate mechanism for memory storage, it is difficult to reconcile these results with the proposal that

PS-1 expression contributes to the learning deficits that are characteristic of AD. Even more problematic, Janus *et al.* found that expression of the human PS-1 gene was associated with a significant improvement in the performance of mice in the spatial version of the water maze task. Furthermore, Chui *et al.* showed that mice that expressed the human form of PS-1 failed to develop senile plaque. Based on this pattern of observation, it seems that mutant PS1 alleles may require coexpression of human versions of other AD-associated genes in order to promote a behavioral phenotype indicative of AD.

Thus far, mice expressing single AD-related transgenes have been less than adequate as models for the elucidation of AD, prompting several attempts to generate mice carrying mutant APP and PS-1 genes. This double-transgenic approach has begun to yield promising results, including a line of mice that expresses neuropathologies in early adulthood (3 months), as well as a concomitant emergence of impaired choice performance in a t-maze. However, even at 9 months of age, these same mice exhibit no impairment in spatial water maze learning, suggesting that even this double-transgenic manipulation is inadequate to capture the fundamental essence of the Alzheimer's pathology, even though it may more adequately model the etiology of the disorder. However, Puolivali *et al.* reported significantly impaired retention in the spatial water maze in these mice by 11–12 months. Furthermore, these impairments correlated with the total amount of A β . The failure to capture the full range of AD symptoms in double-transgenic mice have led researchers to create so-called triple-transgenic mutant mice (3xTg-AD) which express APP, PS-1, as well as Tau. This final gene is associated with the formation of neurofibrillary tangles, a neural pathology also seen in AD. 3xTg-AD mice develop AD-like plaques and neurofibrillary tangles in the hippocampus, cortex and amygdala in a hierarchical fashion that mimics the progression of AD. Unlike the double-transgenic mice, 6-month-old 3xTg-AD mice showed impaired memory retention in the spatial version of the water maze as well as contextual fear conditioning. However, memory retention was unimpaired in young 3xTg-AD mice, at an age at which AD-like pathology is not yet present. These findings demonstrate the usefulness as well as the difficulties in the use of transgenic animal models in behavioral neuroscience.

Conclusion

The great expectations that arose after the advent of the genetic revolution and transgenic technologies in particular remain to a degree unrealized. Nevertheless, these genetic tools have proven to be of great use in behavioral neuroscience. The ability to selectively over-express or

under-express genes has allowed for modeling of disorders in animals that otherwise would not be possible. However, great care must be taken when interpreting the behavioral results from such animals, as manipulating individual genes can cause cascading effects that have far reaching influences. This is especially true when the genes are altered during or prior to development. As mentioned earlier, a genetic mutation may affect a behavior for reasons unrelated to that which was intended. To some extent these drawback can be overcome by using animals whose gene mutations are present only in certain brain subregions or are only present during adulthood. However, even in these cases behavioral results must be interpreted with some caution. For these reasons using only one behavioral paradigm is not ideal (it is noted that in the majority of studies described above, the spatial water maze served as the sole index of cognitive performance). A clear picture of the effects that a gene mutation has on behavior can only emerge after observing behavior across a wide battery of tasks. A recent study by Matzel *et al.* investigating the role of the cell adhesion molecule Nr-CAM demonstrates this nicely. Nr-Cam is thought to be involved in susceptibility to addiction in humans. Matzel *et al.* found that mice deficient in this adhesion molecule showed no overall learning deficits across four distinct learning tasks (including the water maze). However, these animals did display impaired performance in a fifth cognitive task that was based on learned passivity (passive avoidance). These same animals also exhibited increased propensity to enter stressful environments and a higher sensitivity to heat and shock. Taken holistically, a picture emerges from this study which demonstrates that Nr-CAM deletion may increase impulsive behavior in animals that are hyper-responsive to aversive stimulation, a characteristic thought to underlie a susceptibility to drug addiction. If these various performance measures were assessed only in isolation, no such overall conclusion would likely have emerged. So while transgenic mice have been a boon to understanding brain systems and impairments, like any other tool they are best applied in conjunction with comprehensive testing and analytic strategies.

See also: Animal Models of Learning and Memory; Brain Aging: Structural Imaging Biomarkers of Risk of Alzheimer's Disease; Cognitive Decline in Laboratory Animals: Models, Measures, and Validity; Genes and Behavior: Animal Models; Knock-Outs: Learning and Memory; Memory and Aging, Neural Basis of; Mouse Genetic Approaches to Psychiatric Disorders; Role of Gene Transcription in Long-Term Memory Storage.

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V

Value of Animal Models for Predicting CNS Therapeutic Action

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Quanti exempla animalium valeant in morbis mentalibus humanis tractandis

Glossary

Biomarker – An indicator of a normal or pathological biological process based on scientific evidence and can be objectively measured. They can provide information regarding the health status of the subject, the degree and progression of the abnormality, and response to a pharmacological treatment.

Clinical endpoints – A potential drug must be shown to be safe and effective, that is, to be of clinical benefit to the patient. They may be defined as the absence of a pathogen, the reduction or disappearance of an abnormality, or reduction in the physical or behavioral manifestations of the disease/disorder, including survival and/or quality of life.

Construct validity – Closely related to the pathology and the underlying biology of the disorder, and the accuracy with which changes in the model organism reflects that in the human.

Endo and exo phenotypes – Terms defined originally by John and Lewis and later developed by Gottesman and his colleagues for heritable characteristics of a disorder present primarily in affected individuals as well as in family members regardless of whether the disorder is active or not. The complexity of the endophenotype increases as a function of distance from the gene and gene product.

Ethological validity – The use of natural or species-relevant stimuli for manipulating behavior in animals, or the approximation of the natural environment of the model organism.

Etiological validity – Etiology refers to the causes or origins of pathology. When etiology of a disease can be established, as say, for example, a viral or fungal infection, modeling the effects of such an infection in animals can be extremely useful to discover and develop antiviral or antimyotic agents to combat the infection.

Experimental medicine – Studies in human volunteers or specific samples of a patient population to (1) obtain mechanistic and pharmacological information of compounds entering into development, (2) explore and define biological markers with which the state and progress of a disorder can be monitored, as well as the effects of pharmacological interventions on its progress, and (3) establish models and procedures with which to obtain initial signals of efficacy.

Face validity – The phenomenological similarity between the behaviors exhibited by the animal model and the specific symptoms of the human condition.

Forward genetics – The process by which the genetic basis of a phenotype is investigated (phenotype to gene).

Good laboratory practice (GLP) – Principles that provide a standard for planning, execution, reporting, and archiving of laboratory studies. GLP studies are mandatory for safety and toxicological assessment of a new chemical entity (NCE). GLP, however, is not a Food and Drug Administration (FDA) requirement for preclinical studies to characterize the NCE.

Nonetheless, it is a common practice within the pharmaceutical industry to require preclinical researchers to conduct their experiments in as close a GLP fashion as possible.

Investigational new drug (IND) – The first stage of seeking approval for the marketing, use, and distribution of an NCE in the United States is the application for an IND in which the sponsoring company presents a document detailing the proposed clinical studies with the NCE, the chemistry, manufacturing, and control information of the NCE; and a summary report of the preclinical studies carried out with the NCE describing the pharmacological effects of the NCE, mechanism of action and the absorption, distribution, metabolism, and excretion (ADME) of the NCE; if these effects are known.

Model – A model of a human disorder attempts to recapitulate its aspects in a simpler organism, or in different species. A model is based upon the inducing conditions thought to reflect the etiological sources of the disorder. These inducing conditions may be genetic, ethological, developmental, mechanical, or pharmacological, and the establishment of the model depends on different levels of validity. Once a model is established reliably and is shown to be valid, it serves as an extremely important tool with which to study normal and abnormal human functions. Models of disorders are also important tools with which the effects of pharmacological or behavioral interventions can be assessed.

New chemical entity (NCE) – A potential drug with a novel chemical structure. An NCE is also referred to as an NME or new molecular entity.

Pharmacological isomorphism – In terms of drug development, a special condition of predictive validity is usually determined through pharmacological validation, that is, a comparison of the normalization of abnormal or impaired biology/behavior in animals by the NCE following application of clinically effective drugs used in the treatment of pathology in humans.

Predictive validity – The ability to predict changes in the human subject based upon changes in the model. No common underlying mechanism is assumed.

Procedures – The experimental manipulations by which a model of a human disorder is established and its effects can be measured. Procedures are not necessarily synonymous with the model. For example, aspects of state anxiety in humans can be ethologically modeled in rodents by taking advantage of the rodents' preference for dark, sheltered places; presumably as a defense against predators. Various procedures such as placing the rodent in a light-dark environment and observing behaviors such as the exploration of the light areas can be used to measure this experimental state anxiety. On the other hand, some procedures can be synonymous with the model. The effects of prolonged and chronic stress on physiology and behavior can be modeled directly by subjecting a rodent to an unpredictable series of mild stressors over a period of time. While the individual stressors chosen, frequency of appearance, and unpredictable nature of their application are procedures, chronic mild stress can be reasonably considered as a model of aspects of environmental stress in humans.

Proof of concept – Demonstration that the IND has therapeutic activity through the hypothesized mechanism in a target indication.

Proof of mechanism – Demonstration that a novel molecule is able to interact with the desired target and produce a desired functional effect through the hypothesized mechanism.

Proof of principle – Demonstration that a hypothesis is validated. This hypothesis may or may not be the ultimate therapeutic activity of the IND.

Proofs of mechanism, principle, and concept – Drug targets are continuously subjected to examination to determine whether the theoretical relationship of the target and associated lead molecules to the human disorder can be supported and sustained. Consequently, throughout the drug discovery and development process, objective proofs are considered milestones and the criteria with which further progression of the lead molecule are judged.

Reverse genetics – The process by which function is ascribed to a gene or gene product through the manipulation of that gene through deletion, silencing, or overexpression (gene to phenotype).

Translational research – The process through which information and insights flow from clinical observations to refine the development of animal models as well as the complementary flow of information and insights gained from animal models to the clinical setting, be it through improved diagnosis, disease management, or treatment; including pharmacological treatment.

Value and Validity: Two Sides of the Same Coin?

When first approached by the Editor to write this commentary for the *Encyclopedia of Behavioral Neuroscience* on the topic of the value of animal models for human mental disorders, I was slightly nonplussed by the choice of the word *value* in the title that I was assigned. Value implies economic or moral worth. According to *Merriam-Webster's Dictionary*, value is a noun defined, *inter alia*, as being "... a fair return or equivalent in goods, services, or money for something exchanged," or having "...relative worth, utility, or importance." Indeed, even as a transitive verb, value denotes "...the estimation or assignation of monetary worth, or the high consideration of something." Throughout my professional career examining the validity of the animal models to study the neurobiological substrates of behavior and the probable therapeutic utility of a novel molecule or new chemical entity (NCE), it never seriously occurred to me that I was not doing something of value. Nevertheless, perhaps value is a correct term to use when considered in the context of the pharmaceutical industry, the ethical use of animals for biomedical experimentation and especially the present re-examination of reasons for the apparent lack of novel therapeutics to be used in the treatment of central nervous system (CNS) disorders.

The present controversy regarding the presumed value of animal models for CNS disorders has its origins in the publication of a short, but very influential white paper on innovation or stagnation in the discovery and development of drugs by the US Food and Drug Administration (FDA) in 2004. This document analyzed the growing expenditure of medicinal research and development coupled with the parallel decrease in the number of submissions of NCEs registered from 1993 to 2003. Animal models in general were singled out as a point of concern. In addition to the more obvious reasons for registration failure, such as unexpected toxicity, lack of efficacy, that is, lack of therapeutic action has been cited as the most frequent reason for failure. NCEs submitted for the treatment of CNS disorders, in particular, have seen some of the worse attrition rates of 50–60%. This depressing picture was not helped by notable failures of NCEs with novel mechanisms of action such as Glaxo's serotonin 5-HT₃ receptor antagonist ondansetron (cognitive disorders) and Merck's NK1 receptor antagonist, aprepitant (MK-869 for the treatment of depression). Both these INDs suggested therapeutic potential from their effects in preclinical models. Subsequent examples of novel targets that failed to live up to their clinical promise include Pfizer's CRH-1 receptor antagonist CP-316,311, also indicated for depression, Pharmacia's dopamine D₄ receptor antagonist antipsychotic soneprazole (PNU-101387G). Potential drugs for the treatment of neurological disorders have also been withdrawn from further development for lack of efficacy in clinical trials. These include Novartis' antiparkinson compound TCH346 or AstraZeneca's antistroke compound NXY-059.

This climate and apparent lack of efficacy of potential CNS therapeutics have helped to reinforce a negative attitude toward the value of animal model use in CNS drug research in general and within psychiatric research specifically. This is not a new phenomenon. Louis Lasagna, one of the founders of controlled clinical trials for example, recalls a Merck executive's lack of confidence in animal models to predict what a drug would be good for. This attitude is very much alive today. Indeed, within the biopharmaceutical industry, one could even question the necessity for preclinical demonstrations of efficacy. When an IND is filed, the FDA only requires a summary of preclinical data, if available. On the other hand, the FDA is very specific on the type and detail of safety and toxicological studies that have to be done under certified GLP conditions. Though it is very unlikely that a sponsor would advance an NCE to the IND stage without a preclinical dossier that includes studies of mechanism of action and potential clinical efficacy, it is actually not required.

Validity, on the other hand, also implies worth, but with quite a different emphasis. Validity refers to

something being "...well-grounded or justifiable: being at once relevant and meaningful <a valid theory> b: logically correct <a valid argument> <valid inference>;" as well as "conforming to accepted principles of sound biological classification." Animal models for CNS disorders are endowed with different degrees of validity, of which face, construct, and predictive validity are the most common. In addition, ethological and etiological validity are increasingly important considerations, especially in view of the emerging greater concern with gene and environmental interactions in the development and manifestation of behavioral disorders.

Face validity of an animal model is mainly an intuitive criterion of the reasonableness of the model and is characterized by analogy with human behavior. Models with high validity can be very persuasive on a surface level. Feelings of defeat and worthlessness associated with depression can be modeled, for example, by the immobility developed by rodents when placed repeatedly in a cylinder of water from which there is no escape. One could intuitively argue that, "Yes, I too would feel depressed if all my efforts to change were useless." However, it can also be argued that immobility rather than being a manifestation of defeat could also be a very effective adaptive strategy.

Face validity is neither necessary nor sufficient to establish a model. Other forms of validity such as etiological, construct, and ethological validity are essential to raise an experimental manipulation of animal behavior from a reasonable and analogous description of abnormal human behavior to a more powerful heuristic and predictive tool with which to study the neurobiology of behavior and help predict the potential therapeutic effects of novel drugs. Etiology refers to the causes or origins of pathology, and ideally, if one knew the causes of psychiatric or neurological disorders and could reproduce them in an animal, the discovery and development of novel therapeutic agents would be a much more straightforward endeavor. Unfortunately, the causes of complex behavioral disorders are unknown. Indeed, even within neurological indications where the causes of neurodegeneration are better, though incompletely understood, causal relationships between neurodegenerative factors and behavioral changes, including cognition, are not clearly established.

Although the etiology of complex disorders is poorly understood, one could argue that one of the great strengths of animal models of human mental disorders, and hence their great value, is their ability to associate biological processes with changes in behavior. Though variously defined by various investigators, construct validity deals with the consistency or the relationship of a given psychological construct, such as intelligence, anxiety, and depression, with scientific rationale thought to reflect the construct. Construct validity is closely related to the concept of behavioral homology, which is the

commonality of animal and human behaviors dependent upon similar molecular, cellular, and system biology. For example, schizophrenia is associated with abnormalities in dopaminergic, serotonergic, glutamatergic, and nicotinic function. These functions can be measured in man and in animals neuroanatomically, biochemically, electrophysiologically, and behaviorally and can be subsequently or contemporaneously associated with changes in behavior in schizophrenic individuals. An animal model that recapitulates these biological changes reliably can be said to be a model endowed with increasing construct validity.

Predictive validity of an animal model of a behavioral disorder, as discussed above in the context of clinical pharmacological efficacy, has lately become an unknown quantity. It has not always been so. The major pharmacological interventions for depression and schizophrenia may well have been discovered by serendipitous observations of clinicians of the effects of drugs had on the mood or behavior of their patients. However, knowledge of and subsequent examination of the mechanism of action of these drugs established the importance of the role of the monoamine neurotransmitters, dopamine, norepinephrine, and serotonin in the expression and treatment of these disorders. The biogenic amine theory of mood disorders proposed by Schildkraut and Kety in 1967 was based upon the mechanisms of action of drugs such as iproniazid and imipramine, which potentiate the effects the monoamines dopamine, serotonin, and norepinephrine. The dopamine-blocking properties of antipsychotics such as chlorpromazine and haloperidol are related to the positive, that is, hallucinations, illusions, and delusions, symptoms of schizophrenia. Having clinically effective drugs and knowledge of their mechanism of action provide the basis of the major tool to test the predictive validity of animal models for predicting CNS therapeutic activity of NCEs. This process of validation is called pharmacological isomorphism, and it consists of the comparison of the effects of an NCE with that of a clinically effective drug. Pharmacological isomorphism validation takes place throughout the drug discovery and development process from first establishing a proof of concept showing similarities in outcome in an animal model of a disorder of the NCE compared with the clinically effective drug to clinical trials of the IND where its effects upon clinical endpoints are always compared to the clinical golden standard.

The Drug Discovery and Development Process: Where Animal Models for CNS Disorders Add Value

In many ways, drug discovery is the process of describing function to a novel molecule, the ultimate function being that of efficacy in the treatment of the disorder for which the NCE is intended. Drug discovery and development

can be usefully described as a linear process illustrated in **Figure 1** through which a suitable molecular target is first identified and chosen, a lead compound from many that interact with this target is characterized and progressed for safety and toxicology studies and then submitted to regulatory authorities as an IND for entry into humans. There is a continual process of validation that takes place from the moment that a drug target is identified and selected to well beyond a drug candidate being finally registered and is in clinical use. Animal models add value to this process by intervening at various points. Furthermore, animal models and procedures are not static. As more information about the drug (and the disorder the drug is designed to treat) becomes available, this information feeds back to preclinical drug discovery and associated academic collaborators to help develop new, or refine existing, procedures in a reiterative process.

Target Identification

Drug targets are primarily proteins or protein products associated with a disease and with which a small molecule interacts to produce a beneficial effect. For a target to be druggable, a molecular target should be associated with disease and capable of interacting with the potentially therapeutic NCE. Drug targets can be chosen on the basis of literature, location in target organs, and the presumed relationship with the disorder. In addition, the selection of a particular target by a pharmaceutical company is also dependent upon strategic and economic considerations.

Traditionally, drug targets have focused on G-protein-coupled receptors, enzymes, and ion channels, and these comprise more than 80% of marketed drugs. The publication of the human genomic code plus molecular biological tools, such as microarray technology, gene deletion, and overexpression, or random mutagenesis, all contribute to the identification of novel targets. High-throughput screening methods are now commonly used to provide the initial hits or suitable starting compound(s) for the medicinal chemist to start a synthesis program. Many hundreds of molecules from propriety and/or public compounds are screened for affinity with the molecular target of interest (protein, RNA, or DNA), and those molecules that satisfy the physicochemical criteria imposed upon them are then progressed for validation.

Animal models and procedures developed over the past five decades can play an important role in the identification of novel targets. Forward genetics is the process by which the genetic basis of a phenotype is identified and investigated. Environmental or developmental models of abnormal behavior are being used successfully to induce reliable behavioral phenotypes to which genetic techniques such as qualitative trait locus analyses can be applied to identify and study the genetic and environmental basis of such phenotypes.

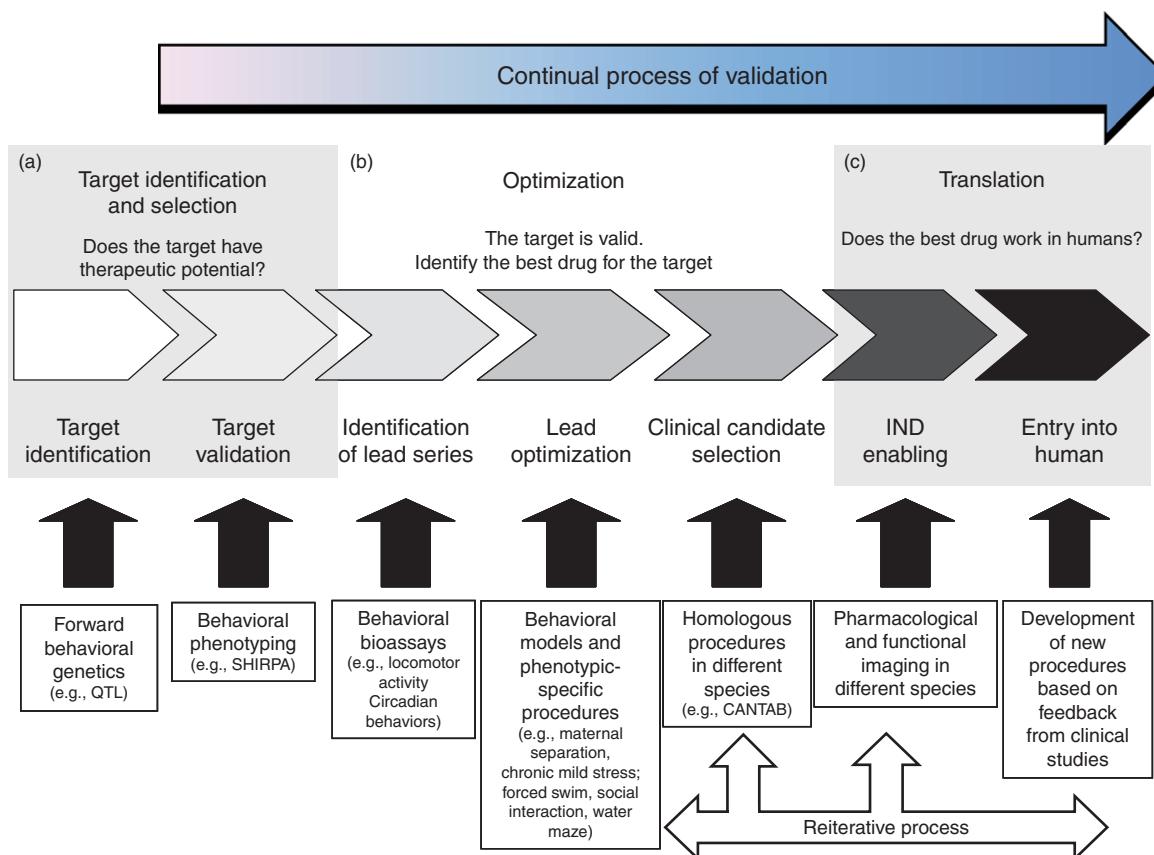


Figure 1 Points of intervention of animal models and behavioral procedures that add value to the drug discovery process during stages of drug target identification and validation, lead compound optimization, and translation. Adapted with permission from an unpublished slide provided by Dr. Guy Higgins.

Target Validation

Early target validation is essentially an interaction between the medicinal chemist and *in silico* and *in vitro* biologists, who will work together to identify the most promising lead series upon which to base the drug discovery efforts. Important activities at this stage include receptor-binding studies, interactions with relevant intracellular messengers, *in vitro* responses in isolated organs, etc. Animal models, particularly genetic models, play a very important role during this early stage of target validation. Once a gene or gene product is identified as a drug target, it is almost canonical that a mutant mouse will be engineered that will have the gene either deleted or over-expressed, and a process of reverse genetics of ascribing function to a gene is started. Gene-deleted mice, or knock-outs, have posed interpretational problems as compensatory changes can develop throughout the life span of the mouse. Technical modifications such as conditional knock-downs or the use of gene silencing through RNA interference have emerged as powerful tools to understand the contribution of that gene to behavior. Random mutagenesis is another technique through which changes in behavior are produced and need to be related to normal

behavior. Regardless of the molecular biological technique used to produce the mutant animal, behavioral procedures are used to identify and characterize the putative function of the gene. This process is known as behavioral phenotyping, and various protocols such as SHIRPA have been developed to standardize this process. A couple of words of caution, however, regarding the predictive ability of either the forward or the reverse behavioral techniques used for target identification or early validation. The background strain of the species used is a very important factor in determining the type and strength of the phenotype produced, and the behavioral specificity of the changes must be carefully evaluated. This latter caveat is particularly germane in assessing cognitive function as apparent cognitive impairments may be more parsimoniously ascribed to behaviors other than cognition that may also be modified by the mutation.

Animal models of function or behavioral bioassays are used in close cooperation with biochemistry and electrophysiology to identify the most promising chemical series to work on and to optimize one or two lead compounds that will eventually be proposed for further progression.

Certain pharmacological experimental manipulations can produce very clear and consistent functional consequences in behavior. For example, stimulation of locomotor activity can be used as a read-out of molecules interacting with the dopaminergic or serotonergic systems. Behavioral bioassays are routinely incorporated into the screening cascade of drug discovery projects. They are devised to measure the behavioral effects of an experimental molecule and help establish *in vivo* proof of mechanism. Bioassays are also extremely useful for revealing unexpected changes in behavior that could affect how the experimental molecule could affect the results of more complex models later on during optimization of a lead compound. For example, long-term recording of circadian behaviors such as feeding, drinking, locomotor activity, sleep-wake cycles, and their modification by drugs can be extremely informative. Bioassays therefore complement *in vitro*, *in vivo*, and *ex vivo* preparations; provide important information for structure-activity relationships of the molecule; and reveal potential side effects or alternate indications.

Lead Optimization

Once a promising lead is identified, the role of *in vivo* animal models assumes a more prominent role in characterizing function to the molecules followed by early examination of potential efficacy in a therapeutic indication. These models include early ADME studies to ensure that the lead compound is optimized in terms of getting to the right place, at a suitable concentration, for an appropriate amount of time, and that the metabolic fate of the compound is understood before it goes into man. It is also at this point that the animal models for CNS behavioral disorders are used intensively to provide the first evidence not only of mechanism, but also of potential therapeutic efficacy. It is very unlikely that model development and testing of lead compounds are done without reference to neurological and molecular correlates. Consequently, experiments are routinely planned to include imaging, biochemical, or electrophysiological measures hopefully on the same animals that have been assessed behaviorally.

As indicated previously, there is considerable controversy as to the predictive value of these animal models. Others and I have argued that some of this disillusionment is due to the siren call of models and procedures with high face value; added to this are the very clear differences in measures of outcome between animal and human testing. Clinical outcomes are measured in terms of reductions in the physical or behavioral manifestations of the disease/disorder. Clinically meaningful changes are usually measured by scales of clinical impression. Preclinical endpoints are typically alleviation or reversal of abnormal behavior or neuropathology by the NCE

compared to a clinically active standard. There is no way that we can get our animals to speak, but we are striving to find measures of behavioral change that are comparable across species.

A major change in biomedical research of mental disorders is the revision from syndrome-based to etiological and pathophysiological criteria. As part of this move away from syndrome-based criteria is the focus of identifying reliable phenotypes that underlie many types of behavioral disorders and can even be shared among them. This endophenotypic approach is helping to break down complex disorders into more manageable parts that can be studied and manipulated. Consequently, it is nonsense to speak of an animal model of a particular behavioral disorder, but rather one should consider these experimental manipulations as modeling homologous behavioral phenotypes. Behaviors indicating social withdrawal, lack of pleasure, or avolition are not only characteristic of depression, for example, but also of schizophrenia. The construct of anhedonia can be modeled, for example, in animals through the following prolonged experience of mild stressors (environmental model of depressed-like behaviors) or by neonatal hippocampal lesions, or juvenile social isolation; these latter considered neurodevelopmental models of schizophrenic-like behaviors. Changes in preference for sweetened solutions, rates of electrical self-stimulation, social interaction, or anticipatory hyperactivity are common readouts measured in these experiments.

In furthering the construct validity of these models, it is not enough to have analogous changes in behavior between the model organism and human. Ideally, these behaviors and underlying neurobiological substrates should extend across various species, from rodents, non-human primates to humans, and that the behavioral procedures used to measure changes should be tapping into the same processes across species. A clear example of the systematic development of procedures through which cross-species comparisons can be made has been provided by Robbins and his colleagues. Building upon the long tradition of modelling the effects of human brain lesions in primates they developed cross-species procedures using nonverbal stimulus modalities such as the presentation of geometrical shapes and use of touch-screen responses that enable the measurement of the effects of these manipulations. These procedures have been subsequently adapted for rodents, making them excellent translational tools as the independence of language based testing allows for meticulous cross-species studies of biological correlates and the effects of drugs on cognitive behavior. Furthermore, these procedures have been instrumental in tracking the course of disease progression in a number of neurodegenerative disorders such as Alzheimer's and Parkinson's, as well as providing preliminary indications of clinical efficacy.

Imaging and Biomarkers

The search for cross-species biomarkers is a major focus in translational research as these can be used to direct pre-clinical and clinical studies and to give early indications of potential clinical efficacy as well as potential toxicity of candidate drugs. Noninvasive imaging techniques such as positron emission tomography (PET) or magnetic resonance imaging (MRI) have become leading translational tools providing *in vivo* information about brain areas that are activated in response to mental states and processing. Imaging techniques can also provide information about disease state and progression. Wherever possible, once a lead compound is identified, PET radioligands are developed to investigate CNS penetration, target exposure, and occupancy *in vivo* and to relate this information to biological function. PET studies are also used in microdosing studies in which minute amounts of the radiolabeled compound can be administered in humans, and valuable cross-species relationships between animals and humans can be established such as dose adjustments and early ADME information. PET studies can also provide a proof of concept for novel targets.

In vivo imaging studies indicating the availability, distribution and exposure of an IND can be of immense help in deciding whether a compound should be progressed for further clinical testing, or in retrospect, exclude reasons for a clinical lack of efficacy. There is a high failure rate in psychiatric clinical trials due to response variability and placebo responses. It is not clear from these failed clinical trials whether the failure of the IND is truly lack of efficacy, or other factors such as whether target exposure had been achieved to exert a biological effect. For example, aprepitant was associated with a failed trial in which the active comparator, paroxetine, was not significantly different from the placebo. This lack of separation from placebo made it difficult to determine the true efficacy of aprepitant. The NK1 radioligand [¹⁸F]SPA-RQ was used to measure NK1 receptor occupancy in various species and eventually in human. These latter studies confirmed that the doses of aprepitant used in the clinical studies were sufficient to reach the brain, displace [¹⁸F]SPA-RQ, and block the receptor. These results indicated conclusively that the aprepitant was able to interact with its drug target and that this interaction was insufficient to produce an antidepressant effect. Aprepitant was subsequently withdrawn from further clinical development as an antidepressant.

Parallel to the advances being made in human imaging, small animal imaging studies are being conducted. Imaging studies reveal changes in brain structure and function underlying neurological and psychiatric disorders in humans that can be translated back in animal models. Small animal brain imaging can be used to examine the effects of drugs on brain structures or provide pharmacodynamic information of drug action. These effects can be related to the behavioral,

physiological, and temporal changes in the animal models and thus providing further construct validity. One major caveat, however, is those animals need either to be anesthetized or fixed in position during the procedure. Consequently, the results are influenced either by a pharmacological background or stress. Another caveat for both human and animal studies is that the observed changes in blood flow are only a poorly understood indirect measure of neuronal activity. With proper controls, however, small animal imaging studies are proving their worth in many phases of the drug discovery and development process.

The establishment of experimental medicine units within biopharmaceutical companies is providing an interface at which preclinical and clinical researchers interact. Biomarker development is an important activity that can involve both preclinical and clinical investigators. For example, imaging techniques are being used as possible biomarkers of neurological and psychiatric progression of disorder. Small animal imaging has been used in conjunction with genetic models of amyloidosis in order to observe the development of amyloid deposition in the brains of these animals, for example. These same animals could conceivably be used to track behavioral impairments as well as the effects of novel pharmacological interventions that can subsequently be related to changes in man. Another example of close collaboration between investigators is in the early detection of behavioral impairments or other forms of toxicity due to the novel compound. For the past decade, there has been considerable interest in the possibility of using immunological interventions for the treatment of disorders ranging from tobacco addiction to Alzheimer's disease. Initial enthusiasm for an active immunization approach targeting brain amyloid clearance that will maintain, or hopefully improve, cognitive function in Alzheimer patients was dampened by the development of meningoencephalitis in some patients. This has led to developing passive immunization approaches that should minimize this risk. However, immunological studies of passive anti-amyloid immunization in an appropriate genetic animal model indicate that meningoencephalitis can also develop in these mice.

Pharmacogenetics, or the individual responses to drug treatment, is a serious concern (but also an opportunity) to the medical community as well as the biopharmaceutical industry. Aside from the fact that some patients may not benefit from particular pharmacological treatment, individual responses during a drug trial may provide such variance as to suggest that the IND has no efficacy and consequently had be dropped from further clinical development. The recent phase II clinical trial of rosiglitazone (Avandia[®]) in Alzheimer's patients is an oft-cited example. This antidiabetes drug showing promise in a genetic model of amyloidosis, including improved cognition, failed to show any overall significant effect on ADAS-Cog scores. However, following stratification of the subjects according

to their APOE4 genotype, very clear improvements in ADAS-Cog were observed in APOE4-negative subjects. These results have justified further development of this drug in phase III clinical trials due for completion in 2009.

Established behavioral animal models of depressed-like behaviors such as the forced swim test, tail suspension, social defeat, and suppressed responding have been instrumental in identifying candidate genes, including *TREK1* (*KCNK2*), for antidepressant drug activity. It is significant that initial confirmation in a patient population is providing forward genetic validation of these models and the use to which they are put. Indeed, to quote the authors, "... this study is one of the first to examine candidate genes derived from mouse models of antidepressant response, validating the utility of such models for understanding the genetic basis of human antidepressant response" (Perlis *et al.*, 2008:2816).

It is apparent throughout this article that the topic of the value of animal models for predicting therapeutic action has focused strongly on animal models for behavioral disorders to the exclusion of neurological disorders. This is not to say that animal models for behavioral disorders have any more or less value than those for neurological disorders. Neurological animal models for symptoms of neurological disorders have been crucial for the discovery and development of pharmaceuticals for their symptomatic treatment. One can cite numerous examples of novel treatments for the control of seizures in the epilepsies, dopaminergic-based treatments for the tremors associated with Parkinson's, or cholinergic treatment for treatment of Alzheimer's disease. Nevertheless, the relief of these symptoms is transitory and can be related to side effects that limit their usefulness. Neurological disorders are closely related with neurodegeneration, and animal models, mostly genetic, have contributed greatly in the understanding of neurodegenerative processes. However, the clinical efficacy of novel treatments developed following activity in these models is still to be demonstrated.

In posthumous answer to the apocryphal Merck executive, and in response to the very present and concerned present day executives of biopharmaceutical companies, one would be hard pressed to demonstrate any real value of animal models based on the criteria of ultimate therapeutic activity outlined above. Nevertheless, a straight yes or no answer would be facile as there are many reasons why an IND fails to show efficacy in clinical trials. In a recent analysis of the apparent lack of congruence between pre-clinical and clinical outcomes of efficacy, using depression as an example, Borsini and I were able to identify at least six factors that can account for INDs being withdrawn from further development. These include an overreliance in the face value of behavioral models and lack of internal replication of results, design of clinical trials, placebo responses, genetic variations in response to drugs, and species differences in bioavailability and toxicology. Furthermore, at

least two further factors can be considered to account for the disappearance of INDs from the development pipeline: (1) lack of interest of pharmaceutical sponsors to continue developing certain drugs and (2) the reluctance or lack of interest in publishing negative results by the sponsors. Publication bias and the reluctance of pharmaceutical companies to disclose the reasons for withdrawal of individual INDs make a thorough analysis of the true predictive ability of preclinical animal models and clinical efficacy virtually impossible.

I hope, therefore, to indicate in this overarching commentary on the use and value of animal models that animal models have and have always had a very valuable and valid role in the drug discovery and development process. Without indication of *in vivo* function, it would be extremely difficult for novel compounds to be identified and optimized. Activity of a novel compound in animal models of abnormal behaviors provides an indication of the potential therapeutic activity of the compound, and as models simulate aspects rather than the totality of poorly understood disorders, it would be nonsense to suggest that activity in one test, or even five, guarantees activity in heterogeneous population of patients. What one can say with certitude, however, that without a much closer interaction between preclinical and clinical investigators sharing their expertise in a continuous process of model validation, the predictive power of any model will not improve.

One could conclude, therefore, that invalid models have no value at all, but that valid models have their limited value, and that is determined by the purpose to which they are being used.

Plus ça change, plus c'est la même chose.

(Alphonse Karr, 1808–90)

Summary

The value of animal models for predicting CNS therapeutic action has been questioned on the basis of a lack of clinical efficacy of potential drugs with novel mechanism of action despite promising preclinical activity. The causes of psychiatric and neurologic disorders are poorly understood, and models that attempt to simulate an uncertain etiology are imperfect at best. Animal models are being redefined in terms of genetics and phenotypes rather than syndromes. Within their limitations, the reliability and validity, particularly construct validity of animal models, are demonstrating their value by identifying novel targets and validating targets throughout the drug discovery and development process. Closer ties between clinical and pre-clinical investigators ensure the continual refinement of animal models not only in describing function to novel compounds, but also in the establishment of biomarkers and the study of pharmacogenetics.

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See also: Animal Models of Bipolar Disorder; Animal Models of Learning and Memory; Brain Imaging; Cognitive Decline in Laboratory Animals: Models, Measures, and Validity; Depression; Drug Addiction; Effects of Stress on Learning and Memory; Fear, Anxiety, and Defensive Behaviors in Animals; Feeding; Genes and Behavior: Animal Models; Human Fear and Anxiety; Knock-Outs: Learning and Memory; Maternal Deprivation; Measuring Stress; Mouse Genetic Approaches to Psychiatric Disorders; Motivation; Neural and Pharmacological Substrates of Aggression; Neural Basis of Recognition Memory in Nonhuman Primates; Neural Basis of Working Memory; Neural Substrates of Conditioned Fear and Anxiety; Neural Substrates of Unconditioned Fear, Defense, and Anxiety; Neurogenesis and Memory; Role of Gene Transcription in Long-Term Memory Storage; Schizophrenia; Stress and Brain Morphology; Stress and Emotionality; Stress and Energy Homeostasis.

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Genetics of Language

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Glossary

Abnormal spindle-like microcephaly-associated (ASPM) – A gene involved in neurogenesis that regulates brain size.

Developmental dyslexia or reading disorder (RD) – A disorder in children characterized by deficiencies in decoding written language.

Forkhead box P2 (FOXP2) – A gene that encodes a regulatory protein belonging to the Forkhead box group of transcription factors. Mutation of this gene has been implicated in an inherited speech disorder and anomalies of brain development.

Klinefelter's syndrome (KS) – A syndrome related to an extra X chromosome (47,XXY) in which the behavioral phenotype is characterized by language-based learning difficulties.

Microcephalin 1 (MCPH1) – A gene implicated in neurogenesis that regulates brain size.

Specific language impairment (SLI) – A neurodevelopmental disorder characterized by problems in understanding or producing language in the absence of causal factors such as hearing impairment, mental retardation, neurological deficits, primary emotional disorder, or social deprivation.

Speech sound disorder (SSD) – A neurodevelopmental disorder marked by developmentally inappropriate errors in speech production caused by articulatory and phonological processing impairment and resulting in significantly reduced intelligibility.

Williams syndrome (WS) – A genetic disorder caused by a rare spontaneous mutation affecting chromosome 7, associated with mental retardation but spared language function and verbal short-term memory.

languages, and the fact that language is acquired with such remarkable facility and regularity in children without explicit instruction. More recently, psychologist Steven Pinker has suggested that natural selection might have shaped the evolution of a human's 'innate grammar' and posited that this knowledge must be represented in the microcircuitry of the brain. According to this strong 'nativist' viewpoint, other animals do not have the capacity for language, and therefore language marks a preeminent trait of human evolution.

Opposing 'emergentist' views contend that many of the same observations attributed to innate knowledge of language can be observed in a system which is not innately structured. Proponents such as Elisabeth Bates and colleagues underscore the remarkably plastic and experience-dependent nature of cortical microcircuitry development and specialization, particularly with regard to language. For example, 95–99% of right-handed individuals are genetically predisposed to left cerebral hemisphere dominance for language, yet infants and young children who sustain damage to left hemisphere language areas succeed in developing normal or near-normal language in the right hemisphere of the brain. From an evolutionary standpoint, it has been argued that the qualified successes by Sally Savage-Rumbaugh and colleagues in teaching symbol-based communication systems to Bonobo chimpanzees (who share 98% of the same genes with humans) suggest that the divide between humans and our closest primate relatives may not be as wide as some believe, but rather reflect a gradation of communicative skills.

Models of the relationship between genes and language must make sense of a complex and temporally dynamic interaction between the genotype, environment, and epigenetic factors and must do so with reference to their influence on the brain. Conservative estimates have suggested that 40% of all genes in the genome are expressed in the brain; hence, thousands of genes may have direct or indirect functional implications on various aspects of language development. While a wealth of new information has emerged in recent years, our understanding of genetic factors that influence language and its development remains rudimentary, related in part to the complexity of language itself, as well as to changing tides in our understanding of how genes influence human behaviors. In the following sections, we review current evidence suggesting that genetic factors may play a significant role in the ontogeny of language in humans.

Background

Discussions of the genetics of language can be traced to Charles Darwin, who in 1871 remarked that "Man has an instinctive tendency to speak, as we see in the babble of our young children, while no child has an instinctive tendency to bake, brew or write." Advancing this notion nearly 100 years later, the linguist Noam Chomsky claimed that humans are born with an innate or hardwired knowledge of grammar that is apparent in observations that common grammatical principles are shared by all

Specific Language Impairment

SLI and the Genetics of Language

A prevalent approach to understanding genetic contributions to language has involved the identification of genes associated with disturbances of language development in children. Approximately 5–7% of school-aged children are designated as having a clinical condition known as SLI. This developmental disorder is characterized by problems in comprehending or producing language that occur in the absence of clear causal factors such as hearing impairment, mental retardation, gross neurological deficits, primary emotional disorder, or social deprivation. Although a broad consensus on standardized diagnostic criteria for SLI is currently lacking, the disorder has been a focal point for discussions of the genetics of language because of the relative specificity of the disorder to language development. This interest is based on the following rationale: if anomalies in a gene or a set of genes can be associated with SLI, which by definition implies normal cognitive development in other domains, then this provides some evidence that these genes are particularly important for language. However, the relevance of this approach to elucidating the genetic contribution to normal variation in language function remains open to question.

SLI: Heredity Studies

A first step in establishing whether or not genetic factors are involved in SLI requires establishing the heritability of the disorder. Several decades ago, Eric Lenneberg and others noted that delayed or deviant language development tended to run in families. Subsequent family studies have suggested that 24–63% of children with SLI have at least one family member who is also affected. While the familial occurrence of language impairment may reflect genetic influences, it may also be due to shared environment. A commonly used method to tease apart the relative contribution of genetic or environmental factors in language acquisition is to ascertain whether monozygotic (MZ) twins, who share 100% of their DNA, are linguistically more similar to one another than dizygotic (DZ) twins, who share only 50%. If MZ twins are more similar in language ability than DZ twins, then it can be inferred that this may be related to their greater genetic similarity.

When applied to SLI, the approach has typically involved examination of whether or not concordance rates for SLI differ between MZ and DZ twins. Twins are concordant if both have SLI, and are discordant if only one twin is language impaired. Comparing concordance rates provides an estimate of the contribution of genetic factors to the emergence of SLI. Theoretically, if SLI was

completely determined by genetic factors, MZ twins would have 100% concordance while DZ twins would have 50% concordance. Twin studies of SLI to date have almost uniformly indicated higher concordance rates for MZ twins compared to DZ twins. A metaanalysis by Karin Stromswold showed that for spoken language disorders, concordance rates averaged around 84% for MZ and 50% for DZ twins. The resulting overall heritability estimates from these and subsequent studies have ranged from 0.5 to 0.75, indicating a moderate to strong genetic contribution to risk for SLI. In general, concordance is higher the more severe the disorder although moderate heritability (39%) has also been observed for language ability in the normal range. Concordance is significantly higher for males compared to females.

While twin studies have proven to be a powerful technique, the heritability estimates derived from them are susceptible to a broad range of methodological issues (e.g., methods of ascertainment, diagnostic criteria, and definition of ‘impairment’) and theoretical assumptions. For example, twin studies assume equal exposure to environmental influences. However, some evidence suggests that MZ twins may have greater similarity of experience than DZ twins, potentially biasing estimates of the genetic contribution. Heterogeneity in the phenotypic manifestations of language impairment in children is a major complicating factor in their interpretation. Binary classification of ‘affected’ or ‘unaffected’ does not adequately reflect phenotypic variations since some siblings may fail to meet the criteria or cutoff for a diagnosis of SLI, yet demonstrate subthreshold disturbances of speech and language development.

The extent to which aspects of language (phonological, lexical, semantic, syntactic, and pragmatic) are compromised in individuals with SLI can vary. Recent studies suggest that genetic factors may not exert a general influence on language development but instead may play a role in the emergence of specific forms of language impairment that differentially involve distinct components of language. For example, reports suggest that language impairment associated with speech deficits of sufficient severity to warrant clinical referral may involve a stronger genetic component than language problems with preserved speech. Some aspects of language development appear more influenced by environmental factors than shared genetic influences. For example, data from the Twins Early Development Study (PI: Robert Plomin) suggest that at 2 and 3 years of age, vocabulary development and grammar share genetic influences but variation is more strongly accounted for by environmental factors.

In attempting to provide a more precise characterization of the SLI phenotype, some researchers have found it helpful to forego the use of global measures of language to define SLI and instead examine endophenotypes using biological or cognitive markers of vulnerability to the

disorder. This approach assumes that a complex behavior such as language may reflect the influence of genetic factors on one or a number of elemental abilities or neural processes that are more directly related to genetic influences and thus serve as better indices of the genetic risk of developing the disorder. Theoretical accounts of the essential problems underlying SLI have emphasized deficits impacting phonological and morphosyntactic development. These are evident in difficulties repeating meaningless but phonotactically legal words (e.g., 'perplisteronk'), deficits in auditory perception of rapid changes in speech signals, problems producing verb inflection, and errors in comprehension of marked syntactic structures such as passive voice phrases. Using such an approach, Dorothy Bishop and others have highlighted deficiencies of phonological short-term memory and processing of grammatical inflections. Both appear highly heritable but are subject to genetic influences that do not overlap significantly.

SLI: Karyotype Analysis

Karyotype analysis is used to detect specific chromosomal deletions, duplications, or translocations that may be associated with language impairment. Classic karyotype analysis can detect anomalies in the order of 10 megabases in size, while fluorescence *in situ* hybridization (FISH) can detect smaller anomalies in the order of 100 kilobases or less. In 2001, scientists at the Institute for Child Health in London reported that karyotype analyses (using FISH) in three generations of a British family (the KE family) with an inherited speech disorder disclosed an autosomal-dominant mutation in half of the members. The 15 affected individuals presented with verbal dyspraxia, an impairment in the ability to produce the coordinated oromotor movements required for speech. Their speech was effortful, inflections marking tense or number were often incorrect, and word order was commonly confused. In addition, they demonstrated poor comprehension of syntactically complex structures and performed poorly on a lexical decision task. The mutation involved a single gene in region 7q31 referred to as FOXP2, which stands for Forkhead box P2.

This first account of an association between a specific speech/language disturbance and an identified genetic deficiency created tremendous excitement in the scientific community, and it was argued that the finding was strongly supportive of the existence of a 'language gene.' However, subsequent investigations have discounted these claims. The apparent language problems appeared closely tied to their poor neuromotor control of facial muscles and difficulty pronouncing words. In addition, observations of nonverbal learning disabilities and significantly lower IQ in affected members compared to

unaffected members suggested that the effects of the mutated gene were not specific to speech or language.

The significance of FOXP2 mutations to understanding the genetics of SLI has also been questioned because it is relatively rare in the broader population of SLI children. Moreover, the affected members had structural brain abnormalities that are not normally seen in SLI and may even preclude the diagnosis. These anomalies involved portions of the left inferior frontal gyrus (Broca's area, a classic language center), the caudate nuclei, and cerebellum. Nonetheless, FOXP2's probable role in determining variation in oral facial skills required for articulate speech has been supported by findings that mutations of FOXP2, somewhat different from those observed in the KE family, also result in verbal and orofacial dyspraxia. Overall, current evidence suggests that FOXP2, like other FOX genes, may regulate the transcription of target genes (essentially turning other genes on or off), particularly genes involved in normal brain development. Mutations may indirectly cause a form of speech and language impairment because of resulting abnormalities in the development of frontostriatal and frontocerebellar networks required for speech production.

Consistent with this, the normal human-specific variant of the gene has been observed in Neanderthal DNA and thus dates to an evolutionary period associated with primitive vocal communication systems and the appearance of a human-like vowel space. In animals, disruption of copies of FOXP2 affects cerebellar development and vocalization. However, FOXP2 is active in several regions of the CNS and in other aspects of embryogenesis and neurogenesis (e.g., developing lung, and cardiovascular/intestinal and neural tissue). Vernes and colleagues have recently suggested that FOXP2 may downregulate CNTNAP2, a gene that makes a protein that enables intercellular interactions during brain development. Other genes relevant to language development that are active during neurogenesis and are developmental regulators of brain size are the MCPH1 (Microcephalin 1) and ASPM (abnormal spindle-like primary microcephaly-associated) gene.

SLI: Genetic Linkage and Association

Large research studies use a method known as genetic linkage analysis to determine which chromosomal regions contribute to the development of language impairment. In these studies, genotypes from multiple members across several generations of affected families are examined in an attempt to identify a genetic 'marker' that segregates with the disorder. A marker is a fragment of DNA sequence at a unique location in the genome that shows variation within the population. Markers commonly involve differences in single bases called single nucleotide

polymorphisms (SNP's). If linkage studies succeed in implicating a chromosomal region in a disorder, then genetic association studies are used to compare the frequency of specific markers in samples of affected individuals to the frequency of those markers in matched but unaffected controls. The goal of this comparison is to identify those markers that are more frequent in affected individuals.

The first genome-wide screen of SLI, completed in 2002, studied 98 families and found evidence for linkage of SLI phenotypes to 16q24 and 19q13. These findings were confirmed in a replication study in 2004 involving 86 new families. In a separate study with five extended families with SLI, evidence was found for linkage to 13q21. However, this localization was based on the analysis of reading discrepancy assessments in these families rather than a language-impairment phenotype.

Related Disorders

Speech Sound Disorder

Speech sound disorder (SSD) frequently overlaps with SLI and affects approximately 4–16% of children between 3 and 6 years of age. The condition is marked by developmentally inappropriate errors in speech production caused by articulatory and phonological processing impairment and results in significantly reduced intelligibility. These errors occur despite a well-formed oromotor tract, normal intelligence, and intact hearing, and are distinct from problems associated with mutism or stuttering. Synonymous terms for SSD have included articulation disorder or, more recently, phonological disorder. Quantitative measures of SSD symptoms are often based on assessments of consonant articulation, and repetition of nonsense and complex multisyllabic words. Recent genetic studies have linked SSD to 6p22 and 15q21. The linkage peak on chromosome 15 was located in region 15q14, adjacent to regions such as 15q11–13 that have been implicated in the Prader–Willi syndrome and in Angelman's syndrome. Both of these disorders are associated with articulatory difficulties and poor oromotor skills, but also include impairment in other domains. Other studies have shown linkage to a chromosome 3 locus (3p12–q13) which had previously been implicated in RD.

Developmental Dyslexia

Developmental dyslexia, also commonly referred to as reading disability (RD) or specific reading disorder, has a prevalence of 5–10% in school-aged children and has long been regarded as a familial and heritable disorder of neurological origin. Often related to SLI, multiple deficits have been proposed to underlie the disorder, the most

common involving: (1) deficits using grapheme-phoneme (letter-sound) rules (phonological decoding) or (2) deficits recognizing words by letter patterns or lexical units (orthographic decoding). Broadly, deficiencies in phonological decoding correspond to a type called 'phonological dyslexia' and orthographic decoding problems are characteristic of 'surface dyslexia'. Like SLI, heritability estimates differ according to how the phenotype is defined. Heritability is higher for phonological than for surface dyslexia. Genetic contributions to performance on tasks sensitive to problems with phonological processing (e.g., phoneme awareness and nonword repetition) are about 60–70% across different measures, with 30–40% accounted for by environmental factors.

Phonological processing problems have been most closely linked to a region on the short arm of chromosome 6. At least four other areas have been implicated in the genetic transmission of risk for dyslexia by genome-wide linkage studies. The first of these candidate genes was identified in 2003 on chromosome 15q21 and titled *dyslexia susceptibility 1 candidate 1* (DYX1C1, also referred to as EKN1). In 2005, three new candidate genes were proposed: ROBO1, DCDC2, and KIAA0319. DYX1C1 is expressed in several tissues in the brain, including cortical neurons and white matter glial cells. Beyond this, the function of DYX1C1 remains unclear. ROBO1 was discovered by serendipity in an individual with RD who demonstrated a translocation involving the 3p12–q13 region. This region is involved in the guidance of dendritic connections and interhemispheric axons. DCDC2 and KIAA0319 are located in the region of 6p22. They are thought to have a role in neural migration, and are expressed in brain areas that have been implicated in reading, particularly frontal, temporal and inferior parietal cortex in the left hemisphere. Overall, disruption to normal patterns of regulation of these genes, through deletion or duplication, appear to result in inadequate guidance of cortical neuronal migration as well as the development of axonal and dendritic connections during brain development.

Language Disorders in Genetic Syndromes

In addition to genetic studies of SLI and related conditions, advances in our understanding the genetics of language have emerged from studies of *de novo* occurring genetic variations. Specific genetic anomalies or syndromes, where language development has proceeded along a different developmental trajectory than other aspects of development, provide important opportunities to study the effects of genes on structure and function of the brain. Among these, Klinefelter's syndrome (KS) and

the Williams syndrome (WS) have been especially informative.

Klinefelter's Syndrome

KS is a relatively common genetic disorder (1/1000 males) defined by the abnormal chromosome karyotype 47,XXY. In contrast to X-linked conditions such as fragile X or Aarkog syndrome, which can result in mild to moderate mental retardation respectively, KS results in rather selective cognitive impairments particularly involving language-based learning difficulties. Delays in language and speech development are often detected by age 2–3 years as difficulties in articulating and structuring language output. These difficulties remain evident in some form at all ages. Later in development, significant impairments are frequently observed in higher-order aspects of expressive language, particularly in deficits with word retrieval, expressive grammar, and narrative formulation. Problems with receptive processing of language are evident in relative deficiencies in phonemic discrimination and in comprehension of syntactic and morphological aspects of language, as well as slow verbal processing speed. These early language impairments tend to predict language-based learning disabilities, including problems with reading, spelling, and writing.

Behavioral studies suggest that individuals with XXY show reduced hemispheric specialization for language, and recent structural neuroimaging studies have shown cortical thinning in left inferior frontal as well as temporal regions of the brain. Functional neuroimaging studies have revealed decreased asymmetry in activation of the superior temporal gyrus (STG) and the supramarginal gyrus (part of Wernicke's area). Such findings have suggested that genes on the X chromosome may be involved in hemispheric specialization for language.

Williams Syndrome

WS is caused by a rare spontaneous microdeletion affecting chromosome 7. It is often included in discussions of the genetics of language because, at first blush, it appears to constitute the opposite pattern to SLI. Children with WS have relatively spared language function and verbal short-term memory, in the context of manifest impairments in visual-spatial and social-pragmatic skills, attention deficits, and heightened anxiety levels. Yet, analyses of the linguistic and cognitive capacities in WS have revealed a more complex picture. While children with WS learn to speak fluently and with rich grammatical structure, their lexicon may be somewhat restricted to simple, concrete expressions (i.e., significant reduction in use of abstract relational terms), suggesting that cognitive impairment is also reflected in their language development.

Evolutionary Considerations

Grammatical language is thought to have its origins in genetic changes that influenced brain development between 100 000 and 50 000 years ago. A 'macro-mutational' viewpoint suggests that changes in a single gene less than 100 000 years ago may have resulted in a cascade of neurological changes in the brain that culminated in the emergence of modern language capacities. Alternatively, micro-mutational theories suggest that a series of small or gradual genetic changes resulted in alterations in brain function (e.g., increased computational efficiency) that cumulatively allowed for encoding of human-specific behaviors such as language. Language came about when speech and syntax were added to older communication systems and putatively emerged in the left cerebral hemisphere from a disposition towards right-handedness and the use of gesture.

Interestingly, a new sign language created by deaf Nicaraguan children over the past 25 years has provided an opportunity to examine the inception of universal hallmarks of language. Researchers found that in creating the language, children analyzed complex events into basic elements, which they then sequenced into hierarchically structured constructions according to principles that were apparently independent of gestures accompanying speech produced by hearing individuals in their surrounding environment. Successive learners adopted and extended this procedure, ultimately transforming the signs from a gestural form of communication into a linguistic system.

Contemporary studies suggest that differences in recently evolved versions of two genes may influence preferences in how humans code language in speech. Broadly speaking, 'tonal' languages such as Chinese use pitch changes to convey differences in the meaning of words, while nontonal languages such as English are more dependent on cues present in vowels and consonants and their arrangement to signal comparable changes in meaning. Mutations in genes called ASPM and Microcephalin that began to appear roughly 37 000 years ago and believed to affect brain development (discussed earlier) were more frequent in populations using nontonal languages. As this difference could not be explained by historical or geographic factors, the direction of this effect suggests that tonal languages may have older origins.

Conclusion

Significant advances in genetics coupled with improvements in understanding language and its disorders have together revolutionized our understanding of the genetics of language in the last two decades. The evidence for a moderate to strong genetic contribution to speech and

language development is overwhelming, although recent studies suggest that this may overlap significantly with genetic influences on nonverbal cognitive abilities. As the field rapidly evolves, it is likely to transform our current understanding of these relationships in the coming decade.

See also: Animal Models of Learning and Memory; Development and Language; Developmental Neurogenesis; Dyslexia (Developmental); Evolutionary and Developmental Issues in Cognitive Neuroscience; Human Evolutionary Genetics; Language and Communication – Brain Substrate; Neural Basis of Working Memory; Sex Hormones, Mood, and Cognition.

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Human Evolutionary Genetics

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Human Evolution: Biological and Cultural

Human evolution starts about 6 million years ago, the approximate estimate of our separation from the line leading to chimpanzees, the nearest primates. The first important difference seems to have been the bipedal stance, perhaps accompanying descent from the trees to the savannas of Central Africa. The immediate advantage may have been that of giving higher running speed, useful for catching prey and flying away from dangers, but the freeing of hands it permitted helped greatly a major exaptation (a secondarily appearing adaptation): tool making. The oldest tools collected by archeologists are stones modified by breaking them so as to use the cutting edge thus produced for various purposes, for example, opening bones of dead animals and eating their bone marrow, which is considered a delicacy still today. They are at most 3 million years old. However, tools made of wood and other less durable material could not survive. There were, however, other signs of heightened intelligence: the increase of brain size was already on its way. Starting from 350 ml, which is still standard in all the nearest primates, it increased to the present value of 1300 ml in perhaps irregular ways (e.g., Neandertals, now extinct, had bigger brains than modern humans). There were physical signs that language was already beginning: at the time the first tools were made, five out of six skulls had a larger size on the left side of the brain, in the Broca region. The biological basis of language must have anyhow progressed over a long time, and perhaps the final major steps took place in the small East African tribe that was responsible, before it started for the Out of Africa spread that began 60 000 years ago. The main evidence for this is that any living human population can learn without difficulty any other existing human language, and therefore the necessary biological machinery was already present in basically all members of the founder population before it spread. However, language evolves much faster than genes and the 6000 or so languages in existence today may be the last remnants of the societies (the tribes) that were generated during the great demographic expansions. The first was between 60 000 and 10 000 years ago, at which time most of the world had been settled under an economy of food collection (hunting-gathering), and saturation with that economy had been reached. The world was then populated by roughly 1000 times more than the initial East African tribe that started

and produced the Out of Africa expansion. Demographic saturation of some especially rich areas (Middle East, China, Mexico, West Africa, and New Guinea) stimulated the development of food production (the agro-pastoral era). From these centers the new economy spread around at a rate about double that of the Out of Africa expansion, and today we have reached another saturation level, with a population size roughly another 1000 times greater than that when domestication of animals and plants began.

Language

Language was probably fully mature at the time of the origin of modern humans, and certainly had a fundamental place in the evolutionary success of our species. It would seem that another neurobiological function was also more highly developed in humans than in the other primates: the form of imagination that can lead to inventions that, if accepted, can rapidly change individual and social life. Communication permitted by language increased the efficiency of cultural transmission, the transmission of knowledge, and especially new knowledge created in every generation, and today the rate of spread of new information has practically the speed of light.

It may be useful to specify that the term 'culture' is used to indicate whatever knowledge and behavior is learnt from any sources during life, and thus includes also the novelties that are generated and transmitted. This is basically the simple idea that Lamarck used for explaining evolution, but today we know that biological evolution depends on transmission practically limited to that from parents to children, and therefore it takes many generations before a biological novelty (generated by genetic mutation) can spread to the whole population. While biological change deals with chemical, very simple changes of DNA, which are rare, spontaneous, and random, the cultural hereditary patrimony consists of novelties in our knowledge and behavior, that we can simply call, somewhat vaguely, 'new ideas,' and take, in cultural evolution, the same place that genetic mutations have in biological evolution. A first difference is that, unlike genetic mutations, new ideas are not random but aimed to give a specific result, which is hopefully useful to the people being taught, and/or (perhaps) to the inventor.

The other major difference between cultural, and biological evolution, is transmission. Biological evolution does transfer knowledge only from parents to children;

in culture the same mechanism has an honorable place (vertical transmission). When it is the only cultural transmission mechanism for specific traits, it may prove as conservative as biology. Certain types of information, for example, the religious one, and many other behavioral customs are learnt and more easily accepted more or less irreversibly during early ages of life. However, culture is transmitted also from anybody to anybody (horizontal transmission) irrespective of relationship, age differences, etc. Moreover, there are social institutions like schools or governments that impart instruction to large groups of individuals, and teachers or leaders of great influence because of intelligence, activity, power, or simply charm of the teacher/leader. Technologies such as writing invented between six and thousand years ago, has greatly increased the power of past knowledge: we still learn directly from Aristotle or Moses, but the ease of printing and using other mechanisms of transmitting information like radio and television have enormous power to influence large masses of people.

Natural Selection

The human species, like all others, is under control of natural selection, that is, it is governed by demographic laws such as survival and fecundity: individuals who create more of the next generation are those who survive more easily until maturity and/or are more fecund. The rate of evolution under natural selection depends on the variation of individual reproductivity (darwinian fitness, a measure of the adaptation of an individual, or of a genetic trait), which is a function of survival and fecundity of individuals. Natural selection thus adapts automatically a population (and the ensemble of populations forming a species) so as to guarantee optimal reproduction: living organisms are mechanisms capable of reproducing themselves, and their capacity to prosper is tantamount to their capacity to reproduce, that is, generate descendants that look like the parents in essential traits. For traits that are not affected by natural selection, the rate of evolution is equal to the mutation rate, and mutation is a rare, spontaneous, random event that is mostly neither advantageous or disadvantageous but selectively neutral. Under biological evolution alone, corrections and improvements take place by mutation and natural selection, but today we try to correct with our own medicines and decisions the problems that we can diagnose, and therefore we evolve mostly by cultural evolution. We also produce new problems for ourselves by cultural evolution. The corrections we try may produce benefit but, like any change, they will also have costs. Eventually, also cultural changes fall under the sickle of natural selection, if they decrease survival or fecundity.

A paradigmatic example is a problem called lactose intolerance, that is produced by milk consumption in

adults. Milk contains a sugar called lactose that babies of all mammals can digest and use to exploit a fair amount of the energy contained in milk. This is made possible by an enzyme called lactase that all babies produce, but after weaning everybody stops producing. Animal domestication of mammals by humans has stimulated also adult humans to use milk produced by domesticated mammals, but like all mammals humans do (did) not produce lactase after weaning. It happens that the indigestible lactose contained in milk taken by adults produces medical symptoms of a condition called 'lactose intolerance.' However, genetic mutations have arisen that repress the stopping of lactase production after weaning, and therefore make individuals lactose tolerant for all their lives. One of these mutations originated in the Ural mountains about 6 thousand years ago, in a population of reindeer shepherds who had developed the use of milk as food by adults. In a cold region it was a great benefit to be able to use the extra calories of milk without medical problems, and therefore natural selection greatly favored the custom arisen by mutation and transmitted to direct descendants of the original mutant. Practically all the reindeer shepherds thus became tolerant in a reasonable time because of natural selection, and the adult lactose-tolerance mutation spread to neighboring countries by mixed marriage. Today almost all Scandinavians show lactose tolerance, which is common also in most northern Europe, but is less and less common if we study the rest of Europe. Diffusion takes time and moreover in the south of Europe natural selection in favor of the mutant is not so impressive: in south Italy tolerance is 25%, in Sardinia 20%.

This is an example of biological and cultural coevolution with a relatively uncomplicated interaction. In animals like mammals which live on lactose containing milk lactase, at least in their junior years, production may have lasted all life. However, there is an economic advantage in general by not producing something that is not needed (and may even be disadvantageous). Thus, biological evolution of the mutation determining 'lactase production after weaning' may have spread early enough in the origin of mammals hundreds of million years ago. But cultural evolution generated the use of milk by human adults as a secondary effect of the domestication of medium size animals, a custom which had benefits (continuation of the greater energy production permitted by lactose use, especially in the cold); but also costs (lactose intolerance) for the rest of the population. It could be later eliminated by further genetic evolution. There are probably many other similar examples, and a fundamental cultural process, the introduction of agro-pastoral economies, was probably a major factor in determining genetic or cultural processes, or mixed ones like lactose tolerance, with increasing benefits and decreasing costs. They must have been quite different in the different areas of agro-pastoral economies.

The Four Major Factors of Biological Evolution

All novelty in biological evolution is determined by mutation: natural selection is the key to adaptation and therefore the maintenance of life through survival and fecundity, but there are two other major factors of evolution which also depend on demographic quantities: random genetic drift (or simply drift) and migration. Natural selection is the only source of adaptation, which is formally measured by darwinian fitness, but biologically we are also interested in knowing the specific anatomical and physiological mechanisms that are responsible for greater or lesser reproduction of individuals possessing given traits. Thus, a dark skin color helps resisting higher temperatures determined by more solar radiation, because where the amount of ultraviolet radiation is increased, skin cancer is also increased. Therefore, skin color is darker where latitude is lower. However a peculiarity, white skin, is another case of natural selection due to a cultural novelty. Skin is white in Europe because wheat, the major food of Europeans, introduced somewhat before 10 000 years ago in a small village at the boundary between Turkey and Syria, does not contain vitamin D. Wheat became a major food with agriculture. If vitamin D is not contained in sufficient proportions in the diet, bones do not grow properly, and serious, life-limiting rickets is generated. But wheat does contain a precursor which can be transformed into vitamin D under the skin, under irradiation by enough ultraviolet. A dark skin prevents the sun ultraviolet (UV) from reaching the derma under the skin where the enzyme is found, and mutations to white skins have been formed and spread where they are useful, in the north of Eurasia for wheat eaters.

Drift

Drift is the consequence of statistical fluctuations of gene frequencies due to the finite size of populations. It is measured by the number of reproducers per generation, N , which is about one third of the population census for human populations. The smaller is N , the greater is the genetic variation among populations, estimated by variance measurements, and the smaller that within populations. Variation within one population is also measured in a very simple way also by another quantity called genetic diversity, which is the estimate of heterozygosity of the individuals forming the population, averaged over all genes.

Migration

Migration among populations reduces diversity among populations, that is, it has the same effect as increasing N , and therefore one often considers these two factors together by uniting them into the quantity Nm . When

Nm is high, genetic diversity among individuals of a population is high, and that among populations low.

Founders' Effects

The greatest effects of drift are seen for rare recessive genes. The most famous is Tay Sachs disease, which causes blindness, dementia, and death in the first year of life in children born perfectly normal. Healthy carriers of the disease (heterozygotes) are 1/25 among Ashkenazi Jews in New York, and the incidence of Tay Sachs is one in 2500 births. The disease is absent or extremely rare elsewhere, and also in other Jewish ethnic groups who, after the Jewish diaspora, peopled world parts other than N. Europe. There were many millions of Ashkenazi Jews in N. Europe before the Holocaust, but they probably originated from a very small group that migrated from Rome to N. Europe in the Middle Ages. Fifty percent of them descend from just four women, but there was very substantial growth that generated the several millions of Jews living in N. Europe before the Holocaust. One founder of Ashkenazi Jews, or more probably one of their descendants not many generations after the founding, was a heterozygote for a mutation of the Tay Sachs gene that, being recessive, multiplied undisturbed in children and later descendants of the mutant. Under these conditions, if a heterozygote marries a fully normal individual, their children have a 50% chance of being heterozygotes. At some later stage two heterozygotes may marry with each other and, according to Mendel's expectations, one out of every four of their children is expected to show the disease.

Today this terrible disease has practically disappeared, thanks to prophylactic abortion of fetuses diagnosed as diseased. Conservative rabbis who do not accept prophylactic abortion suggest that heterozygotes should take a DNA test before marriage, and avoid marrying together if they are both heterozygotes. Many other rare genetic diseases can be diagnosed by simple DNA tests. Two populations – Quebec in Canada, and Capetown in S. Africa – were generated by a relatively small number of founders. Both colonies started approximately around 1650, by numbers of colonists of order of 2000. Records of marriages of their descendants exist for both. Among descendants a few genetic diseases or minor traits are found that are very rare or unknown elsewhere. The founders or early descendants who carried the mutants have been identified by the reconstructed genealogies.

Worldwide Phenomena Showing the Quantitative Importance of Drift

Drift is not noticeable only for these observations on rare genetic diseases, but is responsible for much more general phenomena. An effort of whole word analysis of human

populations, the Human Genome Diversity Project, has generated a collection of cell lines totaling about 1000 individuals, coming from 52 aboriginal populations of the five continents. It is deposited at the Fondation Jean Dausset of Paris (HGDP-CEPH). DNA produced from the cell lines is available to research workers. In order to avoid DNA patenting that would hinder research, pharmaceutical factories have not access to these DNAs, and all results must be published. One very clear result obtained with 783 micro-satellite genes, and confirmed with 650 000 single nucleotide polymorphisms of the Illumina microarray is that there is a very regular linear fall of genetic diversity, measured as heterozygosity, as a function of geographic distance from the likely place of origin of the population of ~1000 East African hunter-gatherers that started the Out of Africa expansion and occupied the whole world. The most likely explanation of this fall of genetic diversity is a phenomenon called the serial founder effect. It is assumed that the expansion occurred as a sequence of steps, each of which consisted of the foundation of a small colony of the furthest population, farther away from origin. The number of steps between the East African origin and the most extreme periphery occupied (in Chile) must have been in the hundreds, on the basis of simulations that used demographic data in agreement with archeology and anthropology information. Each step must have determined an episode of founder effect, as the numbers leaving the mother colony for founding a daughter colony in unsettled territory in the immediate neighborhood were probably small.

Migration Rates during the Recent Expansions of the Human Species

The migration m mentioned earlier refers to the proportion of population exchanged between two neighboring colonies. Another migration rate, that of expansions of the human species, can be estimated. The first is the hunter-gatherers (HG) expansion from East Africa to the furthest place, Chile, passing through the Bering strait. This distance is $c.$ 25 000 km, and was covered in 50 000 years, i.e. 0.5 km per year. When the world was settled and the hunting-gathering economy was replaced by at least four independent developments of agro-pastoral (AP) economy (in the Middle East, E. Asia, Mexico and West Africa, in order of time, from 11 000 to 4000 years ago) the rates of migration of km/year of these farmers expansions was greater (1 km a year and more). At the time of the agricultural expansions all technologies were more refined, and movement was not in an essentially unsettled territory like the HG expansion (at that time the world outside Africa was occupied by *H. neanderthalensis* in Europe, and very sparse *H. erectus* and *H. flores* in Asia, which must have had very low population densities. It

seems in both cases there was no sign of conflict or admixture of *H. sapiens* with the earlier inhabitants, in agreement with the hypothesis that they all belong to different species that developed after a very first diaspora from Africa of the genus *Homo* some two million years ago. The farmers' expansions were in territory previously settled by hunter-gatherers, but there was probably frequent intermarriage between the new settlers and the residents, generating a peculiar admixture with a gradient of relative frequency.

The same microsatellite and the 650 000 Illumina data used for the serial founder effect were also analyzed for the correlation between genetic and geographic distance of population pairs. The latter are estimated as the crow flies (but via Bering for comparison between an American and a non American individual). The correlations (0.89 and 0.91) are among the highest observed in any biological phenomenon. Simulations showed they could be interpreted assuming that quantitatively the major evolutionary factors were drift, migration, and mutation, and that the effect of natural selection determining differences among populations was rather modest. Selection examples have been shown to be present but are mostly relatively local events. The adaptive reason of most remains to be explained.

Evolution of Major Neurobiological and Sociobiological Traits and Syndromes. General intelligence

Most normal and pathological traits do not behave as simple mendelian traits, but nevertheless many and perhaps most have at least some partial genetic component. A measure called 'heritability' has been introduced to indicate the percentage fraction of variation of the trait that can be due to genes. It is usually calculated on the basis of correlations between relatives of various degrees, from twins of various types to parent-child, sib-sib, and less close relatives. A simple classical theory shows the expected quantitative relations between these correlations and helps to give a rough analysis of the accuracy of the heritability estimate.

An analysis of the intelligence degree, originally suggested to help the discovery of presumably retarded children needing education in special schools, has later been further developed in the idea that it can evaluate 'general intelligence.' The quantity measured is called IQ (intelligence quotient), and a large number of testing methods were advanced, which tend to give similar results and are correlated with success in schools, as judged by scholastic performance. The IQ test has been widely developed and standardized for age, and also for sex, since girls tended to give higher average scores (perhaps because of higher precocity). Correlations of high IQ

and success in various professions gave rise to over-interpretations, and excessive confidence that it might measure genius. In reality, most interesting geniuses have rather a highly specialized intelligence (e.g., in music, mathematics, philosophy, various forms of science, art and literature, and even politics) and are often pathological in other respects (being schizophrenic, or autistic, or having other behavioral abnormality). One of the two men who revolutionized genetics by discovering DNA structure gave a serious blow to the idea that all geniuses have extremely high IQ, by making it known that his IQ did not even reach the 5% upper level. IQ is correlated clearly with reasoning ability and capacity to learn, but exceptional success in life depends more often on exceptional ability in some specific direction, rather than in the average skill needed in scholastic work.

Study of the heritability of IQ gave very high values, especially if based on data of identical twins. However, the extension of studies showed that socio-cultural inheritance has considerable importance, also for identical twins. Trials to answer this objection were made by testing identical twins reared apart, but they are very few and often far from answering the conditions necessary to accept their validity (e.g., many of those reared apart actually lived in the families of close relatives). The extraordinary interest of identical twins to keep close ties leaves a feeling of doubt that the results are somehow influenced by the desire, probably subconscious, that the resemblance is very high. More accurate tests of the environment and statistical analyses using many degrees of relationship concluded that three causal factors have about equal power in IQ: (1) genetics, (2) socio-familiar environment, (3) special unpredictable events of various nature in individual lives. It is interesting that in the beginning two very high quality research groups who analyzed a vast body of published data with the most advanced methods came to quite different estimates of heritability. Later one group withdrew its conclusions and replaced them with results very near to those of the other group, which are those summarized very concisely above. Very few other intellectual skills were examined so extensively, but the most serious and common psychiatric diseases like manic-depressive syndromes, schizophrenia and autism do certainly have both genetic and socio-cultural components. It may be that in all very common disorders and physical and intellectual traits causal factors may turn to give not very inspiring results similar to the above ones. These methods however rely on one simplifying assumption that may obscure or cancel many real and interesting complications: they assume all factors interact additively. One sobering consideration comes from results in the study of a measurable quantity in yeasts: temperature resistance, which can be important

for the pathogenic effects of individual strains. In an organism genetically as malleable as yeasts, in which all possible combinations of genes can be artificially created and their phenotype tested, it was found that different sites even within one gene can have oppositely interacting, reciprocally neutralizing effects. In the human species, in which experimentation of the same kind would be required, one is obviously more limited to carrying out certain types of analysis at a high genetic resolution. Such complicated interactions will probably be studied also in humans, but will be limited to traits that can be studied *in vitro*, in practice only on human cell lines, or *in vivo* using animals in which one can transfer human genes.

In spite of all these difficulties, in the case of some of these highly polygenic diseases which have strong cultural and environmental components, when the DNA of each individual was analyzed, it was possible to identify some DNA segments that have partial but reproducible phenotypic effects. In this genome era one can expect that progress will be rapid, but the analysis is very expensive and is time consuming even with very powerful computers, because of the number of interacting factors, and of the number of DNA units involved and their size. It will also most probably require large numbers of patients for each disease. Nevertheless, a new field is open for the brave.

See also: Genetics of Language; Genetics of Memory in Drosophila; Mouse Genetic Approaches to Psychiatric Disorders; Sleep Genetics.

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Molecular Psychology of Personality

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Glossary

Gene polymorphism – A variation in the DNA sequence that occurs in more than 1% of the population.

Although quantitative behavioral genetics has long established the heritability of personality traits, the underlying mechanisms responsible for individual differences across a broad set of complex behaviors have been poorly understood. Starting with molecular genetic discoveries in the 1990s, and the advent of genomic imaging or imaging genetics in the early years of the new millennium, these mechanisms are now accessible to study. In this article, we give a broad overview of how molecular biology and neuroimaging have advanced our understanding of the biological basis of personality, and briefly discuss our view of the future direction of the field.

Genetic studies of personality traits have to address four concerns. First, most conceptualizations of traits, for example, impulsivity, are not rooted in biology. Second, any one trait may be affected by multiple genes with small effect sizes quantitative trait loci (QTLs), as illustrated by studies of intelligence and neuroticism, and any one gene may participate in more than one trait. Third, gene expression can be regulated by environmental variables, including life experience, and may involve epigenetic or other regulatory mechanisms that are still poorly understood. Fourth, the scale of these regulatory changes may be very large, affecting multiple hundreds of genes that participate in complex molecular signaling pathways, posing analytical challenges that can only be addressed with advances in bioinformatics. These four concerns are central to the emerging field of ‘molecular psychology,’ which focuses on the molecular mechanisms underlying complex behaviors. In the remainder of this article, we highlight the current state of molecular psychology with respect to the study of personality.

Associations between Personality Traits and Individual Genes

Molecular psychology’s birth year may be 1996, when a set of studies was published that first reported significant associations between specific gene polymorphisms and personality traits. The first study reported an association

between a common variation within the promoter region of the serotonin transporter gene (5-HTT) and the personality traits of neuroticism and harm avoidance. In this study, higher levels of self-reported harm avoidance or neuroticism were associated with the presence of the short allele, caused by a deletion polymorphism that is located in the 5' promoter region of the gene (5-HTT linked polymorphic region, 5-HTTLPR). Functionally, this short allele is associated with reduced levels of 5-HTT, suggesting that the genetic regulation of serotonergic tone is associated with these personality traits. A large number of replication studies have produced conflicting results, but two meta-analyses concluded that the evidence supports a small but significant association between the 5-HTTLPR polymorphism and neuroticism. Indeed, even the original study found that this polymorphism could account for only 3–4% of the total variance, suggesting that many more genes may be involved in the regulation of this personality trait.

Also, in 1996, two other studies reported associations between a common variation in the dopamine D4 receptor gene (*DRD4*) and the personality traits of extraversion and novelty seeking. The polymorphism was a variable number tandem repeat (VNTR) located in exon-III of the gene, with the most common variants being 2-, 4-, and 7-repeats. Functionally, these repeats differ in their efficiency in response to dopamine. The 7-repeat allele was shown to be less efficient than the 2- and 4-repeat alleles and the 10-repeat allele was shown to be more efficient than the 2-repeat allele. The 7-repeat allele, in particular, was associated with elevated levels of extraversion and novelty seeking. In addition to the VNTR, the *DRD4* was also shown to have a single nucleotide polymorphism (SNP) (C-521T) in its promoter region, which causes a 40% decrease in the transcription of the gene with the T allele compared to the C allele. Presence of the C allele was associated with higher extraversion and novelty-seeking scores. Subsequent replication studies produced conflicting results and two meta-analyses concluded that there was no association between extraversion and either of these *DRD4* polymorphisms. However, one of these meta-analyses did conclude that there was evidence for a significant association between novelty seeking and impulsivity traits and the *DRD4* C-521T polymorphism. Similar to the 5-HTTLPR polymorphism, the gene accounted for only a small fraction (about 3%) of the total phenotypic variance, suggesting the involvement of many other genes in these traits.

The search for novel candidate genes for complex traits continues. For example, a SNP within the gene that regulates catechol-O-methyltransferase (COMT), which degrades catecholamines (including dopamine), may be associated with extraversion and/or neuroticism. The SNP is the Val¹⁵⁸Met polymorphism, which results in an amino acid change from Valine to Methionine and a 75% decrease in the enzyme's ability to degrade dopamine. Individuals with the Val–Val genotype were reported to have significantly higher extraversion scores, although no association with neuroticism was reported. However, another study, conducted on a female sample, reported a significant association between harm avoidance (a trait conceptually similar to neuroticism) and presence of the Met allele. Another study is consistent with this association, reporting a higher frequency of the Met allele in highly neurotic females, but not males, suggesting that sex is a critical variable.

Although other polymorphisms have been investigated, the discovery of single polymorphisms that show a consistent association with specific traits remains elusive. What is clear now is that the genetics of personality traits is complex, and likely to be affected by large number of genes and gene networks, any one of which may further interact with other genes or be modulated by environmental stimuli. Thus, researchers are increasingly looking for technological breakthroughs that will allow them to query very large numbers of genes, moving the field from individual candidate genes to whole-genome association studies.

Associations between Personality Traits and Whole Genomes

By current estimates, human genetic variation is about 0.5%, and studies devoted to cataloging and data-basing this variation are being conducted. Technological advances in gene microarrays are fast: in 2007, it was possible to probe for 500 000 SNPs at a time; by 2008, that number had doubled. To date, the cost per array is still relatively high, making it too expensive to use in very large-scale genetic studies. To address this problem, some investigators have used a method that involves pooling DNA samples from individuals that share a characteristic of interest, and compared the results of whole-genome analysis for this pooled DNA with that of a control group. In one such study, pooled DNA from about 2000 individuals (selected out of a cohort of more than 88 000 subjects) was used in a whole-genome association study of neuroticism. Surprisingly, this study failed to identify any SNPs that account for more than 1% of the variance. The paucity of positive results for whole-genome associations with personality traits gives us pause. As is always the case with null results, many reasons come to mind. We

listed four possible reasons in the introduction of this article. Even larger sample sizes, and even more sophisticated bioinformatics and data reduction approaches may be needed to identify relevant gene candidates. In addition, the choice of phenotype measures and the possibility of interactions with environmental variables may be significant factors that obscure a simple gene–trait association that many have sought. We turn to a discussion of these two factors next.

Endophenotypes and Imaging Genetics

The reliance on self-report has been a weakness in single-gene and whole-genome association studies of personality traits. The need for more objective phenotypes that can be linked more closely to genotypes was fulfilled by the use of endophenotypes such as cognitive-affective processes or localized brain measures. The power of the endophenotype approach is illustrated by the rapidly growing literature of functional brain imaging studies of individuals genotyped for the 5-HTTLPR polymorphism, beginning with a study by Hariri and colleagues, in which they reported a significant association between the short allele and increased amygdala activation in response to negatively valenced faces. While the interpretation of this observation continues to be debated, the basic phenomenon is very robust, and has been replicated consistently across many imaging studies, and recently confirmed by a meta-analysis. One reason for such consistency is that the effect size is much larger when genetic variation is mapped onto an endophenotype (which is closer to the level at which a gene operates) than a more distal phenotype, such as self-reported behavior.

Gene x Environment Interactions

In the first molecular genetic study to report a gene x environment interaction (GxE), Caspi and colleagues discovered that a common variation within the gene that codes for monoamine oxidase A (MAOA) moderates the effects of childhood maltreatment on aggressive behavior later in life. It was reported that maltreated children with the genotype that results in relatively low levels of MAOA are more prone to exhibiting antisocial behavior and conduct disorder, and are more involved in violent behavior. A meta-analysis confirmed these results, although the debate on the implications of this finding continues.

Paralleling the findings on early adversity and monoamine oxidase-A (MAOA) genotype affecting later behavior, it was reported that the effects of life stress on later depression are moderated by 5-HTTLPR genotype, such that carriers of the short allele have twice the level

of depressive symptoms, diagnosed depression, and suicidality than noncarriers do. Another study replicated this association finding, although later studies suggest additional moderating factors, such as social support, age, or sex. Indeed, a recent meta-analysis concluded that the GxE interaction may not be valid for studies using clinical phenotypes of depression. We think that endophenotypes, such as those based on imaging genetics, may provide more reliable measures of GxE effects.

A GxE interaction for 5-HTTLPR genotype was reported in an imaging study, which reported that carriers of the short allele exhibited greater activation in the amygdala (and elsewhere in the brain) as a function of life-stress experience. In these same individuals, more life stress also correlated significantly with higher levels of rumination, a risk factor for depression. Interestingly, noncarriers exhibited the opposite pattern: more life stress was associated with reduced amygdala activation and less rumination. The data illustrate that both carriers and noncarriers of the short allele may respond to life stress, but in opposite directions.

An attractive candidate for a molecular mechanism of these GxE interactions is ‘epigenetic programming,’ which causes changes in gene activity (turning on or off genes, altering their expression levels) that does not involve DNA sequence alterations. One such epigenetic mechanism involves DNA methylation, in which methyl groups are added to the cytosine bases on DNA, with the functional consequence of reducing or silencing gene expression. Indeed, there is now preliminary evidence for methylation differences as a function of 5-HTTLPR genotype. However, the best evidence for a GxE interaction to date comes from rodent studies that have shown that early maternal experience alters methylation of the glucocorticoid receptor gene in a manner that affects later stress reactivity and that is reversible through cross-fostering or through chemical agents that reverse gene methylation.

Conclusion

In this very brief survey of molecular psychology studies of personality, we present the evolution of a field that has moved from a search for simple associations of single candidate genes toward a search for whole-genomic variation and identification of interacting variables, as well as their underlying molecular mechanisms. Building on a decade-long and rich literature from behavioral and quantitative genetics, the first molecular psychology studies of 1996 and thereafter focused on discovering associations between single-gene polymorphisms and self-reported personality traits. Although there are some successes (most notably the discovery of the 5-HTTLPR polymorphism and its association with neuroticism,

which remains to date the strongest candidate gene after more than a decade of searching for other genes) in this endeavor, it is now clear that the use of endophenotypes may be better suited to discover associations between gene variants and individual differences in behavior or in the neural substrate that regulates these behaviors. We still await breakthroughs in the discovery of more candidate genes by way of whole-genome association studies, but results so far are disappointing.

Attention is now increasingly directed at more complex interactions between genes and other variables that may affect personality traits and individual differences in other behaviors. This is particularly true with respect to the potential role of epigenetic mechanisms, which may regulate gene expression through exposure to life stressors. Exactly how life stress alters epigenetic programming will undoubtedly become a major theme of future genetic studies. Given this focus on reductionistic explanations, we need to remind ourselves that genetic regulation through environmental influences need not be a passive event: the individual too plays a role in shaping an environment as an active agent. It is possible that an individual’s agency may ultimately affect his or her gene expression, closing the circle between nature and nurture.

Acknowledgments

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See also: Brain Imaging; Human Fear and Anxiety; Personality, Temperament, and Behavioral Syndromes.

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Role of Gene Transcription in Long-Term Memory Storage

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Glossary

Central Dogma of Molecular Biology – First articulated by Francis Crick in 1958, it states that biological information flows from deoxyribonucleic acid (DNA) to ribonucleic acid (RNA) to protein. This hypothesis has been a cornerstone of biology for the past 50 years, yet recent findings have challenged the absolute hegemony of this view. For instance, epigenetic modifications represent a mechanism by which environmental information interfaces with DNA to restrict the information flow out of the genome.

cAMP-response element (CRE)-binding protein (CREB) – A transcriptional activator that binds to CRE sites (consensus: TGACGTCA) in genomic DNA. CREB is a basic leucine zipper (bZIP) transcription factor that is phosphorylated at residue serine-133 in response to activation of cAMP-dependent protein kinase (PKA) and mitogen-activated kinases (MAPKs). Phosphorylation-dependent interaction with the co-activator CREB-binding protein (CBP) strongly enhances transcription of CREB target genes.

Epigenetics – DNA-encoded genetic information is identical in the vast majority of cells in the metazoan body, yet a variety of distinct cellular properties are required to generate the multitude of cell types that make up the body. This paradox led C.H. Waddington to propose in the late 1950s that mechanisms other than genetic information can mediate and maintain cell fate decision. A number of developmental epigenetic markers have been defined such as post-translational modification of histone proteins and covalent modification of DNA. Recent research suggests that these epigenetic mechanisms are used in the mammalian brain to guide transcription during memory formation.

Hippocampus – Nestled under the medial temporal lobe of the neocortex, the hippocampus is critical for the formation of spatial and contextual memory. In humans, the hippocampus is required for the formation of episodic memories, a type of autobiographical memory about events set within time and place often summarized as ‘what-where-and-when.’

Histone – The four core histones (H2A, H2B, H3, and H4) form the basis of DNA packaging. These proteins, each in duplicate, are encircled by 146 bp of DNA to form the nucleosome. The amino-terminal tails of histone proteins extend out of the nucleosome and are

sites for post-translational modifications that form the basis of epigenetic information storage. A number of histone amino-terminal tail residues are sites for specific types of modification; for instance, particular lysine residues can be acetylated or methylated to modulate gene expression.

Memory consolidation – The process by which an initially labile short-term memory is converted into a long-term memory in the hours following learning.

Messenger ribonucleic acid (mRNA) – These processed RNA molecules are transcribed from genomic DNA and read by translation machinery into proteins. This biopolymer is the key mechanism by which information is transferred from DNA to protein in accord with the Central Dogma of Molecular Biology. In eukaryotes, sequence-specific transcription factors recruit DNA-dependent RNA polymerase (RNAPII) to genes, which produces precursor RNA (pre-mRNA) copies of DNA. Mature mRNA is produced by removing intronic sequence (splicing), 5' capping with 7-methylguanosine, and 3' poly-adenylation.

Transcription – The process of producing a ribonucleic acid (RNA) copy of a deoxyribonucleic acid (DNA) template sequence. RNA is generated using complementarity of base-pair interactions to specify the appropriate ribonucleotide that is inserted into the growing chain of RNA by the enzyme RNA polymerase (RNAP).

Transcription factor – Broadly defined as any protein required to initiate or regulate transcription. Often, as in this article, this term refers to sequence-specific DNA-binding proteins that bind to regulatory regions of specific genes where they control the efficacy of transcription. By contrast, general transcription factors are required for the transcription of the majority of eukaryotic genes.

Transgene – A gene that is derived from one organism and introduced into another. As with any gene, the transgene includes DNA sequence that is to be transcribed and regulatory elements that control transgene transcription.

Translation – The process by which mRNA is used to guide the production of a protein by the ribosome. The incorporation of each amino acid in a protein is specified by a sequence of three nucleotides referred to as a codon.

Introduction

More than 100 years ago, the psychologists Georg Müller and Alfons Pilzecker discovered that, shortly after learning, memories are unstable and sensitive to retroactive interference from new learning, but with time these labile short-term memories are converted into stable long-term memory through a process that became known as consolidation. Since that time, research in organisms ranging from sea slugs to mice has shown that this consolidation process involves the activation of neuronal signaling cascades that result in the production of new RNA and protein. This *de novo* transcription and translation after learning is a hallmark of the consolidation process that distinguishes long-term memory from short-term memory. Our understanding of the molecular mechanisms of memory formation has evolved considerably over the past few decades largely due to the development of genetic and molecular strategies to study memories, especially those memories known to require specific neuronal circuits, such as hippocampus-dependent spatial and contextual memory.

Stages of Memory: Acquisition, Consolidation, and Retrieval

Long-term memory formation proceeds through three distinct phases: acquisition, consolidation, and retrieval. Acquisition is the process of learning information about the world and initially produces a labile short-term memory that can be disrupted through interference by new learning, seizure, or inhibition of a variety of cellular and molecular processes. Consolidation occurs with time after learning to confer resistance to disruption, thus converting a short-term memory into a long-term memory. Once information has been stored, it must be retrieved to guide behavior. The specific processes that are required for the formation of long-term memory provide insight into the mechanisms responsible for each of these phases. For instance, disruptions that selectively affect long-term memory without impairing short-term memory provide knowledge about the consolidation process. The earliest of these studies showed that pharmacological inhibition of transcription or translation blocks long-term memory, while leaving short-term memory intact. In the 1960s and 1970s, researchers working on organisms ranging from goldfish to mice established that shortly after acquisition there are windows after learning during which transcription and translation are required to consolidate memory. These early pharmacological studies have been substantiated by genetic approaches that demonstrate a critical role of transcription factors in memory consolidation after learning.

The Hippocampus is Critical for Spatial and Contextual Memory

In 1957, the pioneering studies of Scoville and Milner first suggested that damage to medial temporal lobes structures, especially the hippocampus, causes an inability to form new episodic memories in humans. Since that time, experiments in rodent models have substantially advanced our understanding of the role of the hippocampus in memory formation. Two behavioral paradigms, contextual fear conditioning and spatial learning in the Morris water maze (**Figure 1**), have been particularly useful for understanding the role of the hippocampus in memory. The Morris water maze, first developed by Richard Morris, has become the archetypal measure of spatial learning and memory. Rodents are naturally good swimmers but will seek escape from water when placed in a pool. In the Morris water maze, this tendency is used to motivate rodents to find an escape platform submerged below the surface of water that has been made opaque. With repeated training, the rodents find the platform more quickly in a manner that depends on distal cues. Performance in this task can be measured in a probe test in which the platform is removed and the search pattern of the swimming rodent is examined for a bias toward the region that previously contained the platform (**Figure 1(b)**). Hippocampal lesions block acquisition in this measure of spatial memory but leave intact the ability to swim to a visible platform. Because rodents require repeated training sessions to learn the platform location, it is often difficult to dissociate the effect of manipulations on acquisition and consolidation. The contextual fear conditioning task provides an opportunity to disentangle these two processes by producing a robust long-lasting memory in only a single training session with the added benefit that the biochemical consequences of learning can be precisely related to the time of learning. Contextual fear conditioning involves quantifying a species-specific, stereotyped fear response, including activation of the sympathetic nervous system and a characteristic freezing posture, to measure the retention of an associate between a footshock and a particular context (**Figure 1(c)**). Measuring short-term memory in this task provides a key control in consolidation studies by determining whether learning is possibly impaired. Contextual fear conditioning is disrupted by lesions of the amygdala and the hippocampus, but the closely related cued fear conditioning task, in which freezing is measured in response to a discrete cue, is affected by lesions of the amygdala but not the hippocampus. Therefore, cued fear conditioning serves as a control task to suggest hippocampal specificity when using contextual fear conditioning, providing critical information about whether observed changes in contextual memory are likely due to changes in hippocampal function.

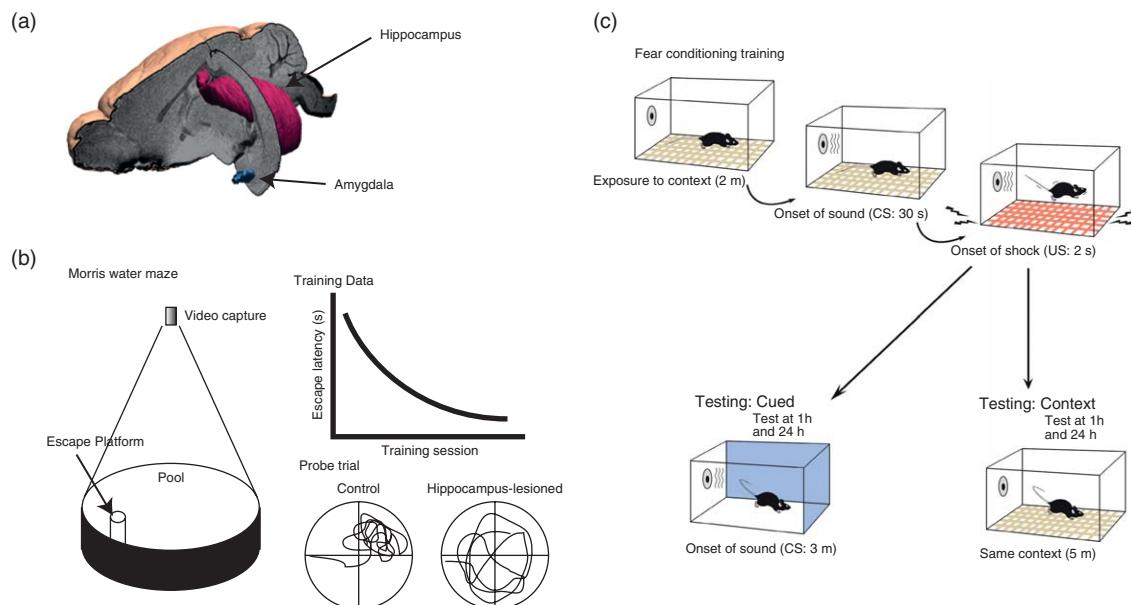


Figure 1 Hippocampus-dependent memory tasks. (a) The hippocampus and amygdala, located in the medial temporal lobe of the cerebral cortex, play critical roles in memory formation. (b) The Morris water maze is a spatial learning and memory task that requires the hippocampus but not the amygdala. In this task, mice learn the position of a hidden escape platform during repeated training session, as measured by a reduced time to find the platform (escape latency). Memory for the platform location is assessed in a probe trial in which the platform is removed. A trained mouse will search extensively within the quadrant that formerly held the platform, whereas a mouse with hippocampal lesions will have no spatial bias to its search for escape. (c) Fear conditioning is an associative memory task that typically involves the pairing of a shock with a novel context and a cue, such as a white noise or tone. During training, a mouse is placed in a novel context and allowed to explore briefly. After the exploration period, a sound is played that co-terminates with a footshock. This training paradigm produces both context-shock and cue-shock associations that can be measured by quantifying a stereotyped and species-specific fear response characterized by immobility, or freezing. The freezing response upon reexposure to the cue in a distinct context measures cue-shock association, while freezing upon reexposure to the training context measures context-shock association. Both of these forms of associative memory require the amygdala, but cued fear conditioning does not require the hippocampus. (Source: Memory, Kandel).

Hippocampal Synaptic Plasticity as a Model for Memory

The existence of well-controlled, hippocampus-dependent memory tasks has been instrumental in establishing a role of individual molecules in memory formation, but another key factor in driving the study of molecular mechanisms of hippocampal memory has been the development of *in vitro* paradigms to examine the changes in synaptic strength after electrical stimulation of neurons, termed activity-dependent synaptic plasticity (Figure 2). The discovery by Bliss and Lomo in 1973 that high-frequency stimulation of connections between neurons in the hippocampus leads to a long-lasting increase in the strength of those connections, termed long-term potentiation (LTP), provided powerful experimental support for Donald Hebb's earlier prediction that learning could emerge from the selective strengthening of connections between neurons firing simultaneously, a key principle of Hebbian learning theory that is often paraphrased as "Neurons that fire together wire together." The initial work by Bliss and Lomo was followed by a flurry of research into the molecular mechanisms of

synaptic plasticity at specific hippocampal synapses using *in vitro* preparations. For instance, a single high-frequency burst of stimulation to the Schaffer collateral pathway from CA3 to CA1 of the hippocampus (Figure 2(a)) produces a relatively short-lived and protein synthesis-independent form of LTP known as E-LTP (Figure 2(b)), but repeated tetani induce a much longer lasting form of LTP (L-LTP) that requires protein synthesis and transcription. The knowledge gained from studies of these and other forms of LTP continue to guide learning and memory research.

Signaling Cascades Involved in Synaptic Plasticity and Memory Formation

By the late 1980s, pharmacological and physiological experiments had paved the way for genetic approaches to study memory formation by suggesting key molecular players in synaptic plasticity and memory formation, such as the *N*-methyl-D-aspartate (NMDA) receptor, calcium/calmodulin-dependent kinases II (CaMKII), and cyclic AMP (cAMP) signaling (Figure 3). For instance, the

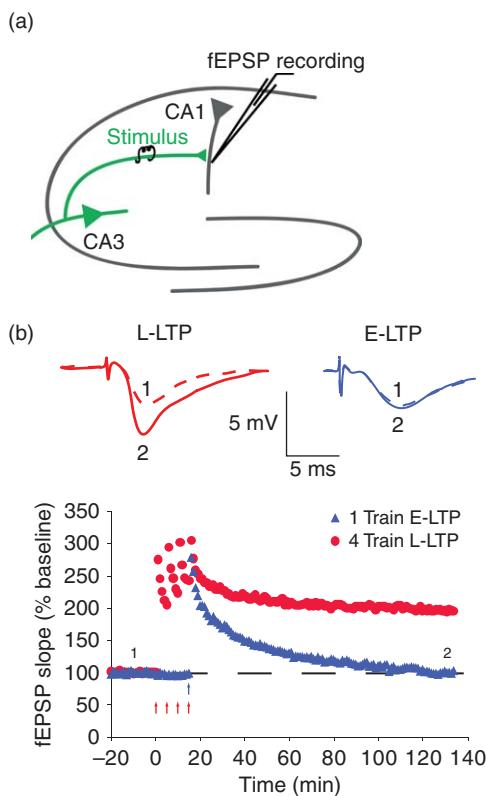


Figure 2 Hippocampal synaptic plasticity. (a) The most heavily studied synapses in the brain are those of the CA3-CA1 Schaffer collateral pathway. This connection is commonly examined using *in vitro* hippocampal slice preparations as illustrated here. A stimulating electrode is placed in the CA3 axon bundles projecting to CA1, and a recording electrode measures field excitatory postsynaptic potentials (fEPSPs) in the synaptic layer of CA1. (b) Representative fEPSPs are illustrated before potentiation with dashed lines (1) and 2 h after potentiation with solid lines (2). Sample fEPSPs are measured by stimulation at 1-min intervals throughout the recording and the initial slope of the fEPSP is plotted relative to baseline fEPSPs prior to potentiation. The slope of the fEPSP increases after both a single 100-Hz tetanus (blue) and four 100-Hz tetani (red), producing long-term potentiation (LTP). LTP induced by a single tetanus, which dissipates more quickly, is referred to as early-LTP (E-LTP), but LTP produced by four tetani, which persists much longer, is called late-LTP. Both E-LTP and L-LTP require NMDA receptor and CaMKII activity, but only L-LTP requires PKA activity.

induction of LTP at CA3 to CA1 synapses, the Schaffer collateral pathway, depends on the activation of NMDA receptors, a type of ionotropic glutamate receptor that requires both the presence of glutamate in the synaptic cleft, glycine binding, and depolarization of the postsynaptic neurons, which releases a voltage-dependent magnesium blockade of the channel pore. Thus, NMDA receptors act as molecular coincidence detectors between pre- and postsynaptic activation, a property that is an important component in the Hebbian theory of associative learning. Unlike other ionotropic glutamate receptor, the NMDA receptor fluxes large amounts of calcium into

neurons. Once inside neurons, calcium activates signaling cascades through calcium-bound calmodulin ($\text{Ca}^{2+}/\text{CaM}$), including type I adenylyl cyclase and CaMKs. Subsequent pharmacological studies showed that inhibitors of CaMKII block the induction of LTP at the Schaffer collateral pathway. Although CaMKII is required for the induction of both E-LTP and L-LTP, cAMP-dependent kinase (PKA) activity is required only for the long-lasting transcription-dependent phase of L-LTP, providing the first suggestion that the PKA pathway may be responsible for initiating the transcriptional program that mediates long-term changes in synaptic strength. Behavioral studies have shown striking co-incidence between the molecular mechanism required for synaptic plasticity and memory formation. For instance, inhibitors of PKA cause long-term memory impairments but leave short-term memory intact. In fact, these deficits emerge several hours after training, paralleling the memory deficits induced by transcription inhibitors. Pharmacological studies provided a critical step in the process of identifying the molecular mechanisms guiding memory formation, but drugs often have off-target effects and only a few of the molecules involved in memory are likely to be good drug targets. Further, pharmacological agents have an impact on glia as well as neurons. Reverse genetic approaches, on the other hand, can readily target an individual gene and can manipulate specific aspects of protein function, even in defined cell types, to elucidate the genetic basis of memory.

Genetic Approaches to Examine the Molecular Mechanisms of Mammalian Memory

The advent of knock-out mice in the late 1980s opened entirely new avenues for the study of mammalian learning and memory. With this technique, the role of an individual gene or isoform in memory formation could be examined without the limitations of drug specificity. Knock-out mice are produced using gene-targeting techniques developed by Cappucci, Evans, and Smithies, for which they won the 2007 Nobel Prize in Physiology or Medicine. This process involves the careful selection of homologous recombination events in mouse embryonic stem (ES) cell lines, which are subsequently injected into developing blastocysts (Figure 4(b)). Homologous recombination, the swapping of DNA between identical DNA sequences, occurs at very low frequency, but a resistance marker in the gene-targeting construct is used to select the few ES cells that undergo recombination for injection into a developing embryo. In addition to allowing for selection, the resistance marker replaces an exon, a piece of the gene coding sequence, leading to elimination of any proteins

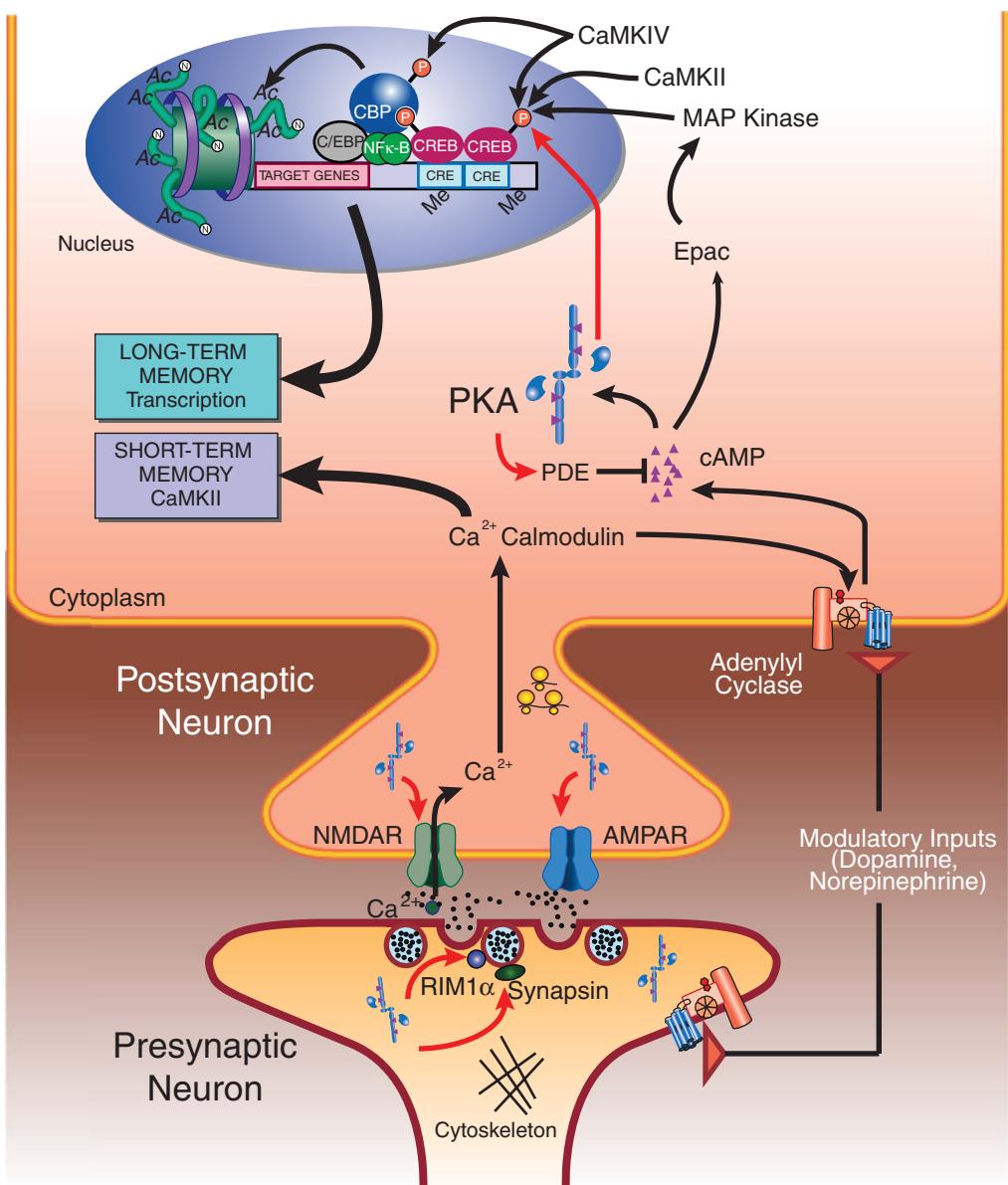


Figure 3 Signaling cascades in memory formation. Many of the signaling cascades that regulate short-term and long-term memory formation have been identified using genetic approaches to study mouse memory. Calcium ions (Ca^{2+}) enter neurons through NMDA receptors and bind calmodulin, which activates kinases including CaMKII to mediate changes in neuronal physiology that underlie short-term memory. Ca^{2+} -calmodulin also activates type I adenylyl cyclase to increase intracellular cAMP levels, which activate protein kinase A (PKA) directly and MAP kinase pathways through exchange factor activated by cAMP (Epac). Signals converge to recruit enhanceosomes consisting of transcription factors (such as NF κ B, CREB, and C/EBP) and transcriptional coactivators (CBP) to cause histone acetylation and transcription. These transcriptional processes are thought to mediate the long-term stabilization of memory that occurs during memory consolidation.

produced from transcripts that include this exon. These conventional knock-out mice have been used extensively to test the requirement for specific genes in learning and memory.

An individual protein is typically composed of multiple functional domains, each of which carries out a specific biochemical function. Because gene knock-out removes all of these functions simultaneously, this

technique is unable to address the role of particular protein domains or biochemical activities in learning and memory. Yet, with a few modifications, the gene-targeting technology used to create knock-outs can generate mice with mutations in individual amino-acid residues, called knock-in mice. The process of producing knock-in mice requires the same homologous recombination process as knock-out, but includes an additional replacement exon

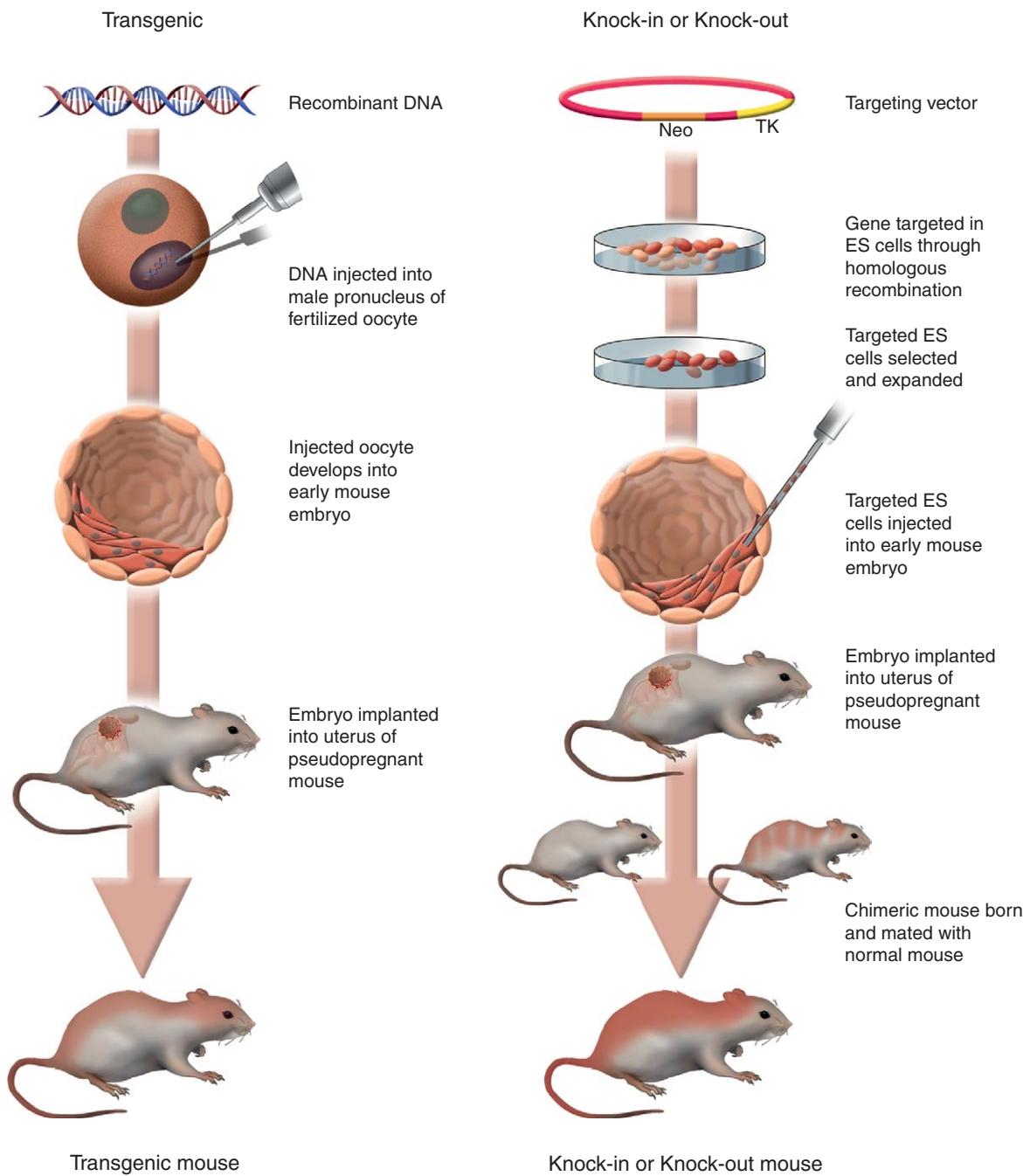


Figure 4 Generation of mutant mouse lines. (a) Transgenic mice are produced by injecting transgene DNA into the pronucleus of recently fertilized oocytes, which integrates into the genome without site specificity. The oocyte develops into an early stage embryo, which is then implanted into a pseudopregnant host female mouse. The offspring of host female are genotyped to identify founders that have incorporated the transgene into their genome. Each founder may have distinct transgene expression patterns, presumably due to the site of transgene insertion. (b) Gene targeting approaches rely on homologous recombination in embryonic stem (ES) cells to knock-out a gene with a selectable marker or knock-in an excisable selectable marker along with the allele of interest. Selection using these markers and a marker against random insertion, typically thymidine kinase (tk), allows purification of only those ES cells that have undergone homologous recombination. The ES cells are injected into a developing mouse embryo from a mouse line with a coat color distinct from the coat color of the mice used to produce the ES cells, and the embryo is implanted into a pseudopregnant female host. The resulting chimeric offspring are derived partly from the original embryo and partly from the ES cells leading to diverse coat color patterns that can be used to assess the contribution of the ES cells to the mouse. These chimeric mice are mated to a mouse with a recessive coat color. If the ES cells have contributed to the germ line of the chimera, some offspring will have the ES cell-derived coat color. These mice will contain the knock-out or knock-in mutation.

bearing the mutations that are to be knocked-in. The selection cassette is then removed in the ES cells by site-specific recombinases prior to injection of the ES cells into the embryo, leaving only the mutated exon in place of the normal exon. The knock-in approach has allowed researchers to go beyond identifying genes that are necessary for memory and to test hypotheses about the biochemical role of particular gene products or protein domains in the machinery of memory.

A major limitation of both knock-out and knock-in mice is that the gene is mutated throughout development and throughout the body. Transgenesis, an approach developed by Palmiter and colleagues, can circumvent these concerns by allowing regulation of transgene expression with a specific promoter, a region of DNA that recruits transcriptional machinery to genes through transcription-factor-binding sites. A transgene consists of a promoter regulating expression of an RNA that encodes a relevant protein. Often, this transgenic protein is a mutated form of a naturally occurring protein designed to interfere with the function of the endogenous protein, such as a dominant-negative or competitive inhibitor. Production of a transgenic mouse lines consists of injection of transgenic DNA into the male pronucleus of fertilized eggs, which leads to random integration of transgene-encoding DNA fragment into the embryonic genome (**Figure 4(a)**). The site of transgene insertion can affect the transgene expression pattern, a finding that has been exploited to identify mouse lines with very refined transgene expression patterns. Because specific protein domains can be mutated in the transgenic protein, this approach often allows identification of the role of specific biochemical functions. Two major caveats of the transgenic approach are that transgenic protein must compete with the wild-type protein and that high levels of transgene expression can interfere with proteins other than the wild-type version of the one that is transgenically expressed. Thus, it can be difficult to determine whether the wild-type gene is required during normal learning and memory.

A substantial advance in gene knock-out technology has been the development of conditional knock-outs using the Cre/LoxP system, which allows the deletion of an individual gene in specific cells and at specific times. This technology was made possible by the discovery of recombinases, enzymes that catalyze the exchange of DNA at specific sequences, particularly the Cre recombinase from bacteriophage P1 that targets the 34-bp LoxP sequence. Using the previously described knock-in strategy, gene exons can be flanked by these LoxP sites. In mice containing two LoxP-flanked (floxed) copies of a gene and a transgenically expressed Cre recombinase, the floxed gene is deleted in cells with Cre expression. The ability to regulate gene deletion through transgenic Cre has been a tremendous boon to learning and memory

research, but there are several major concerns that should be considered when using the Cre/LoxP system. First, with the Cre/LoxP system all deletions are final, so if the Cre transgene is expressed in early development all subsequent cells will lack the gene. This consideration is especially important as some Cre transgenes are unexpectedly expressed in the germ line, which may lead to a full-body gene knock-out. Another concern is that high levels of Cre expression cause cellular toxicity and can produce observable phenotypes. Each of the discussed reverse genetic techniques has been employed to great benefit over the past 20 years of research on learning and memory. In combination, these genetic techniques helped to identify many of the components of signaling cascades that guide transcription during memory consolidation.

α -CaMKII is Critical for Learning and Memory

In 1992, Susumu Tonegawa's lab published one of the first studies of learning and memory in a knock-out mouse, a knock-out of the α -CaMKII isoform. Four distinct genes encode CaMKII isozymes in the mammalian genome, but the α -CaMKII isoform is neuron specific and heavily expressed in forebrain structures, including the hippocampus. Because of this expression pattern and the disruption of synaptic plasticity by CaMKII inhibitors, Alcino Silva generated a knock-out mouse line lacking α -CaMKII. These knock-out mice have impairments both in hippocampal long-term potentiation (LTP) and in the hippocampus-dependent Morris water maze learning, thus suggesting mechanistic overlap between synaptic plasticity and learning. The traditional gene knock-out approach used in these studies has two major limitations: the mutation is present throughout development and all biochemical activities of the protein of interest are eliminated. These considerations may explain the learning-unrelated behavioral abnormalities, including a decreased pain threshold and increased aggression, observed in a later study of this same α -CaMKII knock-out mouse line.

Identifying the mechanisms that guide memory storage requires not only identifying which proteins are important for memory formation, but also how the proteins carry out that role. The biochemical cascades that guide memory formation are likely to provide deep insight into how memories are maintained after learning. For instance, after CaMKII is activated by transient bursts of intracellular calcium, the enzyme becomes calcium independent through autophosphorylation at a particular amino-acid residue, threonine 268. This observation suggests that CaMKII autophosphorylation may temporally store information about recent neuronal activity during synaptic plasticity and learning. Based on this hypothesis, Giese and colleagues generated knock-in

mice with a single-point mutation that converted the phosphorylated threonine residue into an alanine residue ($\alpha\text{-CaMKII}^{T286A}$), which cannot be phosphorylated. In these mutant mice, calcium-dependent CaMKII activity was preserved, but calcium-independent kinase activity was substantially reduced. $\alpha\text{-CaMKII}^{T286A}$ mutant mice have severe deficits both in forms of synaptic plasticity and spatial learning. In addition to these defects, the firing patterns of hippocampal CA1 place cells in response to spatial location were altered. When rodents explore an environment, individual neurons in the CA1 subregion of the hippocampus, called place cells, become tuned to particular positions within that environment. When a wild-type mouse is placed back into a previously explored context, these place cells typically fire in the same location and even come to have a more marked preference for specific parts of the environment. In $\alpha\text{-CaMKII}^{T286A}$ mutant mice, place cells are unstable and shift location with repeated context exposure. Furthermore, the spatial information in place cells, as measured by refinement of place field, does not increase with further exposure. This work showed that a single amino-acid residue on CaMKII is important for learning, highlighting the possibility that biochemical processes in individual neurons may store information during memory formation. Furthermore, these studies provided the first evidence linking synaptic plasticity, spatial learning, and hippocampal place cells by showing that each of these processes is modulated by CaMKII signaling.

Gene knock-out and knock-in are extremely useful tools for studying the mechanisms underlying learning and memory, but these tools have distinct disadvantages. For one, many of the same cellular signaling pathways are used for different processes in different parts of the body. Therefore, components of the molecular machinery of memory may cause decreased overall fitness or even death. In addition, gross changes in behavior caused by mutations can lead to learning and memory deficits that are secondary to other unrelated causes. In some cases, careful behavioral analysis can dissociate specific memory deficit in the face of unrelated phenotypic defects, but other genetic strategies such as transgenesis provide region-specific ways to interfere with gene function. By targeting regions of the brain that are involved with memory, transgenic approaches reduce the likelihood that other processes are disturbed.

The forebrain-specific expression pattern of $\alpha\text{-CaMKII}$ makes this promoter a versatile tool for regulating transgene expression specifically in forebrain neurons. Appropriately, the first use of this promoter was to control transgenic expression of a mutant form of $\alpha\text{-CaMKII}$. Calcium/calmodulin-independent activation of $\alpha\text{-CaMKII}$ by autophosphorylation at threonine 286 can be mimicked by replacing the threonine residue with an aspartate residue ($\alpha\text{-CaMKII}^{T286D}$), resulting in

elevated calcium-independent CaMKII activity. Using the $\alpha\text{-CaMKII}$ promoter, Mark Mayford in Eric Kandel's lab generated a transgenic mouse line expressing $\alpha\text{-CaMKII}^{T286D}$ in the forebrain. These mice have impairments in spatial learning and certain forms of hippocampal LTP, which suggests that dynamics of CaMKII activity are important for effective spatial learning. Furthermore, these results strengthen the argument that CaMKII is involved in the mechanisms of memory itself by showing that its signaling is important in a brain region required for spatial memory, the hippocampus. A major benefit of this work was the identification of a promoter that can regulate, or drive, transgene expression specifically in forebrain neurons of adult mice.

The utility of the $\alpha\text{-CaMKII}$ promoter becomes clear when considering molecules that are vital to mouse survival. For instance, Nr1 is an obligate subunit of a functioning NMDA receptor and traditional knock-outs of the gene encoding Nr1 die shortly after birth. Therefore, Tsien and colleagues used the Cre–LoxP system to remove the Nr1-encoding gene selectively from the forebrain neurons. Cre recombinase was expressed under control of the $\alpha\text{-CaMKII}$. Fortuitous transgene expression patterns, presumably due to the site of transgene insertion, led to an even more restricted expression pattern than that seen with the $\alpha\text{-CaMKII}$ gene itself. When this transgene was combined with two copies of a floxed Nr1-encoding allele, at 3 weeks of age, the Nr1 subunit was deleted predominantly within the CA1 sub-region. Spatial memory and LTP were both severely disrupted in these mice, suggesting that NMDA receptor activity in hippocampal CA1 neurons is critical for memory formation.

cAMP Signaling Regulates Memory Formation

Seminal studies using forward genetics approaches in the fruit fly *Drosophila* and electrophysiological approaches in the sea slug *Aplysia* independently identified a role of the cAMP pathway in learning and memory in the mid-1970s. Nonetheless, evidence supporting a role for the cAMP-signaling pathway in mammalian memory formation awaited the application of genetic approaches in 1990s. The first genetic evidence that cAMP signaling plays a role in mammalian memory came from conventional knock-out mice lacking type I adenylyl cyclase, a calcium/calmodulin-regulated source of cAMP. Daniel Storm's lab observed that these adenylyl cyclase mutant mice have reduced calcium-stimulated cAMP and impaired performance in the spatial version of the Morris water maze. One of the major effectors of cAMP signaling is PKA, but studies with conventional knock-out of individual PKA subunits were confounded by

redundancy and compensation in null mutant mice. The α -CaMKII promoter provided the opportunity to test the role of PKA in memory using a transgenic approach. Forebrain-specific expression of a mutated form of the PKA regulatory subunit that reduces PKA activity (R(AB)) under control of the α -CaMKII promoter impaired spatial memory in the Morris water maze, but both learning and the visual platform version of this task were unaffected. Furthermore, long-term contextual fear memory was selectively impaired with normal short-term contextual and long-term cued fear memory. In wild-type mice, hippocampal CA1 neurons have place fields that are similar during initial exploration to those found with context reexposures 1 h or 24 h later. In addition to defects in long-term synaptic plasticity and long-term memory, place fields in R(AB) mice change upon reexposure to a context 24 h after the first exposure, but are stable between reexposure spaced by only an hour. This finding provides further support for a role of PKA selectively in formation of long-term representations of spatial information. An interesting aspect of this work on cAMP signaling is that, unlike mutations in NMDA and CaMKII, the PKA mutant mice have specific impairments in long-term memory with normal short-term memory. Thus, NMDA receptor activation and CaMKII activity are required for hippocampus-dependent learning, but cAMP signaling appears to mediate subsequent consolidation of learned information into a long-term memory (**Figure 3**).

Memory storage has distinct phases, such as acquisition, consolidation, and retrieval. In addition, memories are subject to changes after initial storage. The study of these processes independently from one another has required the development of conditional transgenic systems with some degree of temporal control. For instance, after contextual fear conditioning, repeated reexposure to the context without reinforcing shock reduces the fear response through the formation of new memories that suppress the original fear response. This process is referred to as extinction learning and experiments in R(AB) mutant mouse lines suggested that the molecular processes guiding the formation of extinction memory are partly distinct from initial fear learning. In *CaMKII-R(AB)* mutant mice, contextual fear memories undergo extinction more rapidly than in wild-type mice. To determine whether this effect was due to a role of cAMP signaling in the extinction process or a consequence of changes in the initial contextual fear memory, an inducible dual transgene system was used that consisted of the α -CaMKII promoter regulating the tetracycline transactivator (*CaMKII-tTA*) and the tetracycline operator regulating R(AB) (*tetO-R(AB)*) (**Figure 5(a)**). The inducible aspect of this expression system is based on the bacterial tetracycline-inducible operon. In *Escherichia coli*, the tetracycline repressor (TetR) binds to tetO sequences at

tetracycline-inducible promoters to repress gene transcription under basal conditions, but when tetracycline binds to the TetR protein, the repressor releases the promoter to allow transcription. This system has been modified by fusing a strong viral activator domain (VP16) to the DNA-binding domain of TetR yielding a hybrid tetracycline-regulated transactivator (tTA) for control of transgenes with tetO sequences in their promoter. The CaMKII-tTA system was developed for the study of CaMKII in memory formation but was applied by Isiegas and co-workers to show that PKA contributes to extinction as well as fear memory formation. In this study, mice were trained in contextual fear conditioning while being fed doxycycline to repress R(AB) expression and were taken off doxycycline after learning (**Figure 5(b)**). Four weeks later, when R(AB) expression was robust throughout the forebrain, the mice were subjected to repeated exposure to the conditioning context in the absence of shock. The rate of extinction learning was facilitated by R(AB) expression, showing that PKA has distinct and opposing roles in initial memory formation and extinction memory (**Figure 5(c)**). An additional advantage to this study was that the role of PKA in memory *per se* rather than developmental process was supported by observation that R(AB) expression in the adult mouse impairs contextual fear memory consolidation. The development of more rapidly inducible transgenic systems will be critical for the separation of effects on acquisition, consolidation, and retrieval.

One strategy to more rapidly manipulate neuronal signaling cascades relies on transgenic expression of receptors that bind ligands not normally present in the brain. This pharmacogenetic strategy combines the cell and region specificity of genetic approaches with the temporal control afforded by pharmacological approaches. A recent study using this strategy provided further support for the role of cAMP in memory formation. In this work, a G_s-coupled octopamine receptor from the sea slug *Aplysia* was expressed in forebrain neurons using the CaMKII-tTA/tetO system. Octopamine is a major neurotransmitter in invertebrates but is present only at trace levels in vertebrates with no known physiological functions. Because the *Aplysia* octopamine receptor is G_s coupled, binding of octopamine by this receptor activates adenylyl cyclase and transiently increases cAMP. In mice expressing the octopamine receptor, octopamine injection increases hippocampal cAMP levels and enhances both short-term and long-term memory. The lack of a short-term memory deficit in PKA mutant mice suggests that the enhancement of short-term memory by this transient burst of cAMP may be a consequence of rapid recruitment of consolidation mechanisms. Alternatively, this enhancement may be mediated by recruitment of other cAMP-regulated proteins, such as exchange-factor activated by cAMP (EPAC), which

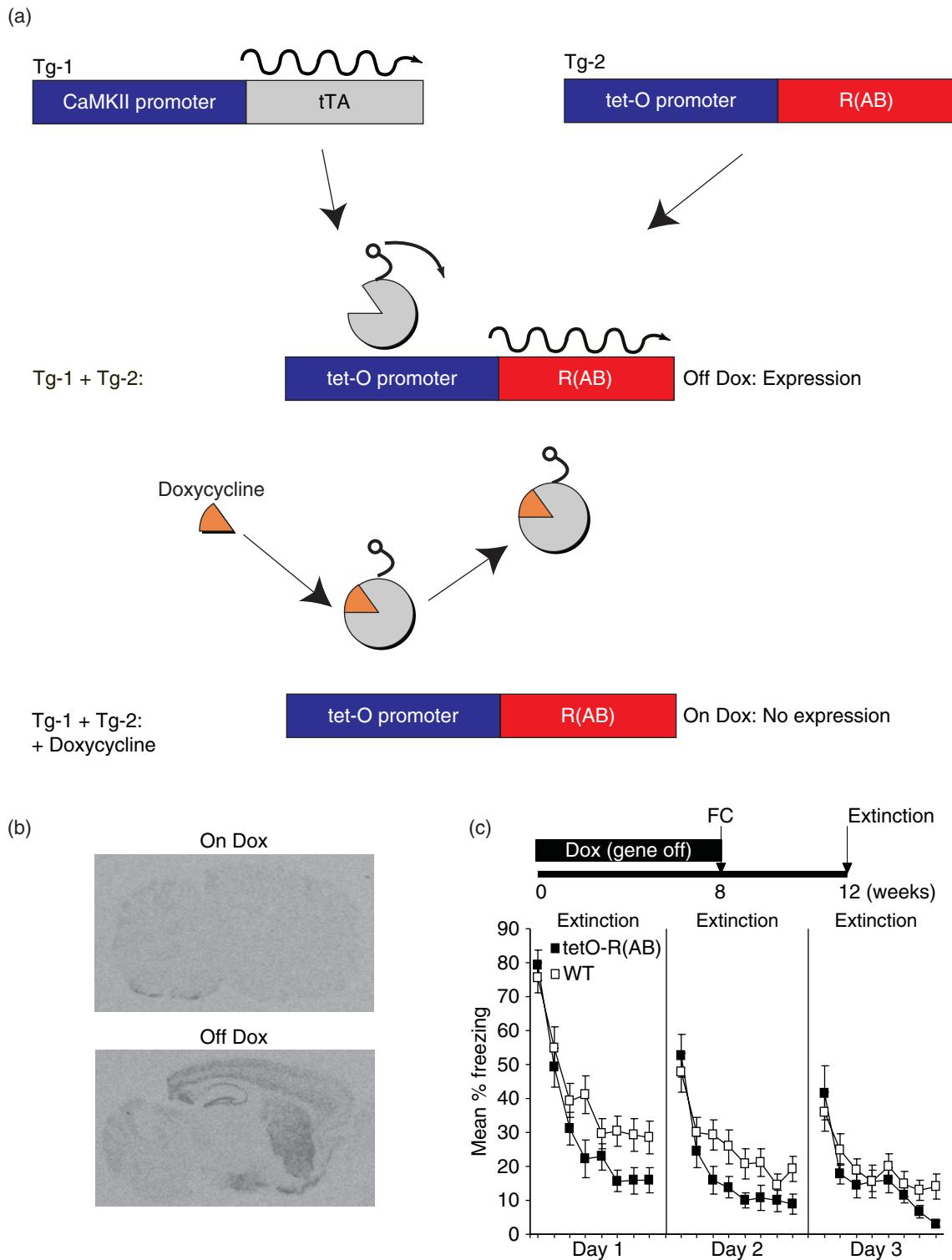


Figure 5 Conditional reduction of PKA activity enhances extinction memory. (a) Temporal and spatial regulation of the PKA inhibitor R(AB) was achieved using a dual transgenic tTA/tetO system. The specificity of this system stems from the tetracycline transactivator (tTA) under control of the α -CaMKII promoter, which drives forebrain-restricted transgene expression. The tTA protein binds tetO sequences in the promoter of the R(AB) transgene to induce expression of R(AB) in forebrain neurons. In the presence of the tetracycline analog doxycycline, tTA cannot bind the tetO sequence, allowing temporal regulation of R(AB) expression by feeding the mice doxycycline. (b) *In situ* hybridization shows that administration of a doxycycline diet efficiently suppresses R(AB) expression (On Dox). In addition, the transgene is specifically expressed in forebrain structures when the mice are removed from the doxycycline diet for 28 days (Off Dox). (c) Inhibition of PKA activity facilitates extinction of contextual fear memory. Wild-type and R(AB) transgenic mice were raised on doxycycline, trained in contextual fear conditioning, and taken off a doxycycline diet 24 h after training. Contextual fear extinction was performed after 28 days on a doxycycline-free diet. Repeated reexposure to the context formerly associated with a footshock reduced freezing more rapidly in R(AB) transgenic mice than in wild-type littermates. Error bars indicate SEM. Source: Isiegas et al., 2007.

activates mitogen-activated kinases (MAPKs). Because of the precise temporal regulation of cAMP increases, Isiegas and colleagues were also able to show that increasing cAMP signals during memory retrieval enhances performance, suggesting that cAMP signaling modulates retrieval as well as consolidation. In complementary studies, several other approaches that increase cAMP signals after training also enhance memory consolidation. For instance, pharmacological blockade of cAMP degradation produces long-lasting L-LTP after a stimulation paradigm that normally evokes short-lasting E-LTP and improves contextual fear memory. Similarly, overexpression of calcium-responsive adenylyl cyclase produces LTP and memory enhancement. It is important to note that these approaches increase cAMP dynamics after learning, leading to an increase in the cAMP signals. By contrast, manipulations that chronically increase basal cAMP or effector levels often lead to impairments in long-term memory.

The CREB Family of Transcription Factors Regulates Memory Formation

The similarity between the memory and synaptic plasticity deficits caused by PKA inhibitors and transcriptional inhibitors suggests that PKA may regulate memory formation by inducing transcription. In many physiological contexts, cAMP signals are converted into long-lasting changes in cellular physiology by activating the transcription factor CREB. PKA and MAPKs phosphorylate CREB on serine residue (S133), causing CREB to bind its coactivator CBP. CREB is constitutively expressed but is activated through phosphorylation to drive expression of genes containing cAMP-response elements (CREs) in their promoters. This inducibility after neuronal activity is a key feature that made CREB an attractive candidate for initiating transcriptional cascades after learning. Both spatial and contextual learning are accompanied by increases in CREB phosphorylation within the hippocampus, and the windows of CREB phosphorylation after learning coincide strikingly well with the windows of sensitivity to inhibitors of PKA, protein synthesis, and mRNA synthesis. Once again, research in both *Aplysia* and *Drosophila* foreshadowed subsequent research in mammalian memory by demonstrating a role of CREB in long-term memory. The first of these invertebrate studies by Pramod Dash in Eric Kandel's lab found that injection of excess DNA containing the CRE sites blocks a form of long-term synaptic plasticity at *Aplysia* synapses known as long-term facilitation (LTF). The first mammalian CREB mutant was a conventional knock-out of the α and δ isoforms, the major CREB isoforms in the brain, and the history of these CREB $\alpha\delta$ knock-out mice illustrates many of the risks and benefits associated with

conventional knock-out mice. To reduce the effect of genetic variability on experimental results, mice are studied on inbred genetic backgrounds, but these different strains of mice can have very different behavioral phenotypes. The initial examination of memory in CREB $\alpha\delta$ mice on a mixed B6- and 129-genetic background showed impaired cued and contextual memory formation, as well as impaired spatial memory formation in the Morris water maze. Shortly after these mice were generated, it was discovered that the β isoform of CREB and the CREB family member cAMP-responsive element modulator (CREM) were upregulated in CREB $\alpha\delta$ mice. Although the consequences of these compensatory mechanisms are still subject to debate, it appears that the overall genetic background may determine whether a CREB $\alpha\delta$ mutant mouse has memory deficits. After initial studies in a B6-129 mixed genetic background, CREB $\alpha\delta$ mutation was backcrossed into two inbred mouse strains, C57BL/6 (B6) and FVB/N. These B6 and FVB/N heterozygous CREB $\alpha\delta$ mutants were mated to produce CREB $\alpha\delta$ knock-outs in a defined F1 hybrid. Surprisingly, these F1 hybrid CREB $\alpha\delta$ mice lacked the memory deficits previously observed on the B6-129 mixed background. Yet, when mice from this same F1 hybrid background contained one CREB $\alpha\delta$ mutant allele and one complete CREB knock-out allele, the memory deficits were observed even on this hybrid background, suggesting that strain-specific differences in compensation by the β -CREB isoforms could account for the effect of genetic background. Later studies confirmed that B6-129 F1 hybrid CREB $\alpha\delta$ mice have fear memory deficits. These findings highlight both the risk of compensatory changes in gene regulation after gene knock-out and the possibility that the genetic background of an individual inbred mouse strain may make a single gene appear more or less pivotal for memory formation.

Because the gene in question is not removed from all cells throughout development, transgenic approaches suffer to a lesser extent from compensatory changes in gene expression. The role of CREB in long-term memory has been further studied using transgenic approaches. For instance, Chris Pittenger in Eric Kandel's lab generated a transgenic mouse line expressing a strong dominant negative allele of CREB (KCREB) that heterodimerizes with CREB family members and blocks DNA binding. Using the α -CaMKII promoter, this dominant-negative transgene was expressed in the striatum, limited cortex, and the CA1 subregion of the dorsal hippocampus. In support of a role for CREB in long-term memory formation, these mice had deficits in the spatial version of the Morris water maze, but surprisingly no deficit was observed in contextual fear conditioning. Either the absence of KCREB expression in the ventral hippocampus, which is more directly connected to the amygdala than the dorsal hippocampus, or the level of impairment

of CREB function by this transgene may account for the absence of fear memory deficits in these mice. In the same year, Alcino Silva's lab produced a transgenic mouse line expressing a form of CREB that lacks the activating residue at serine 133, once again under control of the α -CaMKII promoter. This mutant form of CREB was fused to a variant of the estrogen ligand-binding domain that preferentially binds to the estrogen analog tamoxifen (ERT). The ligand-free ERT domain inactivates the protein by sequestering it into an HSP90 complex, which dissociates upon tamoxifen binding. In this inducible CREB repressor (CREB^{IR}) line, a few hours of tamoxifen treatment causes severe impairment in long-term contextual and cued fear memory tasks, but leaves initial learning and short-term memory intact. The CREB^{IR} transgenic line confirmed the role of CREB family members in long-term memory and, importantly, showed that serine 133, the site of PKA phosphorylation, is central to this role. Because the levels and regions of CaMKII-regulated transgene expression vary considerably due to the effect of transgene insertion site, it is difficult to directly compare behavioral outcomes from distinct transgenes, an effect that is further confounded by redundancy and compensation among CREB family members, but the overall message from the past 20 years of research suggests that CREB or CREB family members play an important role in long-term memory formation.

NF κ B Family Members are Latent Transcription Factors that Regulate Memory Formation

The ability of CREB family transcription factors to lie latent in neurons prior to activation by extracellular signals is a key feature that places this family at the interface between environmental signals that encode information about the world and the most fundamental information storage system in biology, the genome. Another transcription factor family with similar activity-dependent regulation is the Rel/nuclear factor κ B (NF κ B) family, which consists of five members (p50, p52, p65, c-Rel, and RelB) that can act as homo- and hetero-dimers. In the basal state, I κ B inhibitory proteins sequester NF κ B in the cytoplasm by masking a nuclear localization sequence (NLS), but activation of I κ B kinase (IKK) causes ubiquitination and degradation of I κ B, thus freeing NF κ B for nuclear import and activation of target genes. Two kinases thought to play a role in memory formation, CaMKII and protein kinase C (PKC), can activate NF κ B by phosphorylation of IKK. The earliest studies of NF κ B found increased protein levels for p50 and p65 after high-frequency stimulation, whereas I κ B levels decrease. It was subsequently shown by Meffert and colleagues that neuronal activity causes a calcium-

dependent increase in DNA binding by p65-containing NF κ B dimers accompanied by increased nuclear translocation of GFP-labeled p65 and that mice lacking the p65 subunit have deficits in the spatial version of the radial arm maze, a task that requires the hippocampus. Further support for a role of NF κ B family members in memory formation comes from mice expressing a dominant-negative form of IKK (IKK-AA) under control of the inducible, neuron-specific CaMKII-tTA/TetO system. These mice have deficits in the spatial version of the Morris water maze and L-LTP produced by theta burst stimulation. The most direct consequence of IKK-A expression is reduced NF κ B signaling, but expression of IKK-AA also substantially reduces the expression of the catalytic subunit of PKA and impairs forskolin-induced CREB phosphorylation in the hippocampus, suggesting that NF κ B signaling may amplify CREB-mediated transcription after learning.

Microarray experiments are capable of examining the expression of thousands of genes simultaneously and computational analysis using bioinformatics techniques allows the identification of transcription factors that may regulate identified changes in gene expression. Levenson and colleagues used bioinformatics analysis of microarray results after fear conditioning to identify another NF κ B subunit, c-Rel, as a candidate for regulating gene expression during memory consolidation. This finding led the researchers to examine the role of c-Rel in long-term memory using conventional *c-Rel* knock-out mice, where they saw a significant deficit in long-term contextual fear memory, but normal short-term contextual and long-term cued fear memory. Results that are more recent have confirmed this initial finding in *c-Rel* knock-out mice and demonstrated that c-Rel protein is translocated to the nucleus in CA1 neurons at 30 min after contextual fear conditioning. The identification of c-Rel by bioinformatics analysis of microarray data highlights the ability of unbiased screening approaches to reveal important information about molecular mechanisms regulating memory processes.

The analysis of gene expression after learning raises the question of what component of associative memory drives hippocampal gene expression: the unconditioned stimulus (US shock), the conditioned stimulus (CS context), or the CS-US association. In the study by Levenson and co-workers, the vast majority of the learning-induced genes did not have increased expression in the hippocampus when mice were shocked immediately after placement into the novel training context, a protocol that fails to produce associative memory. In a similar microarray experiment, Keeley and colleagues found that hippocampal gene expression 30 min after fear conditioning is very similar to the pattern observed after context exposure alone, suggesting that contextual learning (CS) may drive a substantial amount of gene

expression after contextual fear learning. These findings suggest that the control task can affect the expression profile, and perhaps that the immediate shock control impairs gene expression involved in CS encoding. Yet, certain immediate early genes, such as *Nr4a1/NGFI-B*, have increased expression after context–shock (CS–US) pairing, but not context (CS) exposure alone. Thus, the questions of what role the hippocampus is playing in associative memory processing and how behavioral paradigms affect this processing are still vital open questions that may be clarified by continued studies of gene expression after learning paradigms.

Immediate Early Genes Are Activated by Latent Transcription Factors after Learning

Immediate early gene (IEG) expression is directly induced by latent transcription factors, so protein synthesis is not required for expression of these genes after a stimulus. Interestingly, several IEGs activated after learning and synaptic plasticity are themselves transcription factors, suggesting that cascades of transcription may be critical for long-term changes in plasticity and memory. Notable among these learning-induced IEGs are *Fos*, *C/EBP*, and *Zif268*. As IEGs, the expression of these transcription factors after stimulation does not require translation, suggesting that their expression is a result of activation of latent transcription factors, such as NF κ B and CREB. In fact, there is evidence that each of these three IEGs is regulated in part by CREB. Yet, it is important to mention that the expression of genes after learning is probably not simply regulated by any single transcription factor, but is a result of simultaneous binding of multiple transcription factors to the promoter regions of genes acting as an enhanceosome to cooperatively recruit transcriptional coactivators (Figure 3). For instance, the transcriptional coactivator CBP interacts with CREB, NF κ B, C/EBP, Fos, and Zif268, and cooperative recruitment of CBP by multiple transcription factors is a hallmark of this coactivator. This cooperative CBP recruitment allows computation through the integration of multiple signals of biochemical activation, such as the transcription factors activated after learning, as well as cell-type-specific signals.

C/EBP: A Second Wave of Transcription after Learning

The role of C/EBP in the transcriptional cascade supporting memory was first identified in studies of *Aplysia* LTF, a long-lasting and protein synthesis-dependent form of synaptic plasticity. The *Aplysia* C/EBP gene is

expressed rapidly after repeated treatment with serotonin or pharmacological activation of the cAMP pathway, stimuli known to induce LTF and increased PKA activity. This expression is protein-synthesis independent, further suggesting that a constitutively expressed and PKA-activated transcription factors such CREB drives the expression of this transcription factor. Alberini and co-workers used several approaches to demonstrate that LTF requires C/EBP, and perhaps more interestingly this requirement for C/EBP extends for more than 9 h after LTF induction, suggesting that long-lasting changes in gene expression are required for long-lasting changes in synaptic strength. Subsequent studies in rats showed that after a hippocampus-dependent contextual memory task, long-lasting increases in C/EBP expression are observed for 28 h after learning. Using antisense oligonucleotides to block expression of the C/EBP β -isoform, Alberini's lab went on to show that this transcription factor is required at 24 h after learning for memory retrieved at 48 h after learning. These studies of C/EBP have highlighted two very important aspects of memory formation: transcriptional cascades are likely to mediate waves of transcription during memory formation and the changes in transcription responsible for memory formation are much longer lasting than previously thought.

Fos Expression can be used to Stably Tag of Neurons Activated by Learning

Expression patterns of IEGs have been very useful for addressing systems- and network-level questions about learning and memory. In 1989, Paul Worley's lab examined the expression of seizure-induced genes in the hippocampus after LTP, finding that several IEGs such as *Zif268*, *JunB*, *c-Jun*, and *Fos* are rapidly induced in the hippocampus after LTP-inducing stimuli. *Fos*, an archetypal immediate early gene that is regulated by a wide variety of cellular stimuli, hetero-dimerizes with *Jun* family members to form the transcription factor AP-1. *Fos* gene expression is robustly induced in memory-related structures after acquisition and recall of memory. Because of this expression pattern, Mark Mayford's lab developed a genetic system to stably label neurons that have *Fos* induction during a defined time window, dubbed the TetTag mouse. This bigenic TetTag system uses the *Fos* promoter to regulate the expression of tTA (*Fos-tTA*), leading to activity-dependent production of the doxycycline-repressed tTA transactivator. In addition, a tetO-regulated bidirectional promoter-controlled expression of the reporter enzyme LacZ and a doxycycline-insensitive mutant form of tTA (*tTA**). These mice were raised on doxycycline, so that *Fos-tTA* activation is transient and leaves activated cell unmarked. When these bigenic TetTag mice are taken off doxycycline, any stimulation

that induces the *Fos* promoter regulating tTA activates transcription of the doxycycline-insensitive tTA*, marking these cells with continuous expression of tTA* and the reporter LacZ (**Figure 6(a)**). Therefore, the TetTag mice were trained in the absence of doxycycline. After training, the mice were once again treated with doxycycline to block further marking of neurons. The initial report of the TetTag system focused on the tagging of neurons in the amygdala after fear conditioning. In addition to examining the neurons marked during learning,

expression of the IEG *Zif268* was used as a marker of transcriptional activation after retrieval in the presence of doxycycline (**Figure 6(b)**). More than twice as many neurons were labeled after training relative to home-cage or context-only controls kept off doxycycline for the same interval (**Figure 6(e)**). By examining both TetTag LacZ labeling and Zif268 expression, the researchers asked whether the same neurons activated after learning (LacZ positive) were also re-activated during retrieval (Zif268 positive). The overlap between LacZ

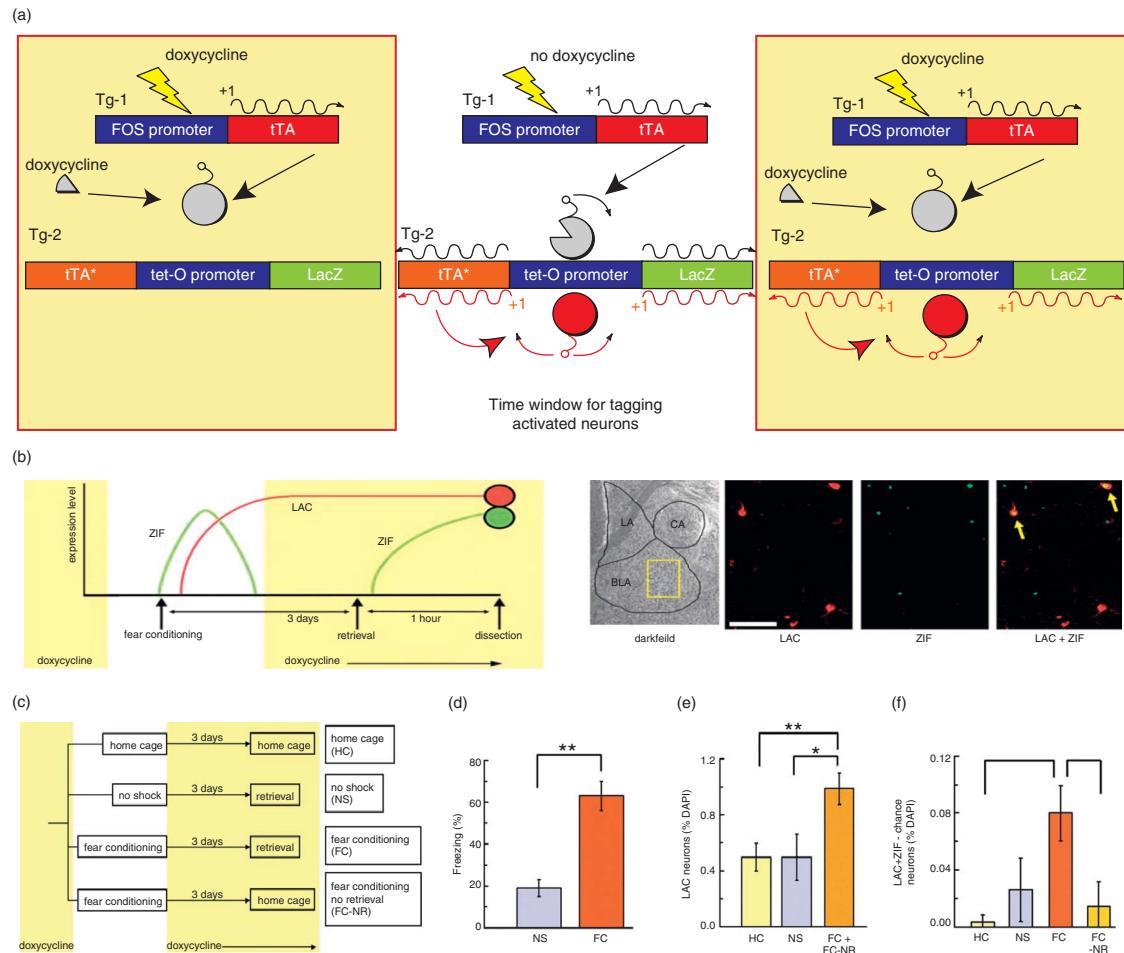


Figure 6 Identifying a memory trace: TetTag system. (a) The TetTag systems combine two transgenes to stably label neurons activated during a defined time window. The first of these transgenes is a *Fos* promoter-regulated tetracycline transactivator (Fos-tTA). The second transgene consists of a LacZ marker and a tetracycline-insensitive version of tTA (tTA*) under control of a bidirectional tetracycline operator (TetO-LacZ-tTA*). In the presence of doxycycline (dox), which can be mixed into the mouse's food, the Fos-tTA transgene is expressed with neuronal activity, but the dox inactivates tTA. In the absence of dox, tTA expressed after neuronal activity binds to the TetO-LacZ-tTA* to induce the expression of the doxycycline-insensitive tTA*, resulting in continued expression of tTA* and the LacZ marker even when the animals are returned to a dox-containing diet. Thus, the window of tagging is limited to when the animals are not being fed dox, but the tag persists after dox is returned to the diet. (b) This system was used in conjunction with immediate early gene labeling of *Zif268* to determine whether the same neurons that were activated by learning were re-activated during retrieval. (c) For these experiments, mice were reared on dox to prevent labeling, taken off dox to allow neuronal tagging for a short window during which the experimental mice received fear conditioning, and placed back on dox for 3 days to prevent tagging during the retrieval. (d) Mice learned the context-shock association as measured by the percent time spent freezing during a retrieval test. (e) Fear conditioning led to significantly more labeling of neurons than exploration of the context alone (NS) or being left in the home cage (HC). (f) Neurons activated during training (LacZ+ neurons) were more likely to be activated during retrieval (ZIF+ neurons) than chance levels, whereas neurons activated in the home cage or simply with context exploration were not. Source: Reijmers et al., 2008.

and Zif268 was significantly above chance in mice that were examined after retrieval and were trained in the absence of doxycycline. In control mice that did not experience retrieval and in control mice that were off doxycycline in the home-cage and context-only group, co-labeling for these two markers was approximately at chance levels (**Figure 6(f)**). In addition, a significant correlation was found between the number of co-labeled neurons in the amygdala and retrieval performance. This approach allows researchers to ask network-level questions about the incorporating neurons into potential memory traces and to use the tTA* transactivator to selectively modulate these neurons.

Local Capture of Transcripts Correlates with Associative Aspects of Memory

The utility of this *Fos*-driven TetTag system for analyzing the incorporation of neurons into hippocampal networks activated after contextual learning has yet to be examined, but the *Fos-tTA* component was used to examine the incorporation of a newly synthesized AMPA receptors into hippocampal CA1 neurons after contextual fear conditioning by combining the *Fos-tTA* transgene with a TetO-regulated EGFP-labeled AMPA receptor subunit (*TetO-GluR1-EGFP*). In this study, roughly 25% of hippocampal CA1 neurons were labeled with GluR1-EGFP after fear conditioning, compared with approximately 5% of neurons in home-cage mice. A similar level of GluR1-EGFP was observed in mice exposed to the context alone, which is in agreement with previous observations that *Fos* and many other IEGs are transcribed in the hippocampus in response to configural learning rather than the associative component of contextual fear memory. Within 24 h, these GluR1-EGFP proteins were trafficked to dendritic spines, but only about 50% of spines were labeled within individual EGFP-labeled neurons. Strikingly, the trafficking to a specific spine type, mushroom body spines, was increased in mice that received context-shock pairing relative to mice that experienced only context exposure. Repeated exposure to the context alone in the absence of reinforcing shock reduces freezing in that context through a process known as extinction, and extinction was accompanied by a reduction in the percentage of EGFP-labeled mushroom body spines relative to other spine types. These findings suggest a startling dichotomy of information encoding in the hippocampus in which neuron-wide transcriptional processes encode information about context whereas spine-specific synaptic capture of transcripts encodes certain associative components of fear memory. If this dissociation exists, a sharp line may not be easily drawn between the two processes because *de novo* transcriptional processes most likely account for the

transcripts that are captured and a few genes appear to be transcribed selectively in response to associative aspects of contextual fear memory, such as *Nr4a1*.

Temporal Dynamics of Arc mRNA Expression allow Examination of Place Representation in the Hippocampus

The finding in TetTag mice that 25% of hippocampal neurons are Fos-activated by contextual fear conditioning echoes fascinating studies carried out by John Guzowski, in which a surprisingly high number of neurons were marked by IEG expression after learning. In these studies, Guzowski used fluorescent *in situ* hybridization (FISH) in conjunction with confocal microscopy to provide extremely high-resolution images of hippocampal gene expression after context exploration (**Figure 7**). For the first 5 min after seizure activity, the transcripts of IEGs *Arc* and *Zif268* were identified in discrete foci within the nucleus (**Figure 7(b)**), but by 15 min after seizure these transcripts had been trafficked to the cytoplasm (**Figure 7(c)**). Similar dynamics were found after the exploration of a novel context. Impressively, approximately 45% of CA1 pyramidal neurons were found to have nuclear *Arc* transcript after 5 min of context exploration, the same number had cytoplasmic *Arc* transcript when examined after a 25-min delay, and very few (<5%) had detectable transcripts in both compartments simultaneously (**Figure 7(d)**). This knowledge of the temporal dynamics of *Arc* trafficking allowed Guzowski to ask a variety of interesting questions about the recent activity of neural networks during context exposure. When the rat was returned to the same context after a 20-min delay, roughly 40% of CA1 neurons had *Arc* transcripts in both nuclear and cytoplasmic, showing that the network of neurons activated by a particular context was stable across time and that these networks are surprisingly large. When the rat was exposed to a distinct context after the same 20-min delay, only 20% of CA1 neurons had exclusively nuclear or cytoplasmic labeling and 16% of CA1 neurons displayed both nuclear and cytoplasmic labeling, suggesting that these representations overlap at roughly the same level that would be predicted by chance (**Figure 7(d)**). The observation that a large percentage of CA1 neurons is activated by single context fits well with observations using electrophysiological and biochemical techniques suggesting that between 20% and 40% of CA1 neurons are involved in representation of a single context. With such a large number of neurons involved in each single representation, how is it possible that the hippocampus is not quickly overwhelmed in the daily life of a wild rat? First, the actual number of distinct representations consisting of 120 000 neurons (40%) within 300 000 CA1 neurons is staggering.

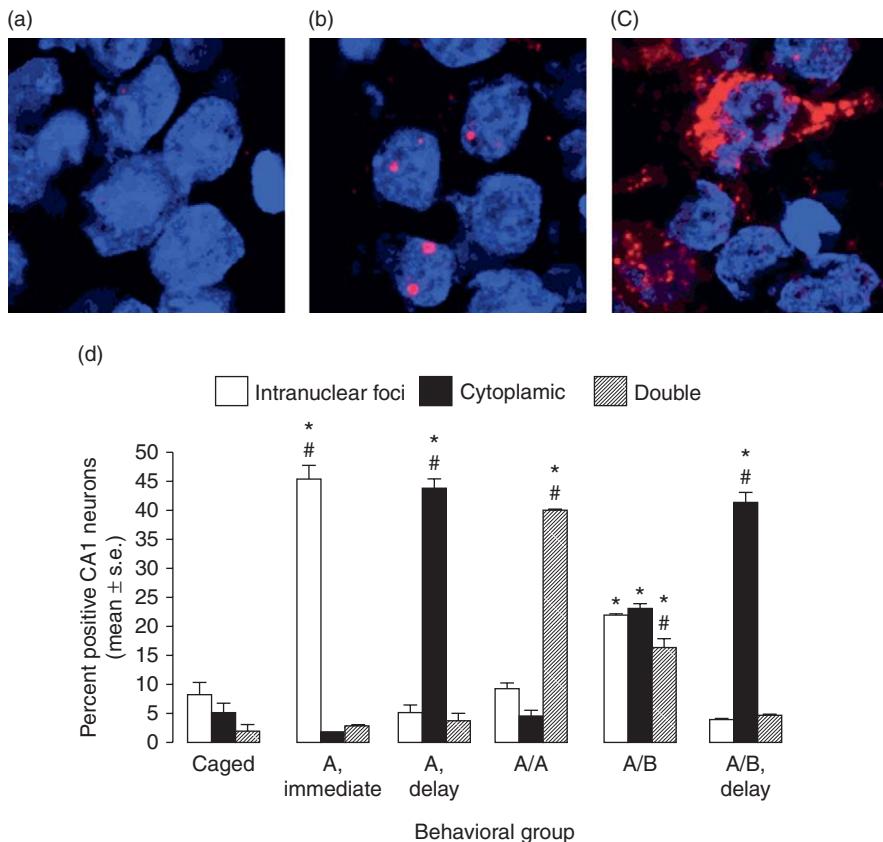


Figure 7 Immediate early gene imaging with catFISH. Fluorescent *in situ* hybridization (FISH) can be used to provide high-resolution images of the localization of mRNAs in neurons. Compartment analysis of temporal activity by fluorescent *in situ* hybridization (catFISH) uses this resolution to identify the neurons activated at two times. (a) Prior to context exploration, *Arc* is largely absent from hippocampal CA1 neurons. (b) At 5 min after exposure to a context, *Arc* mRNA is localized to intranuclear foci that presumably mark the sites of transcription. (c) At 30 min after context exposure, *Arc* mRNA moves to the cytoplasmic and intranuclear foci are no longer found. (d) More than 40% of neurons in area CA1 have intranuclear *Arc* foci immediately after context exposure (A, immediate), and a similar number have cytoplasmic *Arc* labeling 20 min after context exploration (A, delay). Repeated exposure to the same context leads to intranuclear and cytoplasmic labeling in the same neurons, suggesting that the same neurons are activated with repeated exposure to the same context (A/A). When a second context is explored 20 min after the first (A/B), three populations of neurons are identified: 22% with only intranuclear labeling, 23% with only cytoplasmic labeling, and 16% with both intranuclear and cytoplasmic labeling. These results suggest that immediate early gene expression patterns may be context specific and extensive representations of contextual information. Source: Guzowski et al., 1999.

The question really requires knowing how much of a difference between any two representations is needed for faithful discrimination, but by assuming that 10 000 neurons is a sufficient difference to robustly differentiate memories it is possible to estimate the lower limit of this number. If neurons are imagined to function in a bundle of 10 000 neurons, guaranteeing a difference of 10 000 neurons between any two representations, there are still 86 million different representations consisting of 120 000 neurons possible within a CA1 network composed of 300 000 neurons. Second, systems-level consolidation limits the time of hippocampal storage by transferring hippocampal memories to the cortex in representations that may use fewer neurons, a possibility that would be consistent with both systems-level consolidation and the increased generalization that often occurs with remote

memories. Third, it is possible that hippocampal representations become more refined in the days following learning. Such a focusing of memory could account for the basic principle that memory becomes more resistant to interference with consolidation. Fourth, the analysis above ignores the temporal order of neuronal activation, but a given contextual representation is likely to be defined by the sequential activation of neurons representing distinct but adjacent components of the environment. If this sequence of activity matters, then a vastly larger number of firing patterns are possible with the same number of neurons. Lastly, analysis of activation of a few IEGs may not reflect the induction patterns of other IEGs. Perhaps genes other than *Arc* have more selectively expression patterns in the hippocampus, just as some have more selectivity for associative aspects of memory.

Transcriptional Metaplasticity: Recent Experience regulates the Ability to Induce Transcription

Although *Arc* activation occurs in the same neurons with repeated exposure, the ability to induce *Arc* in these neurons is affected by the recent activity history of the neurons. Massed repeated reexposure to a context substantially decreases the number of neurons activated by a final exposure to that context but only mildly affects *Arc* induction in response to a distinct context, suggesting that the recent transcriptional activity modulates the ability to induce further transcription and that this modulation is neuron-wide rather than synapse specific. The firing properties of CA1 neurons measured by electrophysiological methods did not change with repeated context exposure, showing that this change in transcriptional activity is not a consequence of altered electrical activity. Thus, transcription is not only dynamic after learning, but the ability to transcribe genes is altered by experience, a process that could be considered transcriptional metaplasticity. Transcriptional metaplasticity may explain the ability of spaced training to facilitate long-lasting memory more readily than massed training and may allow for better separation of memories by selectively biasing against incorporation of neurons that are involved in recently encoded events. Selectivity of incorporation into neuronal networks based on transcriptional capacity is consistent with recent work by Sheena Josselyn in the amygdala, where she has observed that the level of CREB activity in a neuron can modify the likelihood that it is incorporated into a memory trace. A remarkable aspect of these findings is that if different IEGs show distinct patterns of transcriptional metaplasticity, a wealth of information may be stored by mechanisms that guide the ability to re-induce gene expression based on recent neuronal activity.

Epigenetic Mechanisms in Memory Formation

Together, the existing data on transcriptional regulation after learning suggest that long-lasting changes in gene expression accompany memory formation and that the regulation of transcription during memory may contribute to information storage. Transcriptional activation is a tremendously complex computation that integrates multiple distinct cellular signaling events to determine whether genes are expressed. In developmental biology, the results of these computations are stably stored in the form of epigenetic marks that can alter the future transcription of a gene. Epigenetic marks consist of covalent modifications to the chromatin complex that packages

DNA, as well as DNA itself. The fundamental unit of chromatin is the repeated nucleosome structure, which consists of the four core histones, each in duplicate, tightly encircled by genomic DNA. The amino-terminal tails of histone proteins protrude out of the nucleosome and are sites for post-translational modifications that regulate the ability of the transcriptional machinery to bind to and transcribe the underlying genes. Specific amino-acid residues on histone tails are targets for acetylation, methylation, phosphorylation, sumoylation, and/or ubiquitination. Histone modifications, often associated with another epigenomic mark, DNA methylation, facilitate or repress transcription, and synergistic interactions have been identified among many of these modifications. The exact combination of these epigenomic marks at the promoter and enhancers of a gene is thought to represent a histone code that determines the present rate and future capacity for transcription of that gene. Therefore, epigenomic marks play an acute signaling role that regulates transcriptional activation and an information storage role that dictates the transcriptional programs available for a cell based on prior signaling events. This information storage role of epigenetic modifications may account for the metaplasticity observed in *Arc* studies, and has been shown to stably maintain changes in behavior caused by early life experience.

The Histone Acetyltransferase CBP regulates Memory Formation

The role of CREB in long-term memory formation provides a compelling connection between memory and one particular epigenetic mark, histone acetylation, through the known interaction of CREB with the histone acetyltransferase CBP. Phosphorylation of PKA on serine 133 stabilizes an alpha-helix domain of CREB that recruits the transcriptional coactivators CBP and p300, which are important for transcription of some CREB target genes. CBP and p300 are histone acetyltransferases that modify the local chromatin environment to allow transcription at repressed genes. Acetylation of histone proteins is a type of epigenomic mark that can acutely alter the rate of transcription of underlying genes and potentially function as a type of information storage about past gene activity to regulate future gene transcription. Increased histone acetylation occurs within CA1 of the hippocampus after contextual fear conditioning. Humans with mutations in CBP have a disorder known as Rubinstein-Taybi syndrome (RTS), a genetic disorder characterized by facial abnormalities, broad toes and thumbs, and mental retardation. Although the cognitive deficits in RTS could be explained by developmental abnormalities, the role of cAMP signaling and CREB in memory consolidation suggests an acute role for CBP during memory formation.

This hypothesis has been tested with many of the genetic approaches mentioned earlier in this chapter. Mice with a single knock-out allele of CBP not only have bone morphologies characteristic of RTS, but also have impaired long-term contextual and cued fear memory. Using the α -CaMKII promoter to allow expression of mutant forms of CBP in postnatal forebrain excitatory neurons, two different groups found that expression of mutant forms of CBP lacking the histone acetyltransferase activity selectively impairs long-term memory, suggesting that histone acetylation regulates memory consolidation. In addition, mice homozygous for a knock-in mutation in the domain of CBP that interacts with phosphorylated CREB, the kinase inducible interaction (KIX) domain, have defects in long-term contextual fear memory but normal learning and cued fear memory. This series of papers on the coactivator CBP suggests that not only genetically encoded information but also interactions of the environment with the gene at the epigenetic level are important for memory formation, an exciting possibility that has opened an entirely new avenue of research into the contribution of epigenetic modifications in memory formation. In addition, as discussed previously, CBP may provide a critical role in integrating the activity of multiple transcription factors with the ultimate readout being histone acetylation.

HDACs Act as Memory Suppressors

The role of histone acetyltransferases in memory consolidation predicts that the histone deacetylases (HDACs) opposing histone acetylation may act as memory suppressors and, in fact, inhibitors of these HDAC enzymes enhance long-term memory formation. The first demonstration of a role for HDACs in hippocampus-dependent memory showed that systemic administration of HDAC inhibitors enhances contextual fear memory. In subsequent studies, it was demonstrated that direct injection of the HDAC inhibitor trichostatin A (TSA) into the hippocampus immediately after learning enhances long-term memory without affecting cued fear memory. Thus, HDAC inhibitors act within the hippocampus during memory consolidation to strengthen memory storage. This memory-enhancing effect of HDAC inhibitors requires the CREB–CBP interaction, suggesting that HDAC inhibitors may enhance memory through increased transcription of CREB–CBP target genes. Although many CREB target genes have been identified, TSA was found to effect the expression of only two out of 13 CREB target genes after learning, the orphan nuclear receptors *Nr4a1* and *Nr4a2*. Further studies that identify the subset of CREB target genes that mediates the enhancement in memory by TSA and the individual HDAC(s) that target them promise to provide novel

drug targets for memory enhancement and amelioration of cognitive deficits in neuropsychiatric and neurodevelopmental disorders.

DNA Methylation Dynamics regulate Memory Formation

Histone acetylation is only one of collection of epigenetic marks that includes histone phosphorylation, methylation, sumoylation, ubiquitination, as well as DNA methylation. DNA methylation represses transcription through direct disruption of transcription factor binding sites and recruitment of methyl–DNA binding repressors, such as MeCP2, a transcriptional repressor that recruits histone deacetylases providing a potential link between DNA methylation and histone acetylation. Recent research from David Sweatt's lab suggests that DNA methylation regulates memory formation. For instance, DNA methylation decreases after learning at the CREB target gene *Bdnf*. Surprisingly, intrahippocampal injection of DNA methyltransferase (DNMT) inhibitors blocks the change in DNA methylation and the increase in histone acetylation that accompanies learning in the absence of inhibitor, and impairs long-term memory formation. Although the findings on DNA methylation in memory consolidation are more perplexing than the findings on histone acetylation, they suggest that the dynamics of DNA methylation may be a critical aspect of memory formation. This possibility is supported by the recent findings of Huda Zoghbi's lab that MeCP2 can function as a transcriptional activator as well as a repressor, especially at CREB target genes. Thus, MeCP2 binding through DNA methylation may regulate both basal repression by recruiting HDAC activity and the ability to achieve maximal activity-mediated gene expression during memory consolidation, a possibility that might explain the results observed with DNMT inhibitors and connect DNA methylation directly with histone acetylation.

Conclusion

Research on memory formation has moved through three stages that reflect our growing understanding of information storage in the brain. First, it was discovered that neurons are electrically excitable; allowing for the insight that communication between neurons through these electrical signals can compute information and guide behavior. Second, biochemical changes in neurons were identified that could alter the relative strength of these connections within neuronal networks, suggesting plasticity as a mechanism of learning. Recently, it has been discovered that transcriptional processes guide these

changes in plasticity, allowing for the possibility that one of the most ancient information storage and processing systems in biology, the genome, has been adapted for behavioral information storage through epigenetic modifications.

See also: Active Avoidance and Escape Learning; Amnesia; Animal Models of Learning and Memory; Brain Aging; Structural Imaging Biomarkers of Risk of Alzheimer's Disease; Cognition: Learning and Memory: Pavlovian; Cognition: Learning and Memory: Spatial; Declarative Memory; Effects of Stress on Learning and Memory; Episodic and Autobiographical Memory: Psychological and Neural Aspects; Eyelid Classical Conditioning; Fear Conditioning; Fear: Potentiation and Startle; Genes and Behavior: Animal Models; Genetics of Memory in *Drosophila*; Habituation; Hormones and Memory; Implicit Learning and Memory: Psychological and Neural Aspects; Knock-Outs: Learning and Memory; Learning and Memory: Computational Models; Mechanisms of Memory Formation and Storage in *Hermissenda*; Memory and Aging, Neural Basis of; Memory Consolidation; Memory in *Caenorhabditis elegans*; Memory in the Honeybee; Molecular Neurobiology of Addiction; Mouse Genetic Approaches to Psychiatric Disorders; Neural Basis of Classical Conditioning; Neural Basis of Recognition Memory in Nonhuman Primates; Neural Basis of Working Memory; Neural Substrates of Conditioned Fear and Anxiety; Neuron Excitability and Learning; Neurotransmitters and Neuromodulators Regulating Sleep and Wakefulness; Place Cells; Protein Synthesis and Memory; Short-Term Memory: Psychological and Neural Aspects; Sleep: Learning and Memory; Synapse Formation and Memory; Synaptic Mechanisms for Encoding Memory; Temporal

Lobe and Object Recognition; Transgenic Technologies and Their Application to the Study of Senile Dementia.

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Sleep Genetics

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It has long been known that sleep is under partial genetic control. Studies in the 1930s reported that some sleep phenotypes, such as latency to sleep onset, percentage of REM sleep, and density of spindles in NREM sleep, have higher concordance in monozygotic than in dizygotic twins. Only in the last 15 years, however, at least 10 single genes have either been linked to a human sleep disorder, or have been shown to strongly affect sleep in animals.

Sleep phenotypes (or traits) are complex (quantitative), controlled by many genes, and characterized by strong interactions between genetic and environmental factors. Overall, genes with effects on sleep have been identified in flies, mice, and humans, and can be subdivided in several major functional categories: circadian regulation, neurotransmission and other signaling pathways, and ion channels.

'Circadian mutations' not only affect the timing of sleep but also its homeostatic regulation. For instance, Cry1/Cry2 double KO mice seem to live under a stronger sleep pressure in baseline conditions, when they sleep more and have higher NREM delta activity, but show little further increase in sleep duration and sleep intensity after sleep deprivation. Other mutations of circadian mouse genes (Clock, Bmal1, Npas2, and Prok2) also result in an abnormal response to sleep deprivation, as do mutations of the fly genes Cycle and Clock. Studies in humans, instead, point to an important role for another circadian gene, PERIOD3, which contains variable-number tandem-repeat polymorphisms in its coding region. Relative to PER3^{4/4} carriers, PER3^{5/5} individuals have longer duration of slow wave sleep (SWS), higher NREM delta activity, and after sleep deprivation are more impaired in executive functioning tasks in the early morning. If confirmed in a larger number of subjects, PER3 would be the first gene with a clear role in modulating susceptibility to sleep deprivation.

Many mutations of genes coding for receptors and synthetic enzymes of all major neurotransmitters and neuromodulators have been tested for their effects on sleep. Overall, these experiments have confirmed the arousal-promoting role of the noradrenergic, histaminergic, serotonergic, cholinergic, and hypocretin/orexin systems. One of the novel findings, instead, relates to narcolepsy. In dogs, narcolepsy is an autosomal recessive, fully penetrant, disorder due to a mutation in the gene coding for the hypocretin receptor 2. A narcolepsy-like

phenotype is present in both preprohypocretin/orexin KO mice (where the hypocretins/orexins producing cells are spared but the corresponding peptides are lost) and in hypocretin/orexin-ataxin 3 transgenic rats and mice (where both cells and peptides are missing). Thus, the lack of hypocretins/orexins alone is sufficient to cause a narcolepsy-like syndrome. Genetic studies have also clarified the mechanisms by which some drugs affect sleep. Mice lacking the adenosine receptor A2AR are insensitive to the waking-promoting effects of caffeine. In line with this, in humans a polymorphism in the same gene contributes to individual sensitivity in the effects of caffeine on sleep. Moreover, a functional polymorphism in the human gene coding for the catabolic enzyme adenosine deaminase results in reduced enzymatic activity in blood cells, increase in SWS duration, and increase in NREM slow wave activity. It remains unclear, however, whether this genetic variation also affects the response to sleep deprivation.

Ion channel mutations affect sleep in flies and mammals. In *Drosophila melanogaster*, mutagenesis screenings have identified two genes with striking effects on fly sleep. One is Shaker, which codes for the alpha subunit of a tetrameric potassium channel that passes a voltage-activated fast-inactivating IA current. Homologous channels in vertebrates have similar properties and, in both mammals and flies, IA plays a major role in the control of membrane repolarization and transmitter release. Flies carrying Shaker loss of function mutations sleep only 2–4 h everyday rather than 8–10 h. The second gene is called Sleepless, and Sleepless flies sleep only ~2 h a day (~85% less than controls). Sleepless codes for a glycosyl-phosphatidylinositol-anchored protein with unknown function, and has no obvious vertebrate homolog, but Sleepless flies have reduced levels of SHAKER, suggesting that their short sleeping phenotype is at least in part mediated by the Shaker current. Mice lacking the closest mammalian homolog of Shaker, Kv1.2 (KcnA2), show a decrease in NREM sleep, but their short sleeping phenotype is not as strong as in Shaker flies, perhaps because of redundancy – there is one Shaker gene in *Drosophila*, against at least 16 genes coding for alpha subunits of voltage-dependent potassium channels in mammals. A striking sleep phenotype is also observed in double KO mice lacking the voltage-dependent potassium channels Kv3.1 and Kv3.3, which sleep less (by 40%), have shorter sleep episodes, an overall decrease

in the EEG power spectrum more evident in NREM sleep, and no response to sleep deprivation. These mice are also hyperactive and show motor dysfunction, which may be partly responsible for their sleep fragmentation. The mechanism underlying the effect of these mutations on sleep is most likely different from those involved in Shaker and Sleepless mutants, because Kv3-type channels are mainly expressed in cortical and thalamic GABAergic interneurons, where their presence enables these cells to fire repetitively at high frequency.

There are two sleep disorders with a clear-cut genetic basis: fatal familial insomnia (FFI) and familial advanced sleep-phase syndrome (FASPS). FFI is a rare autosomal-dominant disease due to a point mutation at codon 178 of PRNP, the gene coding for the prion protein. The same mutation is present in patients affected by the familial form of Creutzfeldt–Jakob disease (CJD), another prion disease with extensive cortical, rather than thalamic, damage, and in which dementia, rather than insomnia, is the main clinical feature. In FFI patients, codon 129 on the mutated allele codes for a methionine, while in CJD patients it codes for a valine. Thus, a mutation at codon 178 determines the presence of a prion disease (either FFI or CJD) while codon 129 on the mutated allele determines the phenotype of the disease (CJD or FFI).

FASPS is also transmitted in a highly penetrant autosomal-dominant manner. FASPS subjects have a normal duration of sleep but go to bed ~ 4 h earlier than usual. Some FASPS individuals carry a serine to glycine mutation in PERIOD 2, one of the canonical circadian genes. Other FASPS individuals carry a mutation in the gene coding for casein kinase I delta, and the mutation may affect the ability of the enzyme to phosphorylate PERIOD 2.

While in dogs and mice narcolepsy is genetically determined, nongenetic factors play a major role in human narcolepsy. No association has been found between human narcolepsy and polymorphisms in the genes of the hypocretin/orexin system, and only one case of narcolepsy with very early onset has been associated with a mutation in hypocretin (HCRT). Yet, most patients with narcolepsy–cataplexy have low or undetectable levels of hypocretins, and narcolepsy is strongly associated with the human leukocyte antigen (HLA) allele DQB1*0602. This suggests that the deficit in hypocretinergic neurotransmission in human narcolepsy could be due to an autoimmune attack.

Restless leg syndrome (RLS) is a common sleep disorder often characterized by periodic limb movements

during sleep. RLS can be familial in up to one-third of cases. A recent genome-wide case-control study identified three predisposing loci on chromosomes 6p, 2p, and 15q, which may explain $\sim 50\%$ of the risk for RLS in individuals with European ancestry. Obstructive sleep apnea syndrome (OSAS) is also to some extent under genetic control, but the responsible genes are difficult to identify, because many of the risk factors for OSAS, including obesity and alterations of the craniofacial morphology, are also under genetic control.

See also: Bioenergetics of Sleep; Neuropsychology of Sleep; Sleep: Learning and Memory; Sleep: Medical Disorders.

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Aging and Cognition

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A common but inaccurate view is that with age, cognition deteriorates across the board. Instead, certain cognitive domains show strong effects of aging, whereas others are relatively unaffected, and some even show positive age-related changes.

Cognitive Domains that Show the Greatest Age-Related Declines

Attention

Attention is not a single, monolithic construct, but rather has been fractionated into several subtypes, some of which are more strongly affected by aging than others. On tasks of sustained attention or vigilance, older people generally perform well. When it comes to selective attention (i.e., the ability to focus on information that is currently relevant and ignore that which is irrelevant), older people are more likely to have difficulty. For example, when asked to detect a target against a background of distractors on a visual search task, older adults are usually slower than young people. Older adults seem to be especially impaired on tasks that require switching or dividing attention between perceptual or response sets.

Working Memory

Working memory is a limited-capacity system that is necessary for holding information and using it in the course of decision making, planning, and related cognitive operations. According to the influential model advanced by Baddeley and colleagues, information is held over the short term in material-specific buffers, with a central executive selecting and manipulating output from them. Whereas the ability to maintain information in these buffers remains relatively intact in normal aging, there is deterioration in the ability to manipulate it and apply it to other tasks.

Executive Functions

The executive functions are a family of cognitive processes that allow one to control and guide behavior. These processes, including planning, goal setting, strategizing, and switching, are classically impaired in patients with

discrete lesions to prefrontal cortex (PFC). Older adults are typically impaired on neuropsychological tests that measure these abilities. For example, in a meta-analysis, Rhodes showed that on the Wisconsin Card Sorting Test older people achieved fewer categories and made a greater number of perseverative errors than young people, a pattern of impairment also shown by prefrontal lesion patients. However, not all studies show significant impairment on executive tasks in older adults. Furthermore, poor performance on cognitive tests that are very sensitive to deficits in specific domains is not necessarily a predictor of poor function in practical situations: older adults are often very good at planning, strategizing, and problem solving in the real world.

Episodic Memory

A dominant view of long-term memory distinguishes between explicit and implicit or procedural memory systems, with the former handling information that is available to consciousness, and the latter not so. Explicit memory suffers with age to a much greater degree than implicit memory does. This can be seen in experiments that contrast word-stem cued recall with word-stem completion, a classic paradigm that was used to show the dissociation between impaired explicit and intact implicit memory in cases of organic amnesia. Older people are significantly impaired on word-stem cued recall, but show fewer problems when tested using word-stem completion. Explicit memory can be further subdivided into semantic (i.e., factual knowledge) versus episodic (i.e., recollection of personally experienced events) components. It is the episodic component which is especially vulnerable to aging, particularly in cases where contextual details or associations among arbitrarily linked pieces of information (such as novel names and faces) must be remembered. All other things being equal, the degree of episodic memory impairment displayed by older people is greatest when memory is tested using free recall, is lesser for cued recall, and is least for recognition; this has led to speculation that episodic memory problems in aging can be attributed, at least in part, to faulty retrieval (as opposed to encoding). Well-learned skills, routines, and habits are relatively preserved in aging.

Many researchers posit that age-related impairment in one of the aforementioned cognitive domains may be the reason for impairment in other domains. For example, age differences in episodic memory have been variously attributed to age-related impairments in attention, working memory, and/or executive function. These underlying factors may be at play even in domains that are relatively impervious to the negative effects of age, for example, language. Kemper and colleagues have argued that problems with working memory underlie subtle age-related difficulties in speech production and speech perception (e.g., losing track when listening to an especially long and grammatically complex sentence).

Many Cognitive Functions Are Preserved in Aging

There are some domains in which older adults' performance is as good as, or even superior to, that of young people. For example, general knowledge and vocabulary are accumulated throughout the life span (although there may be a slight decline in the very last years of life).

This accumulation of knowledge and experience (wisdom) may provide a mental scaffolding to help attenuate, although perhaps not completely eliminate, age differences in cognition. For example, older adults sometimes perform relatively better compared to young on tests of attention and memory when tested using materials in which they are domain experts. In daily life, older adults may often do better than expected due to reliance on well-worn routines, or because they have learned to develop and use strategies to reduce cognitive load (including using external aids, such as making a grocery list to compensate for episodic memory decline).

Cognitive Models

Several cognitive theories of normal aging have been advanced. Each of these models has roots in the literature on memory and aging, with either the original theorist(s) or others endeavoring to extend its scope from memory to cognition overall.

Processing Speed

Salthouse has posited that general slowing in cognitive and neural systems is the key mechanism of cognitive aging. This assertion is based on the observation of strong correlations between reaction times and memory: older adults who show longer reaction times tend to show poorer memory. Critics, however, have asserted that general slowing does not easily account for the complex patterns of impaired versus spared cognitive functions in

normal aging. In addition, several objections have been raised regarding this method. For one, the choice of task from which one derives reaction time may be important: tasks that are more difficult and complex tend to show stronger reaction-time correlations with memory. Also, support for the processing speed model may be weaker in longitudinal compared to cross-sectional studies.

Cognitive Resources

Craik has suggested that cognitive aging may best be characterized as the result of a shrinking pool of cognitive resources that are available to an individual. These cognitive resources (mental energy) are especially taxed by self-initiated operations as seen, for example, on memory tests involving recall as opposed to recognition. This notion is supported by studies showing that tying up cognitive resources in young people (dividing attention, by having people perform a concurrent secondary task) yields a qualitatively similar pattern in memory to that produced by aging. However, there appear to be subtle but important differences between the effects of normal aging, on the one hand, and dividing attention in young people, on the other. Further, critics have called for a clarification of the exact nature and neurobiological bases of cognitive resources.

Inhibition

Hasher and Zacks proposed that memory loss in old age primarily involves dysfunction of the ability to keep irrelevant information out of mind, and to remove no-longer relevant information from one's mind (akin to wiping clean one's mental chalkboard). A somewhat related use of the concept of inhibition has been made by Jacoby and colleagues to explain many age-related memory errors as reflecting a failure to inhibit habit by using controlled, deliberative processing (e.g., by automatically taking one's usual route home today, and failing to recollect to stop for milk at the store as one had intended). The concept of inhibitory failure does a good job of explaining some memory failures in older adults, but whether it can be extended to all of cognition is currently unclear.

Although each of the aforementioned models has the virtue of parsimony, at present, no single model appears to offer a complete explanation of the data (although, as mentioned above, many of the original theorists did not intend their theories to extend to all of cognition). Currently, most researchers would posit that multiple cognitive mechanisms go awry in aging, and recently, interest has increased in the brain changes that may underlie these cognitive changes.

Brain Changes

Current studies seeking links between cognitive aging and brain changes most often use magnetic resonance imaging (MRI), which permits inferences about neural structure and function at the molar level. Although no brain region or system is invulnerable to the effects of aging, some are more obviously susceptible than others. Three regions have received considerable interest in particular.

Prefrontal Cortex

Aging of the brain appears to follow an anterior–posterior continuum, with anterior regions especially vulnerable to aging, and posterior regions less so. Both cross-sectional and longitudinal studies indicate that PFC deteriorates more rapidly than most other regions of the brain. This pattern is evident in both gray matter (usually measured volumetrically) and white matter (whether indicated by volumetric measures, counts of white matter hyperintensities, or estimates of tract integrity from diffusion tensor imaging). In general, studies attempting to link decreases in frontal gray matter volumes with poorer performance on cognitive tasks have not yielded strong results (perhaps because the vast majority of such studies is still cross-sectional, or perhaps because of differential age effects within different subregions of PFC). In contrast, white matter integrity appears to be a relatively good predictor of cognitive function in older adults. Several recent studies have shown that, as the number of white matter hyperintensities increases and/or tract integrity decreases, attention, working memory, and episodic memory are reduced. Positive correlations between white matter integrity and cognition have been reported for both interhemispheric (e.g., corpus callosum) and intra-hemispheric connections (e.g., tracts linking PFC with parietal cortex and the striatum).

Across cognitive domains, many functional neuroimaging studies report relatively less activation in posterior regions of the brain in older adults, accompanied by greater activation in anterior regions. Within PFC, patterns of activation are often different between young and older people. Grady and colleagues were the first to report that, whereas young people showed relatively greater left PFC activation during encoding and right PFC activation during retrieval on an episodic memory task, older adults showed less activation in left PFC during encoding coupled with bilateral activation during retrieval. Cabeza has argued that this pattern exists in other domains, including working memory, executive function, and perception, although the functional role of this pattern remains unclear. Some researchers suspect that it reflects compensation on the part of older brains,

whereas others postulate that it reflects inefficiency. To answer this question, a useful and increasingly popular strategy has been to compare brain activation patterns between older adults who show relatively high versus low levels of performance, but the extant data are mixed: some studies have reported that high-performing older adults showed bilateral activation, whereas young people and low-performing elders showed unilateral activation, supporting the notion of bilateral activation being compensatory. Yet, others have found that high-performing older adults showed relatively unilateral activation patterns (similar to young people) and low-performing older adults showed bilateral activation, supporting the notion of inefficiency.

Medial Temporal Cortex

Given the fact that older people show a prominent episodic memory impairment, the hippocampus and surrounding structures in medial temporal cortex have received considerable attention. Volumetric MRI studies show robust declines in medial temporal volume with aging; although some researchers have asserted that different structures may decline at different rates (e.g., hippocampus compared to entorhinal cortex), a clear consensus has not yet emerged. Surprisingly, studies seeking links between medial temporal volume and memory have often failed to show strong correlations. Functional neuroimaging studies of episodic memory have generally shown reduced activation in medial temporal regions in older adults, accompanying their poorer behavioral performance.

Striatum

The striatum is strongly interconnected with PFC and medial temporal regions, and also shows significant structural and functional deterioration with age. Interest in the striatum's role in cognitive aging has been bolstered by assertions that the cognitive profile of normal aging is a less extreme version of that of Parkinson's disease. At present, however, it is unclear what the unique and combined roles of PFC, medial temporal cortex, and the striatum may be.

Neurotransmitter Systems

Many neurotransmitter systems are affected by aging. Historically, a loss of cholinergic cells and corresponding decline in the availability of acetylcholine in the hippocampal system have been considered an early indicator of brain and cognitive aging. Consistent with this view, there is evidence that the degree of memory loss in old age correlates with reductions in brain acetylcholine levels. Further support derives from studies showing that young

adults receiving injections of scopolamine, a drug that blocks acetylcholine activity, show cognitive changes similar to those seen in old age, whereas treatment with drugs that increase acetylcholine supply (e.g. physostigmine) can enhance memory and related functions. The cholinergic depletion is also thought to be a factor in Alzheimer's disease (AD), with current pharmacological treatments (e.g., donepezil) targeting this system in an attempt to slow down the progression of the disease. Several other neurotransmitters are necessary for cognitive function and appear to be linked with cognitive aging. For example, in both the striatum and throughout the cerebral cortex, concentrations of dopamine decline with age, and its binding is reduced. The reductions in dopamine tend to be correlated with impairments in executive functions and memory in older adults.

Neurocognitive studies of aging are still in their infancy, and will be aided by advances on several fronts. First, despite the explosion of functional MRI (fMRI) data, we still do not understand what the fMRI signal reflects about neural activity and how this relation may be affected by aging. Second, perhaps more important than the contributions of individual regions, are the interactions among them. As mentioned above, structural neuroimaging has implicated deterioration of tracts connecting different brain regions in age-related declines in several cognitive domains. In functional neuroimaging, system-level interactions are being explored with new statistical analysis methods; these have suggested that age differences in cognition can be attributed to complex changes in the interactions among PFC, medial temporal, parietal, and other regions. Third, today, most investigations focus on one cognitive domain and one neural technique (e.g., fMRI of working memory). There is a need to examine the potential combined effects of multiple influences (e.g., by combining structural with functional neuroimaging, or by examining the combined effects of working memory and frontal lobe volume on episodic memory).

Potential Confounds

Several factors must be taken into account when performing and evaluating research on cognitive aging. These include: prodromal dementia, sensory decline, affect, and variability.

Prodromal Dementia

Aging is the greatest risk factor associated with AD, and cognitive studies that recruit seemingly healthy older adults risk including participants who are in the early stages of, or on a trajectory toward, AD or another dementia. Predicting whether an older adult will show dementia

in the next few years is very difficult at present, for clinicians and researchers alike. The concept of mild cognitive impairment (cognitive function that is below average, but not impaired enough to qualify as dementia) has been proposed as useful in identifying those who are particularly at risk of developing dementia in the relatively near future. However, a certain proportion of the population will show such a cognitive profile yet remain stable. Many samples of older adults (especially in earlier studies) undoubtedly contain a few people who are on the brink of dementia, and thus such studies risk overestimating the effects of normal aging on cognition. However, the opposite danger exists, in studies in which the inclusion criteria regarding cognitive and physical health are so stringent that only a small proportion of older adults (super-agers) meet them, and the sample is no longer representative of the population.

Sensory Decline

The loss of sensory acuity is one of the first aspects of aging that comes to a layperson's mind, but is an underappreciated factor in cognitive aging research. Studies of perception and memory that add noise to visual or auditory signal can mimic the effects of aging in young people. The influence of perceptual loss may also be indirect – straining to hear or see an unclear stimulus ties up additional cognitive resources (mentioned above), leading to fatigue and a decline in performance. Despite this clear confound, not enough current studies of cognitive aging attempt to equate sensory function between older and younger samples.

Affect

A significant number of older people experience depression (and its effects on cognition can be difficult to tell apart from early dementia), although many studies show that mood grows less negative over the life span. In fact, Carstensen and colleagues have argued that with age comes a change in emotional focus, with older adults paying greater attention to positive and ignoring negative emotional information and experiences. This putative change in orientation is thought to affect processing of stimuli in laboratory studies, as well as reminiscences and social interactions in the real world.

Variability

Traditionally, studies of cognitive aging have been concerned with mean differences between older adults and college freshmen. Recently, however, interest has been revived in the question of individual differences. Not all older adults show the same cognitive profiles. For example, Glisky and colleagues have divided older adults into

those who are above average (high) versus below average (low) on executive function. These groups yield qualitatively different patterns of memory performance, supporting the assertion that executive decline underlies memory decline in aging. Such studies are generally cross-sectional, however, and require additional longitudinal and cohort-sequential data for corroboration – it is currently unclear whether low-executive older adults have always been this way, or have only recently declined to this level. Another factor is circadian arousal patterns: with age, there is a tendency for one's optimal time of day to shift from afternoon to morning. Consequently, if all participants are tested in the morning, memory differences between young and old are much smaller than if both groups are tested in the afternoon. Variability within older adults from moment to moment may also be informative: such fluctuations in cognitive function may be a harbinger of significant cognitive decline in the near future, and perhaps even imminent death!

Lifestyle Factors

Cognitive aging is largely the result of structural and functional decline in particular brain regions (e.g., PFC and medial temporal cortex/hippocampus) but several lifestyle factors also contribute. ‘Use it or lose it’ has become a familiar refrain to remind us of the importance of keeping busy and engaged in stimulating activities in order to preserve cognitive function in old age. Research has given truth to this old adage and there is now abundant evidence from animal and human studies that enriched environments, intellectual stimulation, and physical exercise all contribute to superior performance on tests of learning and memory. The interplay between biological and lifestyle factors is underscored by findings that experientially related cognitive benefits are associated with corresponding changes in brain plasticity as measured, for example, by enhanced neurotransmitter (e.g., acetylcholine and dopamine) function, increased cerebral blood flow, activity-related gene expression, and new cell growth (neurogenesis) in hippocampus.

Other lifestyle factors also affect the rate of cognitive decline in old age. Education seems to be a positive factor, possibly because intelligent, highly educated people have more cognitive reserve upon which they can draw in old age. It is sometimes difficult to determine if the effects of education are direct or indirect, in the sense that educated people are often part of a privileged sector in society that is also better informed on, and able to afford, healthy lifestyles. In contrast, excessive stress is a destructive force to which older adults, because of limited physical and psychological resources, are especially vulnerable. The adverse effects of stress in the elderly extend to cognitive performance, particularly to memory. It is not

surprising perhaps that education and stress influence cognitive function in old age; however, perhaps less predictable is the emerging relationship between cognitive ability and psychosocial status. Older people living at home function at a higher cognitive level than matched counterparts living in institutions but, in both groups, measures of psychosocial health (e.g., personal control, optimism, happiness, and meaningfulness) correlate significantly with performance on neuropsychological tests measuring a wide range of cognitive processes. These relationships have been tracked over long periods of time and the consistent finding is that the changes in one domain (e.g., psychosocial) are accompanied by parallel changes in the other. More work is needed to understand how such relationships are related to other factors (e.g., physical and mental health, and significant life events) that affect the lives of older people in major ways.

A growing body of research indicates that dietary factors significantly contribute to cognitive decline in old age, as well as to various types of cognitive impairment associated with age-related diseases. It is well established that high-fat diets harm cardiovascular and other biological systems but it is now known that high-fat (particularly saturated fat) diets, consumed over an extended period of time, can impair cognitive function. The latter effect can be substantial even in young adults but is exacerbated in older populations and in individuals at-risk for conditions that affect brain function (e.g., type 2 diabetes and AD). With respect to the latter, it has been estimated, for example, that long-term consumption of diets high in saturated and trans-fats may increase the chances of developing AD by as much as 150%. The mechanisms of such dietary effects are not fully understood but possibilities include increased insulin resistance, oxidative stress, and neuroinflammation – all of which are associated with brain aging. As a complement to studies highlighting the risk factors in dietary fat, there is also evidence that low-fat diets, especially those rich in antioxidants, or diets high in omega-3 fatty acids that have anti-inflammatory properties can enhance cognitive function.

The search for lifestyle protective factors that help to slow down the process of cognitive decline is in its early stages but the evidence is mounting that cognitive aging is as much about what happens to us as we age, as it is about getting old.

Cognitive Recovery and Rehabilitation

Historically, research into cognitive aging has been concerned largely with the pattern of lost and spared functions associated with the aging process, but interest is growing in the potential for improving cognition in the

elderly, particularly with respect to memory function. Clearly, older adults can benefit from the use of external aids as reminders (e.g., post-it notes, memory books, and electronic managers) and there seems to be relatively little resistance to this practice. However, for more complex learning and remembering, older people must make a conscious effort to summon and apply the types of internal mnemonic strategies (e.g., visual imagery and deep encoding) that young adults use automatically. In addition, considerable research has focused on developing specialized forms of practice and some interesting techniques have emerged. An example is spaced retrieval, which involves repeated rehearsal at progressively longer delays, and has the advantage of directing attention to the studied material during critical stages in the learning process. Another is the method of vanishing cues, in which the individual attempts to remember specific information with diminishing numbers of cues – a method that has been moderately effective with brain-damaged amnesic patients. These and other domain-specific techniques have been tested in normal, older adults and in older individuals with diagnosed cognitive impairment, but with only limited success. Part of the difficulty is that the benefits observed in artificial lab settings do not transfer well to real-world situations where it is necessary to recruit such techniques in the absence of environmental supports.

Domain-specific techniques typically have a relatively narrow focus but age-related cognitive decline can be pervasive, affecting a range of cognitive processes and influenced by numerous factors. Largely in response to this, the emphasis has shifted to more broad-based, multi-dimensional cognitive rehabilitation programs, designed specifically for the elderly. By far, the most ambitious of these is the Advanced Cognitive Training for Independent and Vital Elderly (ACTIVE) program, launched in 1998 as a multicenter, randomized control trial, involving 2802 independent-living older adults. The study compared three cognitive interventions (memory, reasoning ability, and speed of information processing), each consisting of 10 sessions over a 5–6-week period. Outcome measures, which included practical problem solving as well as standard experimental tests, made extensive use of composite measures to assess overall ability in the respective areas. Over a 2-year period, significant improvements followed each training program but, importantly, participants performed better only in the cognitive domain in which they received training, and there was no evidence of generalization to real-world activities.

A team of scientists and clinicians at the Rotman Research Institute in Toronto took a different approach in developing a comprehensive cognitive rehabilitation program. This program is guided by a general model of strategic processing which assumes that cognitive tasks,

whether they involve straightforward aspects of learning and memory or complex problem solving, require strategic thought directed at achieving a particular goal. The protocol divides into three, 4-week modules – memory skills training, practical task training, and psychosocial training where the aim is to enhance psychological well-being and confidence in participants' ability to follow a strategic approach in problem solving. The trial was conducted on 49 community-dwelling, normal older adults, following a multiple baseline, crossover design that allowed for between- and within-group comparisons of training effects. Abilities in all training modalities were evaluated at baseline, immediately, and at 6 months after the completion of training. Significant gains over the long term were achieved in all three modalities. Notably, there were improvements in performing simulated real-life tasks, which reflected generalization of improved cognitive skills to everyday situations.

The results of the ACTIVE and Rotman trials are encouraging and reinforce the value of the multidimensional approach. At the same time, they raise important questions. Among them is whether, in the long run, focusing on specific processes or following a more comprehensive strategy will be more efficacious. Another question relates to the suitability of the respective protocols for clinical populations. The programs were designed for individuals with age-related cognitive decline or relatively mild impairment and it is not known if they can be adapted to individuals with more severe impairment. If that proves to be the case, significant progress will have been achieved in slowing the rate of cognitive decline associated with normal and abnormal aging.

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See also: Attention and Speed of Information Processing; Brain Aging: Structural Imaging Biomarkers of Risk of Alzheimer's Disease; Episodic and Autobiographical Memory: Psychological and Neural Aspects; Memory and Aging, Neural Basis of; Parkinson's Disease.

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Brain Aging: Structural Imaging Biomarkers of Risk of Alzheimer's Disease

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Glossary

Diffusion tensor imaging – This is an MRI method that can detect microstructural alterations in the brain. It is especially useful for detecting structural changes in normal appearing white matter in demyelinating diseases and neurodegenerative disorders.

Entorhinal cortex – A region of the brain that receives multi-modal sensory information from neocortical regions and relays this information to the hippocampus. The entorhinal cortex is also important for memory function.

Gray matter – Gray matter is the other major component of the central nervous system; it contains neural cell bodies, glial cells (astroglia and oligodendrocytes), and capillaries. In contrast, white matter mostly contains myelinated axon tracts. The color difference arises mainly from the whiteness of myelin.

Hippocampus – A region of the brain that is critical for the acquisition of certain types of new information. The normal functioning of the hippocampus is important for memory about events and things (declarative memory).

Magnetic resonance imaging – A technique that allows us to visualize the structure and function of the body and especially of the brain. With this technique, the anatomy of the brain can be seen in great detail so that changes in given regions of interest can be quantified in patients with AD and in those people who are at risk of developing AD.

Mild cognitive impairment (MCI) – Mild cognitive impairment describes individuals who have cognitive impairments beyond that expected for their age and education, but who do not meet criteria for dementia. The cognitive impairments do not interfere with their activities of daily living. Although MCI can present with a variety of symptoms, when memory loss is the predominant symptom it is termed 'amnestic MCI' and is frequently seen as a risk factor for Alzheimer's disease.

Voxel-based morphometry – A neuroimaging analytic technique for quantifying group differences in MRI scans. It allows the detection of focal differences in brain anatomy using a statistical approach.

White matter – One of the main components of the central nervous system and consists mostly of myelinated axons.

Introduction

Alzheimer's disease (AD) is a brain disorder named after the German physician Alois Alzheimer who first described it in 1906. It is one of the most common neurodegenerative disorders among the elderly with more than five million Americans being affected at present. Although age is a major risk factor for AD, the disease is not a normal part of aging. The pathology associated with AD starts in parts of the brain that are critical for recent memory, such as the entorhinal cortex and hippocampus, and then spreads to other parts of the brain. It is for that reason that one of the earliest behavioral symptoms of the disease is a deficit in recent memory. In the early stages of the disease, patients may have trouble remembering, for example, what they had for breakfast, although they can remember events from their past in great detail. Some pharmacological agents can slow down the progression of AD in some people; however, at present there is no cure for this devastating disease which is the sixth leading cause of death in the United States.

Although a pathological diagnosis is the gold standard for a definitive diagnosis of AD, quantitative structural magnetic resonance imaging (MRI) can provide a proxy measure of the underlying pathology in neurodegenerative diseases. With these techniques, one can visualize, with excellent resolution, the anatomy of the brain in three dimensions in living people and quantify changes in given regions of interest. With quantitative structural MRI, it is possible to (1) examine the relation between alterations in given brain regions and the sequential development of behavioral symptoms in degenerative diseases and (2) develop biomarkers of risk of, for example, AD.

Quantitative Structural MRI Studies in People with Mild AD and in those at Risk of Developing AD

Since one of the hallmarks of AD during its initial stages is a disturbance in recent memory, medial temporal lobe regions important for memory function, such as the hippocampus and the entorhinal cortex, have received special attention in both *postmortem* and *in vivo* investigations on the pathophysiology of AD. It is for that reason

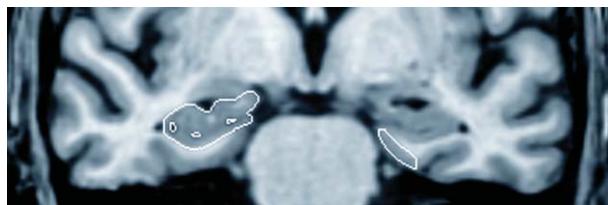


Figure 1 A coronal MRI section of the temporal lobe showing the entorhinal cortex (right hand side outline) and the hippocampus (left hand side outline).

that in this article, which focuses on sensitive MRI markers of risk of AD, we concentrate mostly on investigations of medial temporal lobe structures that show pathology very early in the disease.

Figure 1 shows the hippocampus and the entorhinal cortex on a single coronal MRI section. Atrophy of the hippocampus, a region critical for normal memory function, has been well documented in patients with a clinical diagnosis of AD using quantitative structural MRI techniques. In particular, those studies that evaluated patients with very mild AD have shown that significant hippocampal atrophy can be detected even in the very early stages of the disease.

In addition to patients with mild AD, there is now increased interest in studying elderly individuals who are at high risk of developing AD. One such at-risk group consists of people who are diagnosed with amnestic mild cognitive impairment (aMCI). Such older individuals have a deficit in the memory domain, but do not meet criteria for dementia. A number of centers have now demonstrated that people with aMCI convert to AD at the rate of approximately 12–15% per year. Among structural MRI studies that have examined the volumes of medial temporal lobe structures in older people with aMCI or cognitive complaints, the majority have reported hippocampal atrophy. Thus, aMCI may represent the very beginning stages of the disease.

Postmortem pathological studies have implicated the entorhinal cortex and the trans-entorhinal region as very early sites of involvement in AD. In fact, it has been suggested that early AD-related pathology may start in the trans-entorhinal region and the entorhinal cortex, and then spread to the hippocampus and other parts of the brain. With the development of MRI-based protocols for segmenting the entorhinal cortex, interest in quantifying the extent of atrophy in this structure to track disease progression and to differentiate those at risk for developing AD has increased. A number of structural MRI studies have reported significant entorhinal atrophy in patients with AD. More important, however, is the fact that MRI-derived entorhinal atrophy can be detected even in incipient or preclinical AD, that is, in older people with aMCI and in those with objective or subjective cognitive complaints.

In concluding this section, it should be emphasized that *in vivo* structural MRI studies of brain regions known to be pathologically involved very early in the disease process of AD show results very comparable to *postmortem* tissue findings. Thus, MRI-derived changes in brain regions of interest can be used as surrogate markers of the underlying pathology. Tissue studies, however, are by definition cross sectional and cannot determine who among nondemented older individuals will decline in cognitive status with time and develop AD. Longitudinal MRI investigations provide a unique tool for tracking the progression of the underlying pathology and for detecting those at risk of developing AD.

Age-Related Structural Alterations in Medial Temporal Lobe Gray Matter Regions

The information reviewed above regarding both *in vivo* imaging and *postmortem* tissue studies demonstrates that the so-called pathological aging affects posterior regions of the brain to a greater extent than anterior regions. However, cross-sectional MRI studies have suggested that anterior brain regions (i.e., prefrontal and frontal lobes) are more significantly affected by aging *per se* than temporal lobe regions. A number of investigations on aging have reported age-related shrinkage of hippocampal volume, although not to the same extent as in mild or incipient AD (see **Figure 2** which shows hippocampal

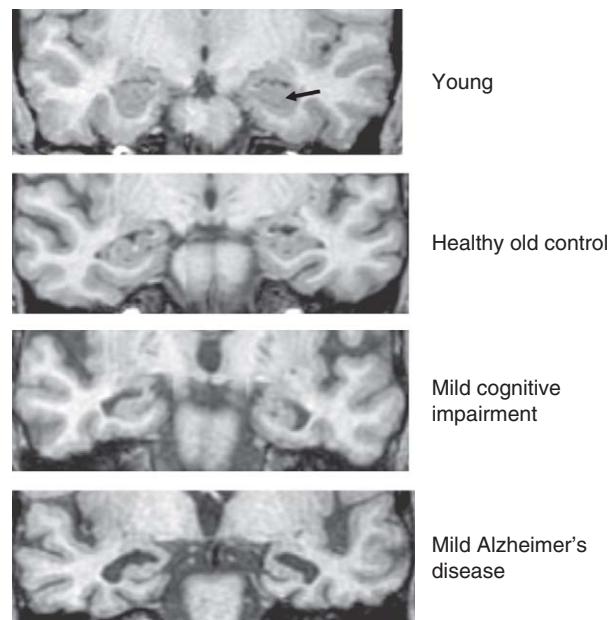


Figure 2 A montage of coronal images showing the effects of age and disease progression on hippocampal size. The arrow points to the hippocampus.

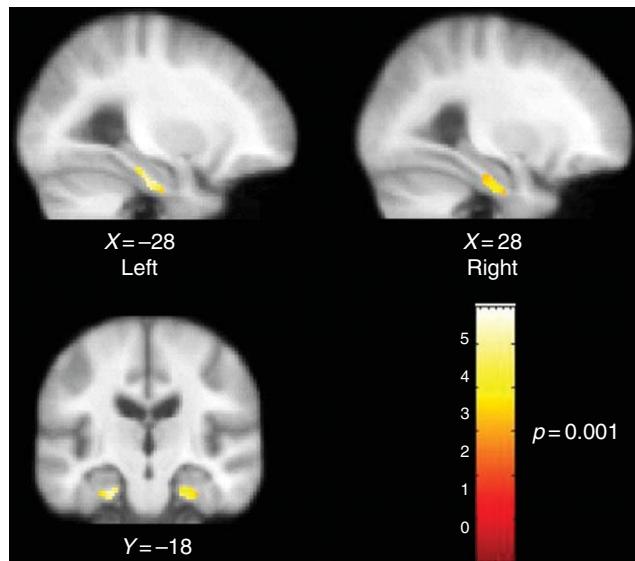


Figure 3 Color map showing significant ($p = 0.001$) voxels of decreased white matter density (volume) in participants with aMCI compared to controls, superimposed on coronal and sagittal slices of a template based on data for all subjects in the study. The image is masked to include white matter regions. The colors correspond to the t values shown on the color bar. Note the bilaterality of significant differences in the white matter of the parahippocampal gyrus. Adapted with permission from Stoub TR, deToledo-Morrell L, Stebbins GT, Leurgans S, Bennett DA, and Shah RC (2006) Hippocampal disconnection contributes to memory dysfunction in individuals at risk for Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America* 103: 10041–10045.

shrinkage as a function of aging and disease progression). The effects of age on entorhinal cortex volume are less clear, with some publications demonstrating lack of an age effect, while others reporting minimal change. Since the entorhinal cortex is one of the earliest brain regions pathologically affected in AD, it may be an important region to track longitudinally in order to develop markers important for differentiating healthy from pathological aging due to degenerative disorders such as AD.

In Vivo Anatomical Markers of Risk of AD

There is now increased interest in developing imaging markers that can discriminate reliably those among elderly individuals who are at high risk of developing AD as determined by longitudinal clinical as well as imaging follow-up. The impetus for this interest stems from the conviction that therapeutic interventions, when available, may be much more effective if initiated when older people are in the prodromal stages of the disease process. An exemplary longitudinal multi-center study is the Alzheimer's Disease Neuroimaging Initiative (ADNI) funded partly by the National Institute on Aging and initiated in 2004. ADNI is following 200 elderly control subjects with no cognitive impairment, 400 individuals diagnosed with aMCI, and 200 patients with mild AD. The aim of ADNI is to develop improved methods for clinical trials and to provide a large database which will improve the design of treatment trials by collecting

information on the trajectory of anatomical, biomarker, and cognitive changes.

Although smaller in scale than ADNI, a number of longitudinal MRI studies that measured the volumes of medial temporal lobe structures such as the entorhinal cortex and hippocampus have shown the annual rate of atrophy of both structures to be greater in patients with AD and in those with aMCI than in healthy elderly controls with no cognitive impairment. Furthermore, longitudinally detected decreases in entorhinal cortex and hippocampal volume were shown to be closely related to the rate of decline in memory function.

Centers that have been following older participants longitudinally have also reported on MRI-derived anatomical markers that predict conversion from MCI to AD. However, the findings are not all concordant, partly due to differences among investigations in regions examined or techniques used in determining atrophy in given structures. A number of laboratories have reported that the volumes of the entorhinal cortex and hippocampus are predictive of who among people with a diagnosis of aMCI will convert to AD in the future. This is the case when the volumes of these structures are derived from the baseline scan at entry into a longitudinal study, as well as when volumes are tracked longitudinally.

Those laboratories that have compared the volumes of the entorhinal cortex and hippocampus in predicting risk of AD have found entorhinal volume to be a somewhat better predictor. This is not surprising since AD-related pathology may start in the entorhinal area before the

hippocampus. In a longitudinal MRI paper from our laboratory, we sought to determine if baseline entorhinal and hippocampal volumes and their rate of atrophy could predict risk of AD. We used proportional odds models to assess the relationship between entorhinal and hippocampal size and risk of AD among 58 nondemented elderly people, 23 of whom had a diagnosis of aMCI. All participants were followed with yearly clinical evaluations and structural MRI scans for up to 5 years (baseline and 5 years of follow-up). Fourteen of 58 nondemented people developed AD during the follow-up period, only three of whom had entered the study as healthy control subjects. Not surprisingly, initial diagnosis of aMCI was found to be a risk factor for incident AD. In addition, both baseline entorhinal volume and its rate of decline were found to be independent predictors of risk of incident AD, but, surprisingly, initial hippocampal size and its rate of decline were not, after controlling for entorhinal volume. These *in vivo* results are in line with *postmortem* tissue investigations suggesting that AD-related pathology affects the entorhinal region before the hippocampus. Structural MRI results indicating that entorhinal volume reductions may precede those of the hippocampus have also been reported by other laboratories.

White Matter Changes in Normal and Pathological Aging: Diffusion Tensor Imaging

Most MRI investigations of medial temporal lobe structures in MCI and AD have concentrated on changes in gray matter regions. However, alterations in white matter can also contribute to age- or disease-induced cognitive changes by partially disconnecting different parts of the brain and disrupting information flow. The white matter of the parahippocampal region is especially important in this respect, since its disruption can partially disconnect the hippocampus from incoming sensory information important for forming memories. The anterior medial parahippocampal white matter region includes the perforant path, known to be pathologically involved very early in AD. The perforant path is a white matter tract that relays multi-modal sensory information from the entorhinal cortex to the hippocampus. Majority of perforant path fibers originate from cells in layer II of the entorhinal cortex. These cells are known to be vulnerable very early in AD and, therefore, their loss may affect the structure of the perforant path.

Using an automated analytic technique called voxel based morphometry, a study from our laboratory demonstrated a significant decrease in the volume of the parahippocampal white matter region that includes the perforant path in people with aMCI compared to healthy control subjects (see **Figure 3**). Furthermore, we found

that both hippocampal volume and parahippocampal white matter volume were significant predictors of memory performance. Thus, in addition to hippocampal atrophy, the disruption of parahippocampal white matter fibers contributes to memory decline in older individuals with aMCI by partially disconnecting the hippocampus from incoming sensory information. Other laboratories have reported similar alterations in parahippocampal white matter in patients with AD.

White matter volume change may reflect not only loss of afferent and efferent fibers in the region of the parahippocampal gyrus, but may also be due to partial demyelination in remaining fibers. These events are accompanied by increased brain water content. Since MRI is based on excitation and relaxation of hydrogen atoms, this increased water content alters the MRI signal. One novel MRI technique that takes advantage of this alteration in signal is diffusion tensor imaging (DTI). This technique allows the examination of alterations in the microstructure of white matter *in vivo*; in addition to volumetric changes, it can determine if the remaining normal appearing white matter is really normal.

DTI is based on sensitizing the MR signal to the movement of hydrogen on the order of several microns (diffusion-weighted MRI) and measuring the direction and magnitude of hydrogen diffusion in at least six noncollinear gradients simultaneously. The three-dimensional geometry of the diffusion in a particular volume element (voxel) can be described by a mathematical construct called a tensor that can be represented by a 3×3 matrix. From the diffusion tensor in each voxel, one can derive three eigenvectors defining the magnitude and direction of the diffusion system, with corresponding eigenvalues λ_1 , λ_2 , and λ_3 . The average of the three eigenvalues represents the mean molecular motion and is referred to as mean diffusivity (MD). MD is affected by barriers to diffusion, but does not provide information on the directionality of diffusion. Based on the three eigenvalues and the mean eigenvalue (λ), the intra-voxel organization of diffusion direction can be measured and is referred to as fractional anisotropy (FA). FA values range from 0 to 1, with 0 indicating completely random diffusion (isotropic diffusion) and 1 representing completely directional diffusion (anisotropic diffusion). Highly organized and myelinated white matter tracts have high FA because hydrogen diffusion is highly constrained by the tract's organization. When the barriers to free diffusion of hydrogen in white matter degenerate, either due to age or degenerative disorders, mean diffusivity increases and fractional anisotropy decreases.

As was the case for gray matter, in general, studies that have used DTI to examine the effects of age on white matter integrity have reported age-related reductions in fractional anisotropy and increases in mean diffusivity

with a tendency toward greater effects in anterior versus posterior regions of the brain. In contrast to the findings of decreased white matter integrity in anterior regions in healthy older people, DTI investigations in people with MCI and AD have reported that posterior white matter regions are more severely affected than anterior ones. Those studies that have especially targeted the parahippocampal white matter region as a region of interest to investigate with DTI have demonstrated that remaining fibers in this region show microstructural alterations in people with AD as well as MCI. Thus, in addition to parahippocampal white matter atrophy, microstructural alterations in remaining fibers would further degrade transmission of sensory information from the entorhinal cortex to the hippocampus in AD and contribute to the memory dysfunction.

MRI-Derived Markers that Differentiate Healthy from Pathological Aging

Recently, there has been increased interest in a handful of centers in determining MRI markers of risk of AD not only among people who are known to be at risk, such as those with aMCI, but also among older people who enter longitudinal projects as healthy controls with no cognitive impairment. Such studies have shown that even at the baseline MRI evaluation, control subjects who decline over time and receive a diagnosis of AD have smaller entorhinal cortex, hippocampus, and parahippocampal white matter volumes compared to controls who remain stable.

Conclusions

In conclusion, the material covered in this article shows that MRI-derived volumes of mesial temporal lobe structures that are pathologically involved early in AD provide sensitive markers of risk of AD. Atrophy in these regions can detect, years ahead of time, not only those who will

convert from a diagnosis of MCI to AD, but also those among healthy older individuals who are at risk of developing AD.

See also: Aging and Cognition; Brain Imaging; Memory and Aging, Neural Basis of.

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Cholinergic Systems in Aging and Alzheimer's Disease: Neurotrophic Molecular Analysis

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Glossary

Acetylcholinesterase (AChE) – An enzyme that degrades (through its hydrolytic activity) the neurotransmitter acetylcholine, producing choline and an acetate group. It is mainly found at neuromuscular junctions and cholinergic synapses in the central nervous system, where its activity serves to terminate synaptic transmission. AChE has a very high catalytic activity – each molecule of AChE degrades about 5000 molecules of acetylcholine per second. The choline produced by the action of AChE is recycled and transported, through reuptake, back into nerve terminals where it is used to synthesize new acetylcholine molecules.

Alzheimer's disease (AD) – Also called 'senile dementia of the Alzheimer type (SDAT)' or simply Alzheimer's, it is the most common form of dementia in the elderly. This incurable, degenerative, and terminal disease was first described by German psychiatrist Alois Alzheimer in 1906. Generally, it is diagnosed in people over 65 years of age, although the less-prevalent early-onset Alzheimer's can occur much earlier.

Choline acetyltransferase (ChAT) – An enzyme that is synthesized within the body of a neuron. It is then transferred to the nerve terminal via axoplasmic flow. The role of ChAT is to join acetyl-CoA to choline, resulting in the formation of the neurotransmitter acetylcholine.

Metalloproteinases (or metalloproteases) – A family of enzymes from the group of proteinases, classified by the nature of the most prominent functional group in their active site.

Neurotrophins – A family of proteins that induce the survival, development, and function of neurons. These substances belong to a class of growth factors which are (secreted proteins) capable of signaling particular cells to survive, differentiate, or grow. Growth factors such as neurotrophins that promote the survival of neurons are known as neurotrophic factors.

Neurotrophic factors are secreted by target tissue and act by preventing the associated neuron from initiating programmed cell death – thus allowing the neurons to survive. Neurotrophins also induce differentiation of progenitor cells to form neurons.

Tau – A microtubule-associated protein, which is abnormally hyperphosphorylated in Alzheimer's disease and accumulates in neurons undergoing neurofibrillary degeneration.

Introduction

Age is the main risk factor for the development of a spectrum of neurological disorders including Alzheimer's disease (AD). AD is a progressive and fatal age-related neurodegenerative disorder manifested by cognitive/memory decline, loss of executive functioning, progressive impairment of activities of daily living, as well as a variety of neuropsychiatric symptoms and behavioral sequelae. Prevalence studies indicate that approximately 18 million people worldwide have AD. In the USA, there are more than 5 million people with AD. The percentage of people with AD increases by a factor of 2 with approximately every 5 years of increased age, meaning that approximately 1% of 60-year-olds and approximately 30% of 85-year-olds suffer from the disease. In many neurological disorders, specific neuronal populations are selectively vulnerable. This is especially true for AD. A consistent pathological feature of AD is the degeneration of cholinergic basal forebrain (CBF) neurons ([Figures 1\(a\)](#) and [1\(b\)](#)). CBF neurons located within the septal/diagonal band complex and the nucleus basalis provide the major cholinergic innervation to the cortex and hippocampus ([Figure 1\(c\)](#)). Pronounced neurodegeneration of CBF neurons is also found in patients with Down syndrome (DS) as these subjects display AD-like pathology by the third decade of life. Over the last three decades, the relationship between CBF function and cognition, especially age-related cognitive decline, has engendered great scientific interest and is a major focus for pharmacotherapeutic intervention in AD. Several reports have demonstrated a significant reduction in CBF neurons, cortical choline acetyltransferase (ChAT) activity, and cholinergic cortical fibers in human aging. In particular, clinical-pathologic studies show that deficits in the basocortical cholinergic system correlate highly with

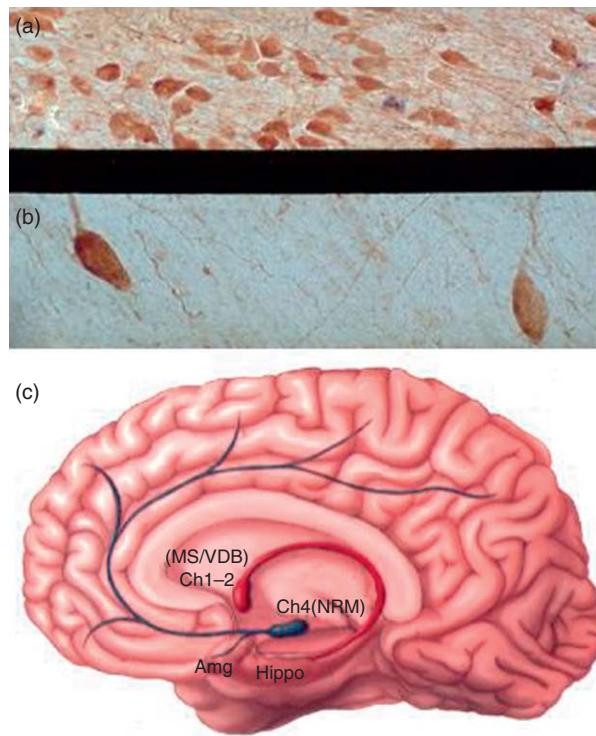


Figure 1 (a) Photomicrograph of human cholinergic neurons with the nucleus basalis in an aged normal subject. Cholinergic neurons were immunohistochemically visualized using an antibody that recognizes choline acetyltransferase. (b) Reduction in cholinergic basal forebrain neurons in advanced Alzheimer's disease. (c) An artist's rendition showing the major cholinergic forebrain projection pathways in the human brain. Amg, amygdala; Ch1-2, cholinergic medial septal (MS) and ventral limb of the diagonal band (VDB) projection neurons to the hippocampus (red); Ch4, cholinergic nucleus basalis of Meynert projection neurons to the cortex and amygdala (blue); Hippo, hippocampus.

disease severity and duration in AD patients. Supporting this concept are studies of the CBF system in nonhuman primates and rodents, demonstrating clear involvement in conscious awareness, attention, working memory, and other mnemonic processes. Taken together with the human findings, this line of research has supported the so-called 'cholinergic hypothesis.' This hypothesis essentially states that a reduction in cholinergic function in the brain contributes to the pernicious cognitive decline seen in senescence and AD. Although degeneration of CBF neurons in AD is well described, there appears to be a capability for neuroplasticity, at least for certain populations of cells. For example, CBF degeneration is accompanied by an increase in the number of nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-d) neurons and an overexpression of the neuropeptide galanin (GAL) which may either exacerbate or slow CBF dysfunction, and this potential for neuroplasticity is a source of extreme interest in the field of AD. While it is clear that many brain regions degenerate in aging and AD,

cholinergic hypofunction is still thought to be central to cognitive decline.

Aging and CBF System

Since age is a major risk factor for the onset of AD, it is crucial to understand the consequences of aging on the CBF system. Mice aged 30 months display a 75% reduction in the biosynthesis of acetylcholine (ACh). In the aged brain, cholinergic neurons are more impaired in ACh release following potassium stimulation than in their ability to synthesize the transmitter. It has been suggested that brain cholinergic neurons function at a normal level until they are stressed or damaged. Furthermore, sustained stress or damage to the CBF neurons is posited to interfere with the ability of CBF neurons to generate sufficient ACh release required for normal cell function during aging. In young rats trained on an attentional task, submaximal chemical lesions of CBF neurons were created and a difference between young lesioned and control rats did not occur until 30 months of age, when the damaged group displayed significant deficits in the task performance. These results are relevant since the age-related changes were related to the dynamic aspects of a second hit of brain CBF neurons. Although animal models of aging provide direct evidence of CBF impairment during aging, data derived from human autopsy material often show dramatic structural age-associated changes. For example, cholinergic axons exhibit axonal and neuronal abnormalities in nondemented middle-aged people, which increased in nondemented older subjects and were reduced in intensity in severe AD. By contrast, these types of axonal abnormalities did not occur in young people, although CBF neurons containing neurofibrillary tangles (NFTs) were found as early as the third decade of life. Perhaps CBF system abnormalities are present very early in aging and progress during the onset of AD and DS.

Cortical ChAT Activity in Early AD

The loss of cholinergic markers within selectively vulnerable neurons and their respective projection sites has been a major tenet in AD. A progressive phenotypic down-regulation of markers within CBF neurons as well as frank CBF cell loss has been observed consistently along with an associated reduction of ChAT and acetylcholinesterase (AChE; a cholinergic degrading enzyme) activity within the cortex in AD. It is presumed that progressive disruption of cholinergic function underlies much of the short-term memory loss seen in AD. In fact, delaying or preventing cholinergic neurodegeneration or minimizing its consequences is the mechanism of

action for most currently available Federal Drug Administration (FDA)-approved drugs for the treatment of AD. Since physostigmine and oral anticholinesterases have beneficial effects on patients with AD, it is suggested that the CBF system is somewhat preserved or has the propensity for neuroplasticity during the progression of dementia, despite a well-documented loss of cholinergic biosynthetic machinery (including ChAT and AChE enzyme deficits) in patients with this disease. Interestingly, ChAT activity is preserved in the hippocampus and neocortex of people with prodromal AD termed 'mild cognitive impairment (MCI).' This suggests that cholinergic enzyme deficits are likely not the primary deficit of memory loss observed in MCI, although this does not eliminate other types of cholinergic dysfunction early in the course of the disease. These findings indicate that the hippocampal and frontal cortical cholinergic projection systems are capable of compensatory and/or neuroplasticity responses during the early stages of AD and can possibly be exploited by well-designed therapeutic regimens with novel drug treatments. This upregulation of hippocampal and frontal cortex cholinergic tone may be important for promoting transmission and/or delaying neurodegenerative defects impacting the transition to frank AD. In this regard, increased ChAT activity in the MCI hippocampus was associated with high Braak scores, a marker for tau pathology that, to some extent, correlates with cognitive decline (Braak III/IV stage), suggesting a compensatory upregulation of ACh production in response to the disconnection of glutamatergic entorhinal cortex input to the hippocampus early in the disease process. In this scenario, increased hippocampal ChAT activity may reflect reactive synaptogenesis, the filling in of denervated glutaminergic synapses by cholinergic axons arising from the septum. The factors underlying the elevation of frontal cortex ChAT activity in MCI are less clear, but may be related to the finding that the anterior cholinergic subfield of the nucleus basalis, which innervates the frontal cortex, is less affected in AD and therefore most likely capable of cholinergic neuroplasticity. AChE, the enzyme that hydrolyzes ACh at the synapse, fails to show a reduction in cortical areas until at least moderately severe levels of dementia. Positron emission tomographic (PET) investigations utilizing a ligand, which labels AChE *in vivo*, suggest only a mild loss of AChE in MCI and mild AD. Moreover, a functional magnetic resonance imaging (fMRI) study of the brain demonstrated that people with MCI treated with the FDA-approved anticholinesterase donepezil showed increased frontal cortex activation relative to untreated controls, which was positively correlated with task performance. In addition to AChE, butyrylcholinesterase (BChE), a serine hydrolase that also catalyzes the hydrolysis of ACh, is found in neurons and glia and is associated with NFTs and senile

plaques (SPs) in AD brain. An AD population-based genetic analysis identified a point mutation that changes alanine 539 to threonine in the K variant of BChE, which reduces serum BChE levels, and could be associated with cognitive decline. BChE activity is increased, whereas AChE activity remains unchanged or declines in AD brain. These data and the observations of cholinergic neuroplasticity in people with MCI support the continued use of cholinesterase inhibitor drugs as a treatment during the onset of AD. Since some patients respond better to a particular cholinesterase inhibitor compared to others, MRI or related noninvasive imaging technologies may be a tool to match patients to an optimal AChE treatment regime or drug design strategy.

CBF System in Early AD

Another tenet of AD is the reduction in the number of CBF neurons in end-stage patients. However, numerous studies using different phenotypic cholinergic cell markers have revealed that CBF neuron alterations during AD progression are more complex than originally proposed. For instance, the vesicular ACh transporter (VACHT), which co-localizes with ChAT in human CBF neurons and participates in loading ACh into synaptic vesicles in cholinergic terminals, is not severely altered in AD. Pharmacological investigations of VACHT in postmortem AD tissue or *in vivo* imaging studies using vesamicol and its analogs suggest that VACHT levels are preserved or minimally reduced coincident with a severe decline in cortical ChAT activity. The discordance between CBF neuronal ChAT and VACHT in projections to the cortex is intriguing in light of the discovery that they are part of a single cholinergic gene locus with shared regulatory elements. Moreover, rodent cholinergic lesion studies and postmortem human brain studies suggest that cholinergic neurons shrink, are depleted of phenotypic markers, and/or persist in an atrophic state after injury or during the pathological process rather than degenerate outright, despite containing NFTs in early AD. Taken together, these observations suggest that CBF neurons may be viable, albeit dysregulated, early in AD, and amenable to pharmacological treatments that may delay cognitive impairment associated with cholinergic deficits.

CBF Neuron Survival – Live and Let Die

The neurotrophic factor, nerve growth factor (NGF), and its cognate high-affinity tyrosine kinase A (TrkA) and low-affinity ($p75^{NTR}$) receptors maintain the survival of CBF neurons (Figure 2). NGF is synthesized from its precursor molecule, proNGF, and is proteolytically

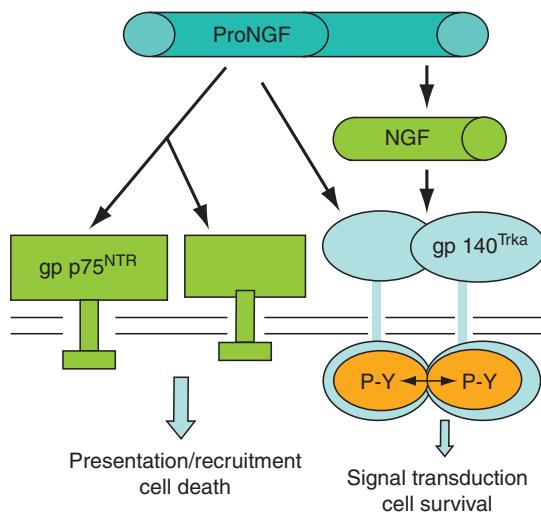


Figure 2 Schematic drawing showing the proNGF-, NGF-, TrkA-, and p75^{NTR} pathways.

cleaved to a mature biologically active neurotrophin peptide (Figure 2). Mature NGF (mNGF) binds to TrkA, which activates signal transduction pathways mediating the survival effects of NGF, and to the p75^{NTR} receptor, a positive modulator of NGF/TrkA binding. Activation of the p75^{NTR} receptor has multiple actions, including apoptosis, which are dependent on interactions with other chaperone molecules. The physiological consequence of TrkA and p75^{NTR} signaling depends upon their interactions with proNGF. The dysregulation of the NGF receptor system plays a key role in CBF neuron dysfunction in AD. In this regard, proNGF activity is increased in the cortex of people clinically diagnosed

antemortem with MCI or mild AD compared to age-matched normal subjects (Figure 3). Several studies suggest that recombinant proNGF binds TrkA and promotes neuronal survival and neurite outgrowth similar to mNGF, but is approximately fivefold less active than the mNGF peptide. Although TrkA-mediated proNGF retrograde transport has not been demonstrated, proNGF accumulation in CBF cortical target sites may be due to reduced cortical TrkA levels and/or impaired retrograde transport of TrkA to CBF perikarya. Furthermore, reduced cortical TrkA levels were positively associated with lower cognitive performance, whereas increased cortical proNGF levels were negatively correlated with a test of cognition.

Studies indicate that increases in cortical proNGF may result in proapoptotic signaling via binding to the p75^{NTR} receptor. Other recombinant proNGF forms bind the p75^{NTR} with high affinity and promote neuronal apoptosis. Perhaps increased proNGF, in combination with reduced TrkA, results in increased binding between proNGF and p75^{NTR}, potentially shifting away from cell survival signaling to apoptotic proNGF signaling (Figure 3). In this regard, *in vivo* studies indicated that the precursor region of the NGF proneurotrophin molecule alone binds to TrkA. This binding differs from another site located on the mNGF molecule that induces downstream TrkA and extracellular signal-regulated kinase 1/2 (ERK1/ERK2) phosphorylation. Since proNGF binds with TrkA to a lesser degree than NGF, it has been proposed that during the progression of AD, the increased proNGF:NGF and p75^{NTR}:TrkA ratios shift in favor of cell death over neuroprotective actions. Moreover, proNGF can initiate apoptosis even in the presence of trophic activation by the

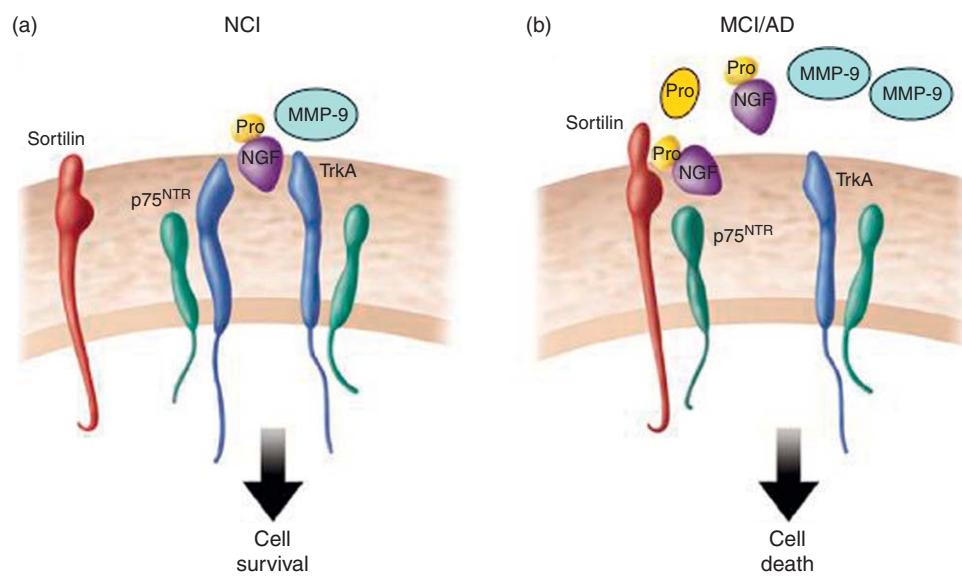


Figure 3 Drawing showing the shift in the balance of proNGF; NGF : TrkA; p75^{NTR}/sortilin : MMP-9 cell survival to death pathways during the progression of Alzheimer's disease (see text for details).

mNGF binding to TrkA, demonstrating that the death signal can override survival, and further exacerbate ongoing neurodegenerative processes. Since aging is also accompanied by increases in proNGF protein levels within afferent targets of cholinergic neurons, a role for proNGF in connectivity-based, age-dependent cell death may exist. In the face of reduced TrkA, p75^{NTR} may activate beta-site amyloid cleaving enzyme 1 (BACE1) cleavage of the amyloid precursor protein (APP), which requires NGF binding and activation of the second-messenger ceramide. Since aging activates β -amyloid (Abeta) generation in the brain by switching from TrkA to p75^{NTR} binding, it is possible that NGF receptor balance is a molecular link for amyloid processing between normal aging and AD. Therefore, drugs that maintain a homeostatic balance between TrkA and p75^{NTR} may slow Abeta accumulation and SP deposition in the aged population and slow the onset of MCI and, ultimately, AD.

Sortilin Regulation of Cholinergic Forebrain Cell Survival

The proapoptotic effect(s) of p75^{NTR}-mediated proNGF signaling is dependent on interactions with the neurotensin receptor sortilin, a Vps10p-domain-trafficking protein, which acts as a cell surface co-receptor with p75^{NTR} to mediate proNGF-induced cell death. Sortilin is required for p75^{NTR}-mediated apoptosis following proNGF treatment and blocking this binding event precludes high-affinity binding of proNGF to p75^{NTR} and subsequent cell death. Sortilin levels are significantly upregulated in aged CBF neurons, but not in the cortex of patients with severe AD (Figure 3). Pro-survival or proapoptotic signaling in the CBF system in aging and during the progression of AD may depend upon alterations in the stoichiometry of TrkA, p75^{NTR}, and proNGF binding, the availability of various co-receptors (e.g., sortilin), and the physiological role of proNGF within these different milieus (e.g., decreased neurotrophism or increased apoptotic signaling). A shift in the ratio of any of these factors during the onset of AD may alter the functional outcome that proNGF binding imparts upon CBF neurons. Ultimately, defining these relationships *in vitro* and *in vivo* in relevant cell culture and animal models along with parallel human postmortem brain-tissue investigations will be a major research effort in the development of neurotrophic mimetics for the treatment of age-related dementia. If proNGF binds with the p75^{NTR} and induces apoptotic cell death, then it is crucial to design drugs that exert neuroprotection by blocking proNGF binding to p75^{NTR}. On the other hand, if proNGF binds with less affinity to TrkA, resulting in cell survival, then the development of drugs that enhance this binding event could provide neuroprotection in aging and AD.

Metabolic Dysfunction in NGF Degradation in AD

The upregulation of cortical proNGF in subjects with MCI and AD suggests that changes occur in the metabolic pathways modulating the maturation and degradation of NGF, which in turn are crucial for the survival of CBF neurons. *In vitro* studies indicate that a protease cascade, which converts proNGF to mNGF and degrades NGF in the extracellular space by the coordinated activity of plasminogen, tissue plasminogen activator (tPA), neuroserpin, matrix metalloproteinase 9 (MMP-9), and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1), may be defective in AD. MMP-9 is a member of the family of Zn²⁺-containing and Ca²⁺-requiring endoproteases capable of degrading NGF in the extracellular matrix. Upregulation of MMP-9 protein levels and activity can occur in both AD and MCI cortex and correlate inversely with cognitive status, suggesting that the dysfunction of the NGF protease degradation cascade plays a prominent role in the dysregulation of the NGF system during the prodromal stage of AD (Figure 3). The protease MMP-9 pathway most likely impacts degradation of proNGF to mNGF and an increase is likely to enhance the breakdown of NGF more readily in AD, leading to CBF neuron death.

Neurotrophin Gene Expression Defects in CBF Neurons in MCI and AD

Single-cell gene expression profiling was coupled with custom-designed microarrays and validation with both real-time quantitative polymerase chain reaction (qPCR) and *in situ* hybridization to evaluate the genetic signature of CBF neurons during the progression of AD. During disease onset, there is a significant downregulation of TrkA, TrkB, and TrkC gene expression in CBF neurons during the development of AD (Figure 4(a)). An intermediate reduction occurs in MCI, with the greatest decrement in mild AD compared to aged controls. Moreover, two separate expressed sequence-tagged cDNAs (ESTs) for each Trk gene, for example, ESTs targeted to the extracellular domain (ECD) and tyrosine kinase (TK) domains, were downregulated. In contrast, there was a lack of regulation of p75^{NTR} expression in CBF neurons. A step-down dysregulation of Trk expression may, in part, underlie CBF neuron demise associated with the clinical presentation of AD. Supporting this concept is the finding that Trk receptor downregulation in CBF neurons is associated with multiple measures of cognitive decline, including a global cognitive score (GCS) and the mini-mental state exam (MMSE).

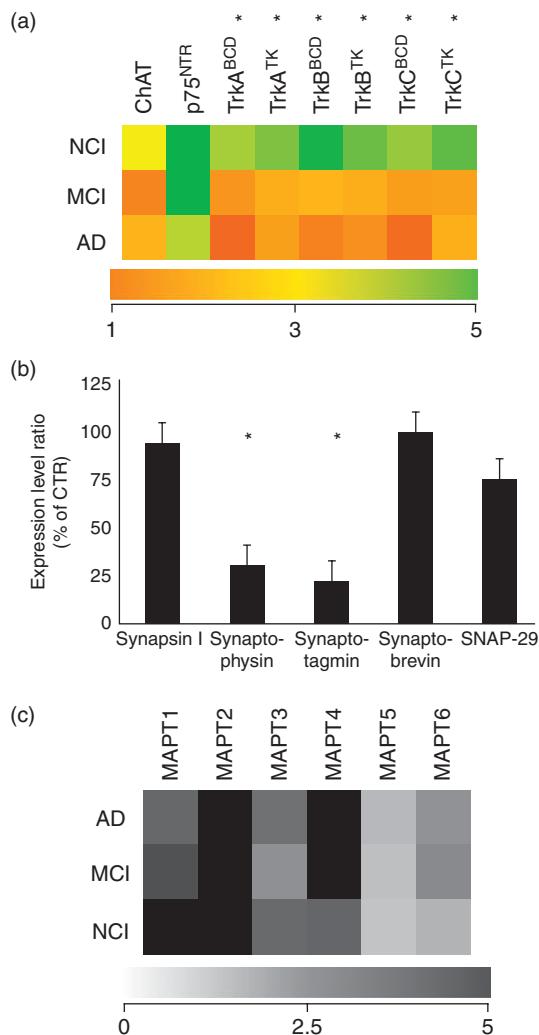


Figure 4 Expression profiling of cholinergic basal forebrain (CBF) neurons during the progression of dementia. (a) Expression profile analysis of p75^{NTR}, TrkA, TrkB, TrkC, and ChAT derived from individual nucleus basalis neurons from NCI, MCI, and AD subjects. Heatmap with a color-coded scale (green = low levels and red = high levels) illustrates relative expression levels. No significant differences are found for ChAT and p75^{NTR} gene expression. In contrast, statistically significant downregulation (* denotes $p < 0.01$) of TrkA, TrkB, and TrkC is observed in MCI and AD as compared to NCI. ESTs identifying the extracellular (ECD) and tyrosine kinase (TK) domains display downregulation. The decrement of Trk gene expression in MCI is intermediate relative to AD, indicating a step-down effect in expression levels from NCI to MCI to AD. (b) Histogram showing selective downregulation of the synaptic-related markers synaptophysin and synaptotagmin observed in AD as compared to age-matched nondemented controls (* denotes $p < 0.01$). In contrast, no significant differences are observed for synapsin I, synaptobrevin, or SNAP-29, indicating a relative specificity of gene expression regulation. (c) Heatmap indicating no overall differences in tau isoform expression in single CBF neurons during the progression of AD. However, a significant shift in the 3Rtau/4Rtau ratio was observed in AD and MCI (CBF neurons) relative to NCI.

Cholinergic Receptor Expression in MCI and AD

Two classes of receptors involved in cholinergic transmission are drug targets for AD: nicotinic ion channels, which mediate fast postsynaptic transmission, and muscarinic G-protein-coupled ACh receptors. The ionotropic nicotinic ACh receptor (nAChR) is a pentameric membrane protein composed of four polypeptide subunits designated nAChRs α , β , δ , and γ . The muscarinic ACh receptor (mAChR) family includes five members, M1–M5. A single-cell expression study via microarray analysis uncovered differential expression levels for nAChR and mAChR receptors as well as ChAT within single CBF neurons obtained from people with no cognitive impairment (NCI), MCI, and AD. Although ChAT messenger ribonucleic acid (mRNA) expression levels did not differ across clinical conditions (Figure 4(a)), there was a significant upregulation of $\alpha 7$ nAChR subunit expression in AD compared to NCI and MCI. No differences were found for other nAChR subunits across clinical groups. This increase in $\alpha 7$ nAChR expression levels within CBF neurons was inversely associated with performance on the GCS and MMSE. CBF upregulation of $\alpha 7$ nAChR is also consistent with reports of increased $\alpha 7$ nAChR mRNA and protein expression levels in hippocampal neurons, astrocytes, and peripheral blood leukocytes in AD. Despite a putative beneficial role for increased CBF neuron $\alpha 7$ nAChR expression in AD (that may be relevant to smoking behavior as well), evidence suggests that increased $\alpha 7$ nAChR expression contributes to cellular degeneration. Notably, $\alpha 7$ nAChR binds and/or interacts with APP and Abeta peptides. Increased CBF neuronal $\alpha 7$ nAChR expression may arise as a compensatory response that is offset by aberrant Abeta- $\alpha 7$ nAChR interactions, leading to cholinergic dysfunction. M1 subunit expression in CBF neurons remains stable during the progression of AD. The M1 receptor is a potentially interesting drug target as it links several of the major hallmarks of this disorder including cholinergic deficiency, cognitive dysfunction, and Abeta and tau pathologies. Therefore, it has been argued that restoring cholinergic tone via activation of M1 mAChR may alter the onset and/or the progression of AD. Despite this intriguing concept, the clinical use of muscarinic agonists in AD has been limited due to adverse side effects that occur at high doses. A novel group of M1 partial agonists, AF102B, AF150(S), and AF267B-i, has been developed and tested in a series of experiments using a transgenic mouse model of AD that recapitulates the major pathologies of AD. Chronic AF267B treatment rescued cognitive impairment and decreased Abeta42 and tau pathologies in the cortex and hippocampus of these mice. These changes were associated with M1 mAChR-mediated activation of the tumor necrosis factor- α converting enzyme

ADAM17/TACE, decreased BACE1 steady-state activity, and inhibition of glycogen synthase kinase 3 beta (GSK3 β). Recently, a report was published, describing a highly selective allosteric activator of the M1 muscarinic receptor, which affects the processing of the APP toward the non-amyloidogenic pathways and decreases Abeta production, *in vitro*. If clinical trials prove efficacious for newly developed M1 mAChR receptor drugs, they may become a viable treatment candidate and the first monotherapy to treat multiple pathologic and biochemical deficits during the progression of AD.

Synaptic, Trafficking, and Neuropeptide Gene Expression in AD

Synapse loss is the strongest correlate of cognitive decline in AD, and requires further effort for pharmacotherapeutic intervention. Single-cell gene array studies report that synaptic transcripts are selectively downregulated in CBF neurons in AD, with significant reductions in synaptophysin and synaptotagmin, but not synaptobrevin or SNAP29 mRNA (Figure 4(b)). Intriguingly, synaptotagmin function is related to vesicle–presynaptic membrane fusion and neurotransmitter release, suggesting that perturbations in presynaptic vesicle trafficking comprise an event in vulnerable neurons in AD. In contrast to synaptic transcripts, mRNAs encoding APP and Notch were unchanged between control and AD subjects, whereas the acid hydrolase cathepsin D mRNA was upregulated in AD. In addition, subunits of protein phosphatase 1 (PP1) (Unigene-NCBI annotation PPP1CA and PPP1CC) mRNAs were downregulated in CBF neurons in AD. This observation is interesting since PP1 can phosphorylate tau on several serine/threonine residues and downregulation of PP1 activity leads to increased tau hyperphosphorylation, which may affect NFT formation in CBF neurons. However, single-cell gene expression of CBF neurons hyperinnervated by fibers containing the neuropeptide GAL, which displays neurotrophic actions and functions via the interaction of three G-protein-coupled receptors (GALR1, GALR2, and GALR3), stabilized levels of mRNAs encoding PP1 subunits and even increased ChAT expression in late AD cases. These observations suggest that GAL overexpression may delay NFT formation and increase cholinergic function in CBF neurons in AD.

Tau Isoform Expression in Cholinergic Forebrain Neurons in Aging and AD

CBF and cortical neurons display NFT formation in the MCI brain, suggesting a concomitant alteration in tau gene expression during the early stage of AD. The human brain contains six isoforms of tau, ranging from 48 to 67 kDa, which are expressed through alternative splicing of a single

tau gene on chromosome 17. Three of these tau isoforms contain three tandem repeats in the carboxy-terminus end of the molecule (3Rtau), while three display four tandem repeats (4Rtau) in this region. Expression levels of the six-tau isoforms within CBF neurons do not differ during the onset of AD. However, there was a significant shift in the 3Rtau/4Rtau ratio with a decrement in 3Rtau in relation to 4Rtau levels for each tau transcript analyzed within CBF perikarya from MCI and AD cases (Figure 4(c)). A similar shift did not occur during normal aging. Shifts in the ratio of tau transcripts may be a basic mechanism contributing to the selective vulnerability of neurons to NFT formation.

Cholinergic Drug Targets

The ultimate goal of translational AD research is to develop therapies to prevent progressive cognitive impairment that occurs during aging and AD, as well as restore memory and cognitive function. The vast majority of clinical trials aimed at treating the cholinergic deficit in AD have concentrated on testing the efficacy of AChE inhibitor drugs (e.g., donepezil, galantamine, rivastigmine, or huperzine A), which are derivatives of tacrine, the prototypical cholinesterase inhibitor. However, evidence suggests that NGF can prevent cholinergic neuron atrophy and correct the behavioral deficits caused by experimental injury or associated with normal aging in animals. This information led to the implementation of an *ex vivo* phase I trial aimed at both protecting CBF neurons from degeneration as well as augmenting the function of remaining cholinergic neurons by delivery of cells genetically engineered to produce human NGF into the area of the CBF in AD. Following a period of 22 months, no long-term postsurgical adverse effects were found and the rate of cognitive decline appeared to be reduced. It should be kept in mind that this was not a double-blind study and further clinical investigations of this gene therapeutic approach is warranted.

In summary, combining basic molecular and cellular research of neurotrophin and cholinergic biology should enhance the ultimate and pressing goal of amelioration of age-related neurologic disease processes. The keys to success include further characterization of the molecular fingerprint of CBF neurons in the nucleus basalis and septal/diagonal band during the progression of AD as well as in relevant animal models of tauopathy, Abeta deposition, and DS.

Acknowledgment

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See also: Aging and Cognition; Brain Aging: Structural Imaging Biomarkers of Risk of Alzheimer's Disease; Memory and Aging, Neural Basis of; Neural Systems of Motivation; Neurotransmitters and Neuromodulators Regulating Sleep and Wakefulness.

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Memory and Aging, Neural Basis of

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Glossary

CA1 region of hippocampus – CA stands for *Cornu Ammonis* (Ammonis horn or the horn of a ram). This name was given to fields of pyramidal neurons in the hippocampus that are curved and resemble a ram's horn. CA1 is one of four *Cornu Ammonis* areas: first CA4 (which underlies the dentate gyrus), then CA3, followed by a very small zone called CA2, and then CA1.

Delay procedure – A sequence of stimulus presentations, in classical conditioning, such that the conditioned stimulus (a neutral stimulus such as a tone or light) precedes and then overlaps with the reflex-eliciting unconditioned stimulus.

Eyeblink classical conditioning – An associative learning paradigm in which a neutral stimulus called the 'conditioned stimulus' precedes at a specified interval an eyeblink-eliciting stimulus called the 'unconditioned stimulus.' The pairing of the conditioned and unconditioned stimuli results in the subject blinking to the conditioned stimulus before the onset of the unconditioned stimulus. This new association is called the 'conditioned response.'

Interpositus nucleus – One of the deep cerebellar nuclei in the center of the cerebellum, also called the 'interposed' nucleus, which is embedded in the white matter. Rodents and langomorphs have three deep nuclei, with the interpositus being the center deep nucleus. In humans, the emboliform and globose nuclei are the 'interposed' nuclei of the cerebellum.

Long-term depression (LTD) – The weakening of a neuronal synapse that lasts from hours to days. It results from either strong synaptic stimulation (as in the cerebellar Purkinje cells) or persistent, weak synaptic stimulation (as in the hippocampus).

Long-term potentiation (LTP) – The long-lasting improvement in communication between two neurons that results from stimulating them simultaneously.

Stereology – Originally defined as 'the spatial interpretation of sections,' it is an interdisciplinary field that is largely concerned with the three-dimensional interpretation of planar sections of materials or tissues. It provides practical techniques for extracting quantitative information about a three-dimensional material from measurements made on two-dimensional planar sections of the material.

Trace procedure – A sequence of stimulus presentations, in classical conditioning, such that the

conditioned stimulus (a neutral stimulus such as a tone or light) precedes and then turns off so that there is a blank period before the reflex-eliciting unconditioned stimulus is emitted.

Brain memory systems and associated brain structures differ in the magnitude of age-related neuron loss. The studies carried out during most of the twentieth century reported significant loss of neurons with aging in many regions of the brain. These scientific findings were translated into the commonly held notion that tens of thousands of neurons were lost on a daily basis in older adults, resulting in impaired cognition and memory in old age. The contemporary perspective of this phenomenon has changed rather dramatically. There has been a growing recognition that neuronal numbers in many brain structures remain relatively constant. Whereas neuron number remains constant in most brain structures, age-related changes in functional activity of neurons have been documented and associated with impairment in some forms of learning and memory.

The variability in performance on learning and memory tasks increases with age. Research on the neural basis of memory and aging has identified correlates of age-related memory impairment at various anatomical and physiological levels, with some individuals showing greater effects of normal aging than others. The inclusion of older adults in the early stages of neurodegenerative diseases in studies of normal aging has also contributed to a perspective of functional decline in the whole population. Both the magnitude of neuronal loss and the degree of functional memory impairment were likely overestimated in previous research.

Changing Perspectives on the Neural Basis of Memory and Aging

Implementation of methods that have become known as 'stereology' came slowly; however, by the late twentieth century these methods had reshaped perspectives of aging and the brain. Stereological counting principles as an unbiased means for estimating the total number of objects were introduced in the mid-1980s. This method involves a three-dimensional counting chamber with inclusion and

exclusion planes that ensure that all objects have equal probability of being sampled and counted only once. Until unbiased stereological methods were introduced, researchers often used counting profiles, which introduced bias because taller objects can be counted in more than one profile, and smaller objects may never get counted at all. In the case of studies of age differences in cell number, differential shrinkage of brain tissue, as it was being fixed for analysis, added additional bias.

Many brain regions assessed with unbiased stereology, including structures, such as the hippocampus, which are critically involved in learning and memory, showed little or no cell loss in rodents and modest cell loss limited to hippocampal regions less essential for learning and memory in humans. Unbiased stereological techniques indicate that neuron number is maintained in neocortex and most regions in the hippocampus. One of the few types of neurons to show significant loss in normal aging is the largest and earliest discovered neurons in the brain, the Purkinje neurons first visualized by Jan Evangelista Purkinje in 1837.

The neural basis of learning and memory in normal aging shows various patterns, depending on the neural structures that are essential for the form of learning and memory. Some forms of learning and memory are preserved well into late life as they are supported by neural systems that are impacted in a minor fashion by normal aging. In addition, neuron loss is not the only means that age-related neural impairment can occur. The subcellular and molecular parameters of neural aging are the focus of many contemporary neuroscientists aiming to elucidate the neural basis of memory and aging.

Model System Perspective of Neurobiology of Memory Aging

To exemplify the neural basis of memory and aging, we draw on examples from the model system of eyeblink classical conditioning. This model system has become one of the best delineated for the study of learning and memory available in the twenty-first century. The neural circuitry is almost completely mapped, and the behavioral and neurobiological parallels in this form of associative learning extend to all mammals that have been studied, including humans. The processes of normal aging affect eyeblink classical conditioning similarly in all species in which older organisms have been tested – mice, rats, rabbits, cats, and humans.

Among the significant advantages of this model system for gerontology are: (1) age differences in the classically conditioned eyeblink responses are large; (2) striking parallels exist between the age differences in eyeblink conditioning in nonhuman mammals and humans; and (3) the neural circuitry is delineated. Behavioral and

neurobiological parallels have been documented that generalize in aging to all mammals studied, including humans.

Brain Circuits and Associative Learning

Cerebellum

A variety of techniques, including electrophysiological recording of multiple and single units, electrolytic and chemical lesions, physical and chemical reversible lesions, neural stimulation, genetic mutations, and pharmacological manipulation, have been used to demonstrate that the dorsolateral interpositus nucleus ipsilateral to the conditioned eye is the essential site for acquisition and retention of conditioned eyeblink responses. Selective lesions (electrolytic or chemical) of the cerebellum prevent the acquisition and retention of conditioned eyeblink responses. Electrophysiological recording of single and multiple-unit activity in the cerebellum indicated that cells in specific cerebellar regions undergo learning-induced changes during eyeblink conditioning. The involvement of the cerebellum in eyeblink conditioning is also supported by studies that show that electrical stimulation of the two major afferents to the cerebellum – the mossy fibers from the pontine nuclei and the climbing fibers from the inferior olive – can substitute for the externally presented conditioned and unconditioned stimuli and can lead to learning. Reversible inactivation of the anterior interpositus nucleus and overlying cerebellar cortex during training completely prevents learning, but when the nucleus is returned to its normal functional status, learning occurs. Inactivation of the efferent output of the interpositus nucleus (i.e., in the superior cerebellar peduncle and red nucleus) does not prevent learning.

A model of the neural circuitry essential for eyeblink conditioning indicates that both the cerebellar cortex and the interpositus nucleus receive information about the conditioned stimulus (CS; that can be a tone or light), conveyed by the mossy fiber system emanating from the pontine nucleus, and information about the unconditioned stimulus (US; i.e., a puff of air to the cornea or a shock to the eye muscles), relayed by the climbing fiber system originating from the inferior olive. Converging signals from the conditioned and unconditioned stimuli are relayed to Purkinje cells in cerebellar cortex and principal cells in interpositus nucleus. The efferent (eyelid closure) conditioned response pathway projects from the interpositus nucleus in cerebellum to the red nucleus and via the descending rubral pathway to act ultimately on motor neurons.

A cellular model system proposed as a mechanism for information storage in the cerebellum is long-term depression (LTD). In this model, coactivation of climbing fiber and parallel fiber inputs to a Purkinje cell induces a persistent, input-specific depression of the parallel

fiber–Purkinje cell synapse. Studies using mutant and transgenic mice with alterations in cerebellar cortex that affected LTD demonstrated a consistent correlation between impaired cerebellar cortical LTD and impaired eyeblink conditioning.

Hippocampus

The hippocampus and the septohippocampal acetylcholine system are normally engaged in basic associative learning of the sort represented by delay eyeblink classical conditioning, and the hippocampus is essential in trace eyeblink conditioning. In a series of studies using delay eyeblink classical conditioning in rabbits, it was observed that activity recorded in the CA1 pyramidal cell region of the hippocampus forms a predictive ‘model’ of the amplitude–time course of the learned behavioral response, but only under conditions where behavioral learning occurs. This response is generated largely by pyramidal neurons. The role of the hippocampus in delay eyeblink classical conditioning is called ‘modulatory’ because manipulations of the hippocampus can impair or enhance the rate of acquisition. The memory trace itself is not in the hippocampus, but the hippocampus can markedly influence the storage process.

Although the hippocampus is not necessary for normal acquisition in the delay procedure, it is necessary for trace conditioning. There is a long-lasting neuronal plasticity formed in the hippocampus following eyeblink conditioning. This change is essential for learning to occur in the trace eyeblink conditioning procedure, at least until the learning is consolidated. Hippocampal slices prepared from animals trained previously in trace eyeblink conditioning had pyramidal neurons showing a marked reduction in the slow afterhyperpolarization compared to neurons in slices from control animals that received presentations of tones and air puffs that were not related.

Delay and Trace Eyeblink Classical Conditioning Procedures

Eyeblink classical conditioning paradigms that have received the greatest research attention are the delay and trace procedures (**Figure 1**). In the delay procedure, a neutral stimulus such as a tone CS is presented before the onset of a corneal air puff US. The organism learns to blink to the tone CS before the onset of the air puff US, and the learned response is called the ‘conditioned response’ (CR). The interval between the onset of the CS and the onset of the US is called the ‘interstimulus interval’ (ISI). The length of this interval affects the rate of conditioning, that is, ISIs greater than 500 ms increase the difficulty level for rabbits and mice. In the trace procedure, the CS is presented and then turned off, and a blank period (trace) ensues before the onset of the US. The trace procedure is called ‘hippocampus dependent’

because organisms with bilateral hippocampal lesions do not acquire CRs. In rabbits, the hippocampus is essential when the trace interval exceeds 300 ms. In humans, the trace interval must be 1000 ms for eyeblink conditioning to be abolished by bilateral hippocampal lesions. In mice, bilateral ibotenic acid lesions of hippocampus abolish eyeblink conditioning in a 500-ms trace procedure with a trace interval of 250 ms. The cerebellar interpositus nucleus is essential in all eyeblink conditioning procedures.

Normal Aging and Eyeblink Classical Conditioning

Since the first studies were carried out in humans in the 1950s comparing young and older adults on delay eyeblink classical conditioning, striking age differences were apparent. When adults over the age range of 20–90 are tested, age-related effects appear in the decade of the 40s. Direct comparisons of a hippocampus-dependent memory measure (California Verbal Learning Test) and a cerebellum-dependent measure (400 ms delay eyeblink classical conditioning) in the same young and older adults revealed a larger age effect on the cerebellum-dependent than the hippocampus-dependent task. These results are consistent with the studies of neural aging that demonstrate significant loss of Purkinje neurons relatively early in the adult life span and age-related stability in hippocampal neuron numbers, with altered hippocampal electrophysiology demonstrated in late life in nonhuman mammals.

Similar to all investigations of normal aging, when participants have subclinical pathology as in the early stages of Alzheimer’s disease (AD), performance is impaired significantly more than it is affected by processes of aging. Indeed, several laboratories have demonstrated that patients diagnosed with probable AD are severely impaired on this task. Disruption of the septohippocampal acetylcholine neurotransmitter system impairs acquisition of eyeblink conditioning in experimental animals. In AD, the cholinergic system is dramatically impaired, such that this dysregulation is the likely cause of severe impairment of delay eyeblink classical conditioning in AD.

Normal Aging in the Hippocampus and Cerebellum

Although there is stability of neuron number in the older hippocampus, electrophysiological investigations of aging in the mammalian hippocampus have revealed that some aspects are compromised. Place cells in the hippocampal CA1 region are less stable in older rats and are associated with poorer spatial learning. In learning-impaired older rabbits, single-unit records of clusters of pyramidal

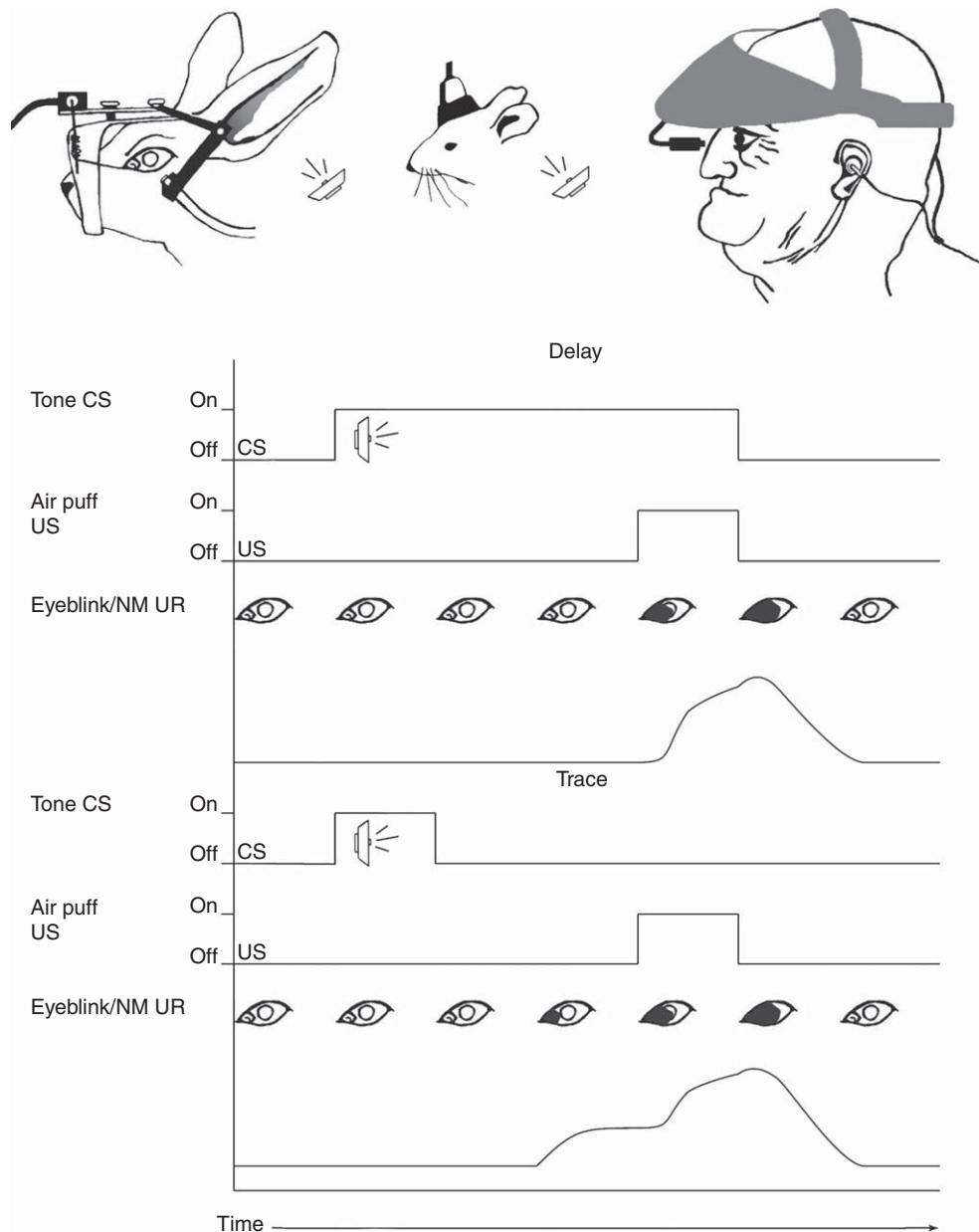


Figure 1 Delay and trace eyeblink classical conditioning paradigms in rabbits, rodents, and humans. (Top) Some examples of apparatus to assess the nictitating membrane (NM)/eyeblink response in rabbits (left), orbicularis oculi muscle activity in rodents (middle), or eyeblink using infrared assessment in humans (right). (Middle row) The delay paradigm involves the presentation of a neutral stimulus such as a tone as the CS, followed by a corneal air puff or orbicularis oculi stimulating US that overlaps and co-terminates with the CS. Below the delay paradigm is a response measure showing a blink after the US, which is called the 'unconditioned response' (UR). (Bottom) The trace paradigm involves the presentation of the CS that is then turned off. A blank (trace) period ensues, and then the US onsets. Below the trace paradigm is a response measure showing a blink after the CS and before the US onset, which is called the 'conditioned response' (CR). When an organism consistently produces CRs, it has learned the association between the CS and US.

neurons in CA1 showed diminished responding. Hippocampal glutamate receptors showed age-related decline. Calcium-dependent afterhyperpolarizations in hippocampal neurons of aged rats were abnormally prolonged. Considerable evidence supports a calcium dysregulation hypothesis of brain aging, and in hippocampus this may be related to elevated postsynaptic calcium

that is closely associated with altered neuronal plasticity. There is a dramatic increase in the amount of LTD induced in the CA1 hippocampal slice in the aged rat. The critical event in the induction of both long-term potentiation (LTP) and LTD is increased intracellular calcium. Dramatic increases in calcium influx (L-type voltage-gated calcium channels) have been demonstrated

in the pyramidal neurons in hippocampal slices from aged rats. Age-related impairments in trace eyeblink conditioning involve an increased afterhyperpolarization in hippocampal pyramidal neurons due, in turn, to an increased calcium-activated potassium current.

In a stereological study of the entire rat cerebellar cortex, 11% fewer Purkinje cells in 23-month-old animals were found. Using unbiased stereological techniques to assess Purkinje cells in the entire cerebellar cortex of mice ranging in age between 4 and 24 months, there is a statistically significant effect of age. There were significantly fewer Purkinje cells in 18- and 24-month-old mice than in 4-, 8-, and 12-month-old mice. Present-day imaging techniques do not have the degree of resolution that enables assessment of human Purkinje cell number *in vivo*. Using human cerebellar tissue at autopsy, it was found that stereological counts of Purkinje cell number were closely related to cerebellar volume. The magnetic resonance imaging (MRI) estimates of cerebellar volume may be an indirect measure of Purkinje cell number. Cerebellar volume is relatively stable in young adulthood, but measurable reductions and increasing variability become apparent around the age of 50 years.

In addition to the loss of Purkinje cells, cellular components show age-related effects. Many Purkinje cells in 26-month-old rats appeared defoliated. Purkinje cell dendrites also showed morphological changes in old mice. In 22-month-old mice, Purkinje cell dendrites had clearly shrunk. One structural reflection of this diminished dendrite was that the thickness of the molecular layer at 22 months of age was reduced in comparison to molecular-layer thickness in younger mice. In addition to anatomical differences in young and older Purkinje cells, other measures of Purkinje cell efficiency have given evidence of age differences. The assessment of Purkinje cell electrophysiology in rats identified a number of cell-firing parameters that were affected. In particular, increasing numbers of aberrant, very slow-firing cells were encountered in older animals. Age-related dysfunction of the cerebellar β -adrenergic receptor was observed to affect spontaneous firing besides modulating the effects of other neurotransmitters such as γ -aminobutyric acid (GABA). This age-related decline in cerebellar β -adrenergic receptor function has been postulated to underlie, in part, age-related deficits in motor learning.

Effects of Aging on Eyeblink Classical Conditioning and Relation to Neural Changes

A strength of the eyeblink conditioning model for research on aging is that humans and nonhuman animals show similar age-related deficits. Humans begin to show age-associated deficits in eyeblink conditioning between 40 and 50 years, while rabbits begin to show age-associated deficits at around 2 years. Based on declines

in reproductive capacity, a 2-year-old rabbit is equivalent to a 35–40-year-old human, which suggests similar onset of age-associated declines in eyeblink conditioning in both species. Results with aging in humans and rabbits generalize to other nonhuman species, such as rats, cats, and mice. Aged mice are impaired in middle age (9–12 months) in 250-ms delay and at an older age (18–24 months) in 500-ms delay eyeblink conditioning just as humans are impaired in middle age (45–55 years) in 400-ms delay and at an older age (70–80 years) in 500-ms delay eyeblink conditioning.

Loss of Purkinje neurons in cerebellar cortex is associated with age-related deficits in eyeblink conditioning in several species: rabbits and mice with direct Purkinje cell counts and humans with an indirect measure, MRI-assessed cerebellar volume.

Present-day imaging techniques do not have the degree of resolution that enables assessment of human Purkinje cell number *in vivo*. Using human cerebellar tissue at autopsy, it was found that stereological counts of Purkinje cell number were closely related to cerebellar volume. The MRI estimates of cerebellar volume may be an indirect measure of Purkinje cell number. Cerebellar volume is relatively stable in young adulthood, but measurable reductions and increasing variability become apparent around the age of 50 years. Two laboratories have reported a positive correlation between delay eyeblink classical conditioning performance and cerebellar volume.

With regard to the hippocampus, afterhyperpolarization recorded in slice preparations from trained rabbits was observed in relation to normal aging. Excitability of CA1 neurons was studied 24 h after the last training session in aged rabbits that reached a 60% behavioral criterion (learning-intact), rabbits trained for 30 days that never demonstrated more than 30% CRs per session (failed to learn), and naive aging rabbits. Aged CA1 neurons from learning-intact animals had significantly reduced postburst afterhyperpolarizations and reduced spike-frequency adaptation compared with neurons from control groups of naive and aging rabbits that failed to learn. No differences were seen in resting potential characteristics after learning. The data suggest that postsynaptic excitability of CA1 neurons is correlated with learning the hippocampus-dependent trace eyeblink conditioning in both young and older rabbits. In young and in learning-intact older rabbits, the data also suggest that a similar level of postsynaptic excitability is achieved at the time that learning has occurred regardless of (1) rabbit age and (2) the actual speed of acquisition of the CR. To identify the current(s) underlying the age-associated learning deficits and decreases in neuronal excitability reflected by an enhanced postburst afterhyperpolarization in CA1 hippocampal pyramidal neurons, whole-cell voltage-clamp recording experiments were carried out using

hippocampal slices in young and older rabbits. Aging neurons had an enhanced, slow outward calcium-activated potassium current. The amplitude of this current was correlated at a significant level with the amplitude of the postburst after hyperpolarization.

Neural Substrates of Individual Variability in Learning and Memory

Neuron numbers vary widely among individuals, in part as a consequence of chance developmental variations. Individual variations are greatest in large populations of neurons arrayed in parallel, which in the neocortex exceed $\pm 50\%$. Individual variations in neuron cell numbers are likely related to behavioral outcomes in normal aging. Learning and memory show many alternative outcomes and great individual variation during normal aging. In comparison to young adulthood, individual variability increases in middle and older adulthood, with some older organisms showing preserved learning and memory and others showing impairment. The cellular basis for most cognitive aging changes is not clear but, in some learning paradigms, could be independent of neuron loss and related to neuron loss in other paradigms. Neuron number in the hippocampus is relatively stable over the adult years.

In the case of hippocampus-dependent learning and memory paradigms, preserved learning and memory in normal older organisms have been associated with electrophysiological functioning comparable to young adult levels. For example, excitability of CA1 neurons studied 24 h after training in hippocampus-dependent trace eyeblink conditioning differentiated good and poor learning in aged rabbits. In general, aged rabbits required significantly more training trials to reach learning criterion than did young rabbits. However, hippocampal CA1 neurons from aged learning-intact animals had significantly reduced postburst afterhyperpolarizations and reduced spike-frequency adaptation comparable to young rabbits. Hippocampal CA1 neurons from control groups of naive and aging rabbits that failed to learn had significantly elevated postburst afterhyperpolarizations and increased spike-frequency adaptation.

Among the mechanisms that support hippocampal electrophysiological function in adult organisms are neurotransmitter and receptor systems. Extensive evidence indicates that nicotinic acetylcholine receptors (nAChRs) act as neuromodulators in communicative processes in the brain and that nAChRs are involved in cognitive and memory functions. nAChRs in the central nervous system are composed of five subunits arranged around a ligand-gated excitatory ion channel. The most abundant nAChR subtypes are those that participate in high-affinity agonist binding associated with $\alpha 4$ and $\beta 2$ subunits, and those sensitive to blockade by α -bungarotoxin and containing $\alpha 7$ subunits. In normal aging, it is the high-

affinity agonist-binding nAChR subunits that show the greatest deficits in human, rabbit, and rodent brain.

Comparisons of 4- and 27-month-old rabbits that learned rapidly or slowly indicated that there was significant variation in the expression of $\alpha\beta$ heteromeric nAChRs in the hippocampus and temporal-parietal cortex in good and poor learners. This result occurred for both young and older rabbits on a task that was hippocampus dependent (trace eyeblink classical conditioning). In rabbits, there was wide variation in $\alpha\beta$ heteromeric nAChRs in brain structures essential for learning and memory, and this variation was associated with differences in learning.

Individual variations in nAChR expression may also be related to outcomes of aging. The magnitude of nAChR loss required for functional impact may be greater in individuals endowed with higher nAChR expression in early life. The 4-month-old poor learners that had fewer $\alpha\beta$ heteromeric nAChRs would likely have been poor learners when tested at 27 months. However, the 4-month-old good learners may have still remained good learners at 27 months. Age-related deficits in nAChR expression in these good learner rabbits may have been postponed to a later point in the adult life span.

See also: Aging and Cognition; Animal Models of Learning and Memory; Brain Aging: Structural Imaging Biomarkers of Risk of Alzheimer's Disease; Cerebellum: Associative Learning; Declarative Memory; Eyelid Classical Conditioning; Neural Basis of Classical Conditioning; Neurogenesis and Memory.

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Parkinson's Disease

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Glossary

Akinesia – An abnormal absence or poverty of movements.

Bradykinesia – Slowing of the executed movements.

Catecholamines – Monoamine neuromodulators and neurotransmitters, including dopamine, norepinephrine, and serotonin.

Dementia – A progressive loss of brain-related abilities.

Hypomimia – An abnormal absence or poverty of facial expressions.

Oxidative stress – Cell damage due to over-production of hydroxyl and other radicals.

PARKIN 1-n – Genes and gene loci associated with Parkinson's disease.

Sialorrhea – Inability to manage oral secretions, drooling.

Epidemiology/Etiology

It is estimated that over 1.5 million people in the United States have PD. It is a disease of the older adults afflicting 1% of the population over the age of 50. Because of the changing demographics of increasing numbers of older adults in the United States and other developed countries its incidence is projected to increase. It is a leading cause of disability, accounting for over 6% of the US nursing home population with the ensuing significant economic burden. While it is fairly certain that genetic susceptibility plays an important role in pathogenesis of Parkinson, many environmental factors have also been identified as causes for Parkinson. Since its formal clinical description in 1817 by James Parkinson, a number of documented endemic outbreaks of parkinsonism related to environmental factors have been reported. For example, it has been known since 1837 that exposure to manganese causes parkinsonism along with other neurological deficits. Outbreaks of parkinsonism among manganese miners and steel workers who were exposed to the inhalant form of Mn⁺ were described in the early twentieth century. The modern debate regarding Mn⁺ relates to its use as a gasoline additive and its release into the environment. There is evidence that manganese interferes with release of dopamine from the vesicles in axon terminals of intact dopaminergic neurons. Another increase in the number of patients with parkinsonism occurred after the flu epidemic of the early twentieth century providing the basis for the popular book by Oliver Sacks, *The Awakenings*. The pathophysiological mechanisms causing parkinsonism in these conditions may not be similar to the typical idiopathic or sporadic PD, but investigation of these cases of environmentally induced disease has contributed to research on the possible mechanisms of genesis of idiopathic PD. In particular, the discovery that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a byproduct of an illicit drug (MPP) that was synthesized in early 1980s, can produce signs and symptoms of common PD opened an important avenue of research that has furthered our understanding of the pathogenesis of PD. Patients with MPTP-induced PD not only exhibit clinical signs and symptoms of PD, but MPTP also mimics the pathogenesis of sporadic PD by selectively destroying the neurons in the substantia nigra. A number of animal models of PD have been generated using this neurotoxin and others. These models have aided researchers in understanding the pathogenesis of PD and development treatments for this disease.

Introduction

Parkinson's disease (PD) is a degenerative disease of the central nervous system characterized by paucity and slowing of normal human movements. It falls in the category of hypokinetic movement disorders, a group of diseases also known as parkinsonism. The primary clinical manifestations of parkinsonism are bradykinesia and akinesia. Conversely, hypokinetic movement disorders are referred to as parkinsonism. Also common to hypokinetic movement disorders is anatomical localization to basal ganglia and its connections. Furthermore, injury to neurons in the basal ganglia and its connections from causes that include, but are not limited to, ischemic, toxic/metabolic, traumatic, and infectious/inflammatory processes, can also manifest as parkinsonism. Similarly, other neurodegenerative diseases of the brain can present with signs and symptoms of parkinsonism. These are considered Parkinson plus syndromes and include multisystem atrophy, cortico-basal ganglionic degeneration, and progressive supranuclear palsy. Majority of patients with PD suffer from sporadic PD. The salient pathological manifestation in sporadic PD is degenerative death of dopaminergic cells in the substantia nigra. This article discusses the epidemiology, clinical presentations, pathophysiology, and pharmacotherapy of PD.

Clinical Presentation of PD

Although PD is a global neurodegenerative disease, its motor manifestations initially overshadow other symptoms. The major motor signs of PD include: tremor, rigidity, akinesia/bradykinesia, and postural instability (acronym TRAP). The acronym 'TRAP' is appropriate since it also defines the experiences of patients with PD as they are unable to move as fast and or as often as they want.

Tremor. Parkinsonian tremor is oscillating movements of the fingers, hands, neck and jaw, and less frequently legs and trunk. Tremors are usually observed when the affected limb is at rest and are typically of a frequency of 3–5 Hz. Although PD is a hypokinetic movement disorder, tremor by nature is hyperkinetic. This clinical paradox indicates that PD has a complex pathophysiology explained by the bursting pattern of firing in the thalamic motor nuclei.

Rigidity. It is an increased resistance to passive movements of the head, trunk, and limbs across joints with or without cogwheel features. Cogwheel rigidity is more prevalent in patients with tremor-dominant parkinsonism; however, many patients exhibit rigidity without cogwheel. The ticking of the cogwheel is often superimposed on the tremor frequency and is usually manifested in the wrist and elbow joints. Rigidity of legs is often manifested in the form of freezing of gait and gait initiation delay. Rigidity of the trunk is the cause for retropulsive falls and produces a reactive stooped posture that can lead to festinating gait.

Bradykinesia/akinesia. It is an essential feature of PD. Bradykinesia refers to slowing of natural body and face movements and is an essential sign for diagnosis of PD. Parkinson patients often assume the bearing of a statue with paucity of natural fidgetiness and slowing of body and limb motions.

Instability of posture. It is the inability to rapidly adapt to changes in stance and posture.

Other motor signs of PD include shuffling gait, festinating gait, and at times freezing of the gait. Gait and posture abnormalities are most refractory to treatment and often the most debilitating signs. Hypomimia, hypophonia, and sialorrhea are secondary motor deficits. These signs develop as a result of bradykinesia and rigidity of the facial and oral muscles and are perhaps more pronounced signs of PD. In patients with PD, eye movements are abnormal and are parallel to the abnormalities observed in limbs. It is well-known that disorders of movements that involve the cerebral cortex, the basal ganglia, and the cerebellum also affect the movements of the eyes. Thus, in patients with advanced PD the eye movements have the same characteristics as the limb movements, that is, the trajectory of the eye movements are broken similar to cog-wheeling movements of the limbs.

While the initial clinical manifestation of PD is in the motor system, it will ultimately affect other areas of the nervous system. Nonmotor symptoms of PD contribute significantly to disability and impaired quality of life. It is increasingly recognized that nigro-striatal degeneration is not the sole pathology in PD; other monoamine neurotransmitter systems are also involved causing the nonmotor signs and symptoms (see below). Recognition of the nonmotor symptoms in Parkinson is essential for its effective treatment. Autonomic complaints are perhaps the most prevalent and neglected symptoms of PD. At one time or another, patients with PD experience cardiovascular, gastrointestinal, urinary, and sexual dysfunction. Depression and anxiety are common nonmotor symptoms of PD and by some counts 45% of patients develop depression during the course of this disease. Depression may be reactive to the disease or a consequence of loss of dopamine, noradrenalin, and serotonin in the brain. It has been reported that PD patients with depression as comorbidity have a higher burden of pathological features, including neuronal loss, gliosis, and accumulation of Lewy bodies compared to the PD patients without depression. Psychosis and deficit in impulse control are other symptoms that are relatively common in PD patients. Up to two-thirds of PD patients can exhibit confusion, hallucination, impulse control disorder, and paranoia. Sleep disturbances including vivid dreaming, excessive daytime sleepiness, and REM sleep behavior disorder are also common. It is likely that most of these symptoms are not constitutional to PD but are manifested as a consequence of long-term treatment with dopaminergic drugs. The risk of developing dementia is nearly sixfolds higher in PD patients than matched population; approximately 40% of PD patients may exhibit dementia. The pathophysiological basis of dementia in idiopathic PD is not clear. Corticobasal degeneration, diffuse Lewy body disease, and progressive supranuclear palsy all present with Parkinson-like symptoms and signs but have distinct pathological features and are mostly refractory to treatments.

Pathophysiology of PD

As mentioned above, various pathological processes in the basal ganglia can result in motor manifestations of PD. Conversely, a single pathological entity, that is, loss of substantia nigra cells and their connections can result in different constellations of clinical symptoms and signs. This assertion correlates with the broad motor and non-motor clinical manifestations of PD. Since the middle of the last century it has been known that loss of dopamine in nigrostriatal system is the sentinel event in the pathogenesis of PD. Cell loss in substantia nigra is precipitated by slow apoptotic cell death, a hallmark of neurodegenerative

diseases. A common thread in the pathogenesis of neurodegenerative diseases is the misfolding/malformation of cellular proteins leading to the production of abnormal intracellular inclusions. These malformed proteins along with environmental factors precipitate cell death in selected areas of brain. Thus, PD similar to other neurodegenerative diseases is a disease of protein misfolding/malfunction. While all of the cellular and molecular factors and the environmental precipitants that contribute to the genesis of PD and its age of onset are not known at this time, it is clear that genetic risks are associated with loss of cells. Linkage analysis studies on cases of familial PD have identified mutations in a number of genes with confirmed roles in genesis of PD, in particular PARK1, PARK7, and PARK8 have been intensively studied. The genes that have been associated with PD are listed in **Table 1**.

Some of the genetic defects were initially discovered in patients with familial PD; however, in sporadic disease these mutations may act as susceptibility factors and increase the risk or the age of onset of PD. For example, mutations in different loci of two of these genes (PARK2 and PARK8) have been noted in various cohorts of patients with sporadic PD. These mutations are thought to represent genetic risk factors in pathogenesis of PD. The exact molecular mechanisms of how an abnormal LRRK2 causes PD are not yet known. Some data suggest that a mutated LRRK2 causes dopaminergic cell death by interfering with translation of the stress response proteins. Another of the identified offending proteins is α -synuclein coded by the gene (SNCA) located in the PARK1 locus. α -Synuclein is present in the presynaptic complexes in the striatum and other areas of brain. Highest concentration of this protein has been reported in the substantia nigra of patients with PD, presumably because of the impaired proteolytic clearance of this protein. α -Synuclein is synthesized either in the form of a helix or a beta sheath and is a significant component of Lewy bodies and Lewy neuritis. It is also found in the plaques that are associated with Alzheimer's disease. Mutations in α -synuclein, parkin, and ubiquitin hydroxylase 1 all

result in reduced degradation of α -synuclein. Reduced degradation of α -synuclein results in the formation of abnormal intracellular inclusions known as Lewy bodies. While deposition of α -synuclein initially causes only minor cell loss in most areas of brain, it is associated with early destruction and significant cell loss in the substantia nigra. The reason for this early cell death is thought to be increased susceptibility of monoaminergic neurons and their axons to other pathological processes.

A vast body of literature chronicling investigations of degenerative cell death via intracellular signaling pathways and apoptosis has accumulated. During development, apoptotic cell death aids in sculpturing the nervous system. In adult organisms the rate of apoptotic neuronal death is relatively low; however, in patients with neurodegenerative diseases apoptotic cell death is accelerated in selected areas of the nervous system. With regards to PD, a number of intracellular pathways leading to apoptosis in Substantia Nigra cells have been explored including oxidative stress, elevation of cytosolic free calcium in neurons and inflammation. Catecholaminergic cells are highly susceptible to oxidative stress because of the enzymatic degradation of dopamine and norepinephrine by the enzymes monoamine oxidases (MAOs) A and B. One of the by-products of this reaction is hydrogen peroxide. Hydrogen peroxide is a source of hydroxy radicals or reactive oxygen species that are associated with oxidative stress and are capable of damaging cellular proteins, lipids, and nucleic acids. MAO enzymes are tightly associated with mitochondrial outer membranes; this proximity may be responsible for damage to mitochondria in adrenergic cells and leakage of cytochrome C from mitochondria, thus initiation of apoptotic cell death. Another culprit in pathogenesis of PD is the calcium overload of dopaminergic cells. The intracellular concentration of calcium is tightly regulated under normal conditions. Under some pathological conditions, calcium leaks into the cytoplasm from both extracellular and sequestered intracellular compartments. Over abundance of free cytosolic calcium activates proteolytic enzymes and is thought to precipitate organelle damage and is thus another mechanism for apoptotic death of adrenergic neurons.

How does presence of a genetic mutation and the resulting aberrant proteins translate into a constellation of clinical signs and symptoms? A significant body of electrophysiological and anatomical data from *in vivo* experimental models has complemented the cellular and molecular information and has illustrated, albeit incompletely, the motor pathways that are responsible for genesis of clinical symptoms. These pathways form the basis for both pharmacological and surgical treatments of PD. Briefly, widely distributed and multimodal sensory and motor signals from the cerebral cortex flow to the

Table 1 Genes associated with PD

Gene	Chromosome/locus	Protein
PARK 1	4 q21–23	α -Synuclein
PARK 2	6 q25–27	Parkin, (ubiquitin E3 ligase)
PARK 3	2 p13	
PARK 4	4 q21–23	
PARK 5	4 p14	
PARK 6	1 p35–36	PTEN-induced kinase-1
PARK 7	1 p36	Oncogene DJ 1
PARK 8	12 p11–q13	Leucin-rich repeat kinase 2 (LRRK2)
PARK 9	1P36	

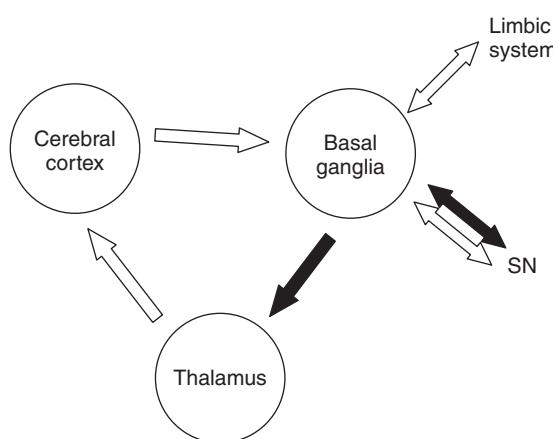


Figure 1 The diagram shows basic flow of information between the cerebral cortex, basal ganglia and thalamus. Each arrow represents a complex set of neural signals representing a variety of modality based information. Open arrows indicate excitatory outputs, closed arrows are inhibitory. Substantia Nigra (SN) and the limbic system have modulatory effects on this circuit, i.e., they are tuning the flow of information.

caudate and putamen nuclei. Caudate and putamen neurons project to globus pallidus, which then projects to motor thalamus and back to the motor areas of the cerebral cortex, completing a circuit. Within this circuit, dopaminergic inputs from the substantia nigra modulate the activity of the medium spiny neurons in the striatum via two classes of dopamine receptors, D1 and D2. At a cellular level, these receptors have opposing effects on the neuronal activity in the striatum, affecting the direct and indirect pathways differentially (Figure 1). Loss of dopamine secondary to cell death in the substantia nigra causes a dysregulation in the activity of striatal neurons that project to the globus pallidus. In turn, this results in a net increased activity of gamma aminobutyric acid (GABA)ergic Globus pallidus *interna* neurons that project to the thalamus. Indeed, electrophysiological single cell recordings from the GPi neurons of patients with PD show that the activity of these neurons increases from a baseline of about 40–60 Hz up to 120 Hz. The resulting increase in the GABAergic output by the GPi neurons affects downstream structures, thalamus and cerebral cortex. Clinical experience in the last decade has validated this circuit and has shown that deep brain stimulation and pallidotomy are effective as surgical therapies for amelioration of some of the symptoms and signs of PD. Pallidotomy refers to a partial destruction of GPi.

Treatment of PD

There is no cure for PD. For the past several decades, treatment of PD has involved replacement of the lost dopamine from the substantia nigra. This has been accomplished by systemic administration (oral) of dopaminergic drugs, for example, Levodopa. It is intriguing

that while loss of dopamine in PD has been thought to involve a small portion of the nervous system, specifically the substantia nigra and nigro-striatal system, diffuse systemic administration of dopaminergic drugs ameliorates the symptoms of PD. This implies that with regards to monoaminergic neurotransmitters and perhaps other neurotransmitters the brain needs only a diffuse supply of these drugs in the neuronal milieu to function seemingly normally. Furthermore, this mode of action of neurotropic drugs provides insights into the global role of monoamines in brain functioning in health and disease. It is conceivable that monoamines function as switches in the sensorimotor continuum of arousal, attention and purposeful motor function, and, that unregulated increase or decrease in their level in the brain result in global diseases such as Parkinson and psychiatric disorders.

Currently, dopaminergic drugs remain the mainstay of pharmacotherapy for PD. Dopamine does not cross the blood-brain barrier and has significant peripheral side effects. However, its precursor Levo-dihydroxyphenylalanine (L-DOPA) can cross the blood-brain barrier and is easily converted to dopamine in the brain by the ubiquitous enzyme, aromatic amino acid decarboxylase (AADC). L-DOPA is administered in conjunction with Carbi-DOPA, which neutralizes the enzyme AADC to block the metabolism of L-DOPA to dopamine in the periphery and thus prevents some of the systemic side effects of dopamine. Administration of C-DOPA also allows a larger quantity of L-DOPA to cross the blood-brain barrier and exert its pharmacodynamic effect onto dopaminergic system. Another group of drugs for the treatment of PD is dopaminergic receptor agonists. These drugs exert their effects directly on D1 and D2 classes of receptors. The older agonists were relatively nonspecific with multiple side effects and consequently are not tolerated well by the patients. This lack of tolerance is attributed to the action of the agonist meds on multiple dopamine receptors, D2, D3, and D4. Although the new meds are thought to be relatively specific, they remain relatively ineffective with different and novel side effects. For example, some of these drugs are reported to bolster addictive behavior, presumably by activating receptors in the nucleus accumbens-septal dopaminergic system. A large number of studies have been performed on pharmacological agents that can stop or reverse degeneration of cells in the substantia nigra; however, as yet no agent has been convincingly proven to change the course of progression of PD. These agents include anti-oxidants, calcium channel blockers, and vitamins. Biological treatments for PD have been extensively explored; however, they are generally in experimental stages. Cell therapies using embryonic, fetal, and adult dopaminergic cells and application of growth factors have shown promise as proof of principle for the treatment of PD. However, before these therapies can be applied for widespread use, much more needs to be done.

See also: Basal Ganglia; Brain Aging: Structural Imaging; Biomarkers of Risk of Alzheimer's Disease; Depression; Hallucinations in Neuropsychiatry and Drug Abuse: From Phenomenology to Pathophysiology; Neural Representations of Intended Movement; Neurotransmitters and Neuromodulators Regulating Sleep and Wakefulness.

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B

Basal Ganglia

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Glossary

Blood oxygen level dependent (BOLD) – BOLD effect uses the endogenous contrast agent deoxyhemoglobin as a source of contrast associated with neuronal activity. BOLD contrast in signal intensity using functional magnetic resonance imaging (fMRI).

Diffusion tensor imaging – Diffusion imaging is an MRI method that measures water diffusion in biological tissues. Diffusion tensor imaging also provides information on the orientation of water diffusion.

Functional MRI (fMRI) – fMRI measures the hemodynamic changes that are associated with neuronal activity in the brain and spinal cord of the human and animals.

Positron emission tomography (PET) – PET is a nuclear medicine imaging technique which uses ionizing radiations. In the brain, PET provides information on brain metabolism, receptors, perfusion, or diagnosis (amyloid binding in Alzheimer's disease).

Tractography (also DTI fiber tracking) – Tractography is an imaging method that is used to reconstruct fiber tracts in the brain of the human and animals.

Tractography is performed using diffusion images.

Introduction

During the past decades, the basal ganglia (BG) were largely considered to be a motor system in which different inputs converged to produce an output specific to the control of motor execution. Series of new findings support the view that the BG are essential for behavioral adaptation, the selection of action commands, and reward-based learning.

Human Basal Ganglia Anatomy

The BG represent an important neuronal system, which consists of four main gray nuclei: the striatum, the globus

pallidus, the subthalamic nucleus (STN), and the substantia nigra. These nuclei are located in the depth of the cerebral hemispheres and are interconnected to each other, the cerebral cortex, and some midbrain structures such as pedonculopontine nucleus through a complex series of circuits.

The Striatum

The striatum includes the caudate nucleus and the putamen that originate from the same telencephalic structure and have the same neuronal organization (**Figures 1** and **2**). Incoming cortical information is processed in the striatum principally by the γ -aminobutyric acid (GABA)ergic projection of spiny neurons. Spiny neurons form synaptic contacts with cholinergic interneurons (the tonically active neurons – TANs) and receive inhibitory feedback from small GABAergic interneurons. GABAergic interneurons receive cortical information. Dopaminergic (DA) terminals arising from the compact part of the substantia nigra form synaptic contacts with striatal spiny neurons.

Thus, cortical excitatory input is received on the spines of the spiny neurons, which are in turn submitted to the inhibitory control of local circuit interneurons, the disinhibiting control of cholinergic TANs and the modulating, excitatory (via D1 receptors) or inhibitory control (via D2 receptors) of DA afferents. Dopamine influences striatal activity by two distinct modes of release – a tonic and a phasic mode. The tonic mode of dopamine release appears to be implicated in the passage of information through the BG, influencing the selectivity in relation to ongoing processes. The phasic dopamine release is crucial for modifying functional synaptic connections and consequently for building stimulus-response or context-habit associations by procedural or instrumental learning.

The Globus Pallidus

The globus pallidus is derived from the diencephalon and is divided into an internal and an external segments

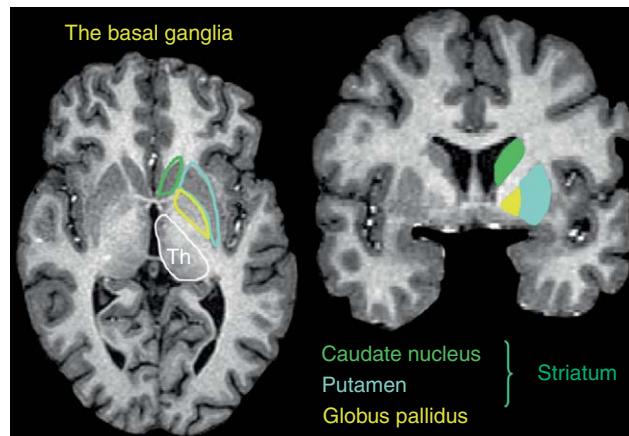


Figure 1 Magnetic resonance imaging of basal ganglia anatomy. T₁-weighted axial slice passing at the level of the anterior commissure–posterior commissure (on the left) and coronal slices passing at the level of the anterior commissure (on the right). The striatum, includes the caudate nucleus (green) and the putamen (light blue); the globus pallidus (yellow). Th, thalamus.

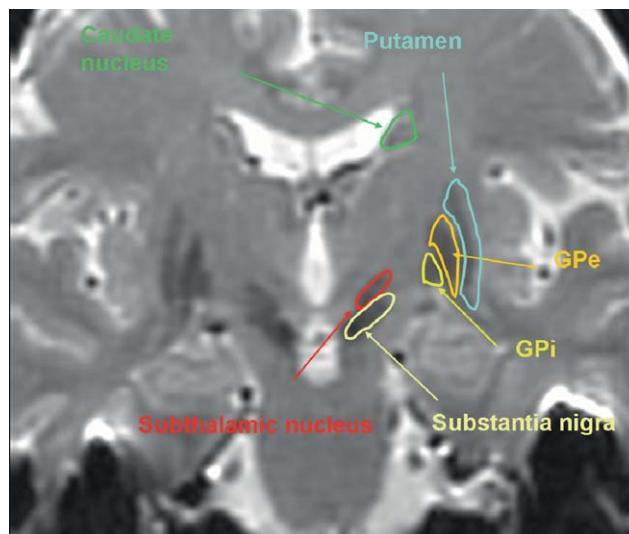


Figure 2 Coronal T₂-weighted image of the basal ganglia. GPe = external segment of the globus pallidus; GPI = internal segment of the globus pallidus.

(**Figures 1 and 2**). Both segments of the globus pallidus contain a single type of neurons. These neurons are characteristically devoid of spines and have very long dendrites (up to 1 mm).

In the globus pallidus, striatal information is greatly reduced through a triple process of volumetric, numeric, and geometric convergence. The number of pallidal neurons is 100 times smaller than that of striatal spiny neurons. A single pallidal neuron receives synaptic input from about 100 striatal spiny neurons. Experimental data in animal models showed that many pallidal neurons could be inhibited by stimulation of more than one cortical area (prefrontal, premotor, supplementary motor, and primary motor areas), and that a significant fraction of pallidal neurons respond to multiple motor, cognitive, or limbic events. This anatomical disposition – which tends to widen

the receptive field of pallidal neurons and thus reduces the specificity of incoming information – is counterbalanced by DA nigrostriatal inputs, which tend to reduce the size of the receptive fields of pallidal neurons.

The Subthalamic Nucleus

The STN is a small ($12 \times 5 \times 3 \text{ mm}^3$), biconvex, lens-shaped structure situated below the thalamus (**Figure 2**). The STN is also derived from the diencephalon. Subthalamic neurons are intermediate in size between the striatal spiny neurons and the pallidal neurons. They use glutamate as their neurotransmitter and have an excitatory action on their target neurons in the internal and external globus pallidus and the substantia nigra pars reticulata.

The STN works as a structure that regulates the level of activity of the output nuclei of the BG (internal globus pallidus and substantia nigra pars reticulata). If hyperactive, as in Parkinson's disease, the execution of motor activity is hampered (akinesia). STN hypoactivity, following vascular lesion or high-frequency stimulation, resulting in motor activity becomes excessive as in hemiballism or hypomania.

The STN can also dynamically control the response threshold as it modulates when a response is executed. This dynamic control results in reduction of impulsive or premature response and provides the support of the STN role in response selection during high-conflict decision-trials.

The STN is also subdivided into functional territories that process motor, cognitive, and emotional information. The STN functional territories enable specific processing in different parts of the nucleus, that is, motor dorsolaterally and associativo-limbic ventromedially. Deep brain-stimulation observations in parkinsonian patients suggest that a complex integration probably occurs at the neuronal level in the STN. Two contacts of a stimulating electrode located in the same associative territory and separated by only 2 mm can produce completely different behavioral effects in the same patient.

The Substantia Nigra

The substantia nigra lies in the midbrain (Figure 2). The substantia nigra contains two zones: a ventral zone, named 'pars reticulata' and a dorsal zone named 'pars compacta'. The majority of cells in the pars compacta are DA

neurons. DA neurons are considered to play a key role in focusing attention on salient and rewarding stimuli. DA terminals modulate the efficacy of corticostriatal synapses – a mechanism that could be implicated in learning. In addition, recent experimental data point to the role of the phasic dopamine signals in contextual regulation of behavior and in response both to novel-neutral, nonrewarded, but behaviourally significant, stimuli and to aversive stimuli.

The afferent control of DA neurons arises from various structures including the striatum, which is believed to be a major source. The precise function of this striatal GABAergic projection to the DA neurons is not known, but some interesting ideas were proposed based on computational approaches. As proposed by Joel et al., the striatonigral projection to the DA neurons may regulate DA neuronal activity and responses to the reward signal.

The pars reticulata of the substantia nigra has striking similarities in cytology and connectivity with the internal segment of the globus pallidus. These two nuclei constitute the major output nuclei of the BG.

Basal Ganglia Circuits

Current Models

The model of BG organization proposed by Delong and Alexander suggested that cortical information is processed through the BG in parallel circuits (Figure 3). This model postulated that the cortical projections from the frontal

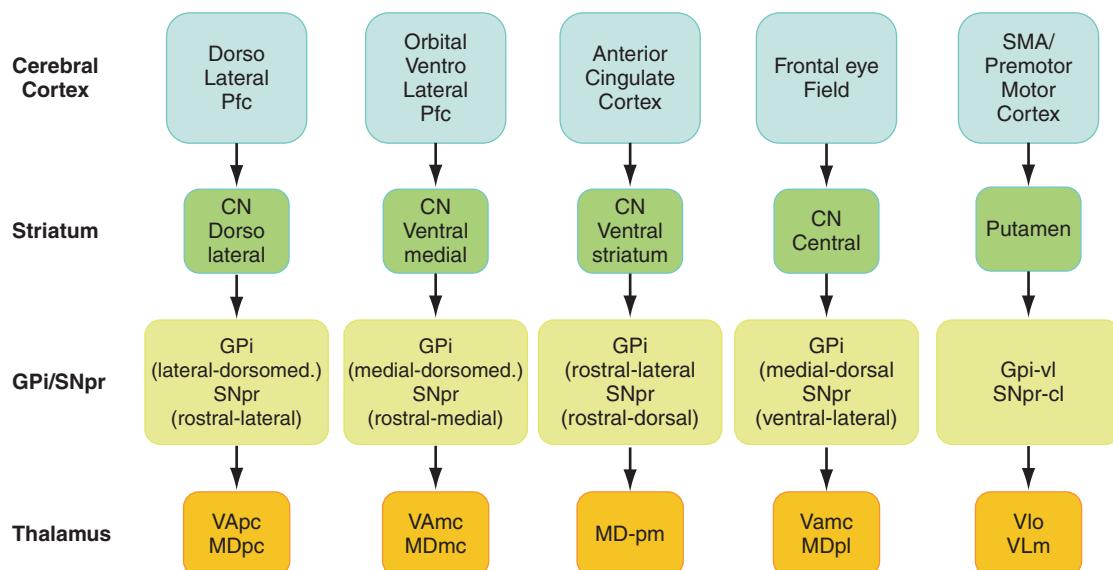


Figure 3 Segregated parallel cortico-BG circuits. Abbreviations. CN = caudate nucleus; GPe = external segment of the globus pallidus; GPI = internal segment of the globus pallidus; mc = magnacellularis; MD = mediodorsal nucleus; pc = parvocellularis; PFC = prefrontal cortex; SMA = supplementary motor area; SNpr = pars reticulata of the substantia nigra; VA = ventral anterior nucleus; VLo = ventrolateral oral nucleus; VLm = ventrolateral medial nucleus. Adapted from Alexander GE, DeLong MR, and Strick PL (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annual Review of Neuroscience* 9: 357–381.

cortex comprise five different circuits – oculomotor, motor, dorsolateral prefrontal, lateral orbitofrontal, and anterior cingulate. This model does not consider integration between motor and nonmotor information in the BG. The assumption that the circuits remain segregated in the BG is contradicted by the anatomical organization of the BG, which exhibits converging properties at several anatomical levels.

Another model, principally developed by Parent and Hazrati, suggests that the functionally organized cortical projections within the BG provide anatomical support in favor of a functional subdivision into sensorimotor, associative, and limbic functional territories, processing somesthetic and motor, cognitive, motivational, and emotional information, respectively. These three functional territories are situated in different portions of the striatum and globus pallidus: the sensorimotor territory in the posterior dorsolateral portions; the limbic territory in the ventromedial portions; and the associative territory in the anterodorsal portions, between the other two territories. The validity of this tripartite model has been demonstrated both in nonhuman primate experiments and in human clinical research.

The model of Mink puts forward the spatial organization of three distinct pathways: direct pathway – striato-pallido-nigro-thalamo-cortical; indirect pathway – striato-pallido-subthalamicopallido-nigro-thalamo-cortical; and hyperdirect pathway – cortico-subthalamic (Figure 4). He also described a center-surround

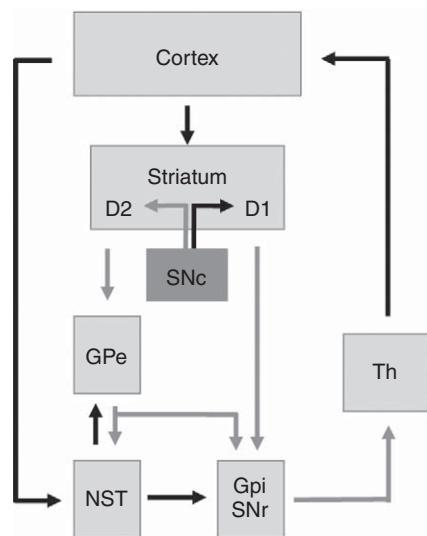


Figure 4 The basal ganglia direct and indirect cortico-striatal-pallido-thalamo-cortical circuits. Excitatory fibers are represented in black and inhibitory fibers in light grey.
Abbreviations: D1 = D1 dopaminergic receptors; D2 = D2 dopaminergic receptors; GPe = external segment of the globus pallidus; GPi = internal segment of the globus pallidus; SNC = substantia nigra pars compacta; SNr = substantia nigra pars reticulata; STN = subthalamic nucleus; Th = thalamus.

organization of information emitted from the cerebral cortex. In this model, the direct pathway provides a centered focalized inhibition of the output nuclei (internal globus pallidus and substantia nigra pars reticulata), allowing a focalized excitation of the thalamus and cortex, thus serving the execution of the desired motor action (Figure 5). In the meantime, the indirect STN pathway provides a large, divergent excitation of the output nuclei, which results in a large inhibition of the thalamocortical projection, leading to an inhibition of competing programs.

A dynamic model of BG function proposed by Nambu expands the center-surround model in the temporal domain. The model privileges the timing aspect of the three pathways. The hyperdirect pathway – which is the most rapid cortico-subthalamo-pallidal projection – first inhibits all motor programs in a reset-like fashion. Then the direct corticostriatopallidal pathway activates the motor sequence to be executed. Finally, the slowest indirect cortico-striato-pallido-subthalamo-pallidal pathway inhibits the motor sequence to terminate the execution. Through this sequential information processing, only the selected motor program is initiated, executed, and terminated at the selected timing, whereas other competing programs are cancelled.

The direct and indirect pathways have been revisited recently in a biologically based computational model. They are interpreted as Go/No-Go devices, which help to choose, among different solutions, the so-called decision-making. The Go pathway is activated by dopamine and helps facilitative responding, while the No-Go pathway is inhibited by dopamine and suppresses responding. Animal models have shown that phasic bursts of DA neurons – which act as teaching signals to learn rewarded behavior – are observed during positive reinforcement, whereas aversive events are associated with DA dips. In the computational model by Frank, dopamine bursts increase synaptic plasticity in the direct pathway while decreasing it in the indirect pathway, supporting Go learning to reinforce the good choice. Dips in dopamine have the opposite effect – supporting No-Go learning to avoid the bad choice.

Thus, from an anatomical point of view, the BG system appears as a device that receives a sample of the three specific functional aspects of cortical information – sensorimotor, cognitive, and emotional – and that processes this information in a convergent manner. However, convergence is not the only aspect of BG processing. A complex integration takes place in each of the BG nuclei that results in the elaboration of a completely new and specific output message that will be sent to the frontal cortex to enable the elaboration of an adapted behavior comprising a motor action executed in a specific cognitive context with an appropriate emotional content.

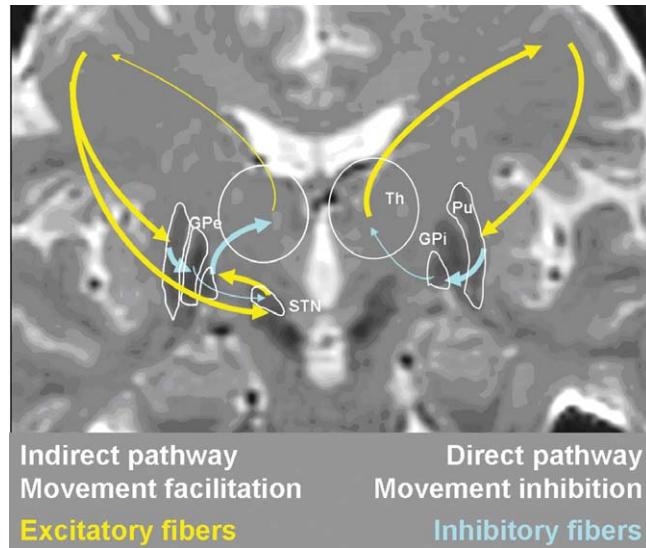


Figure 5 Schematic representation of the facilitatory and inhibitory effects of the direct and indirect pathways. Excitatory fibers are represented in yellow and inhibitory fibers in blue. Thin line means decreased activity, thick line means increased activity. Abbreviations: GPe = external segment of the globus pallidus; GPi = internal segment of the globus pallidus; STN = subthalamic nucleus; Th = thalamus.

Basal Ganglia Circuits in the Human Using Diffusion Tensor Imaging

Tractography-based studies using diffusion tensor imaging (DTI) have reported a strong correlation between human corticostriatal connections and anatomical data from tracing studies in nonhuman primates (Figure 6), in line with the parallel circuit model. Tractography studies showed that each striatal compartment has specific connections with the cortex, and particularly the frontal lobes. The sensorimotor territory of the putamen (posterior part of the putamen) was connected to the primary sensory and motor areas, and to the posterior part of the supplementary motor area – SMA. The anterior striatum was connected to the prefrontal cortex, the frontal pole, and the pre-SMA. The ventral striatum was connected with the limbic system, including the orbitomedial frontal cortex, the amygdala, and the hippocampus.

Although tractography studies showed results that support the anatomical concept of segregated loops in prefrontal, premotor, and sensorimotor networks, evidence of overlapping corticostriatal connections were also observed. Therefore, DTI fiber-tracking studies provided the neuroanatomical correlate of both parallel and integrative networks.

Basal Ganglia Function in the Human, Imaging Studies

Imaging studies in the human have shown that the BG are involved in many aspects of motor control. Location of striatal activation depends upon the nature of the task in a

manner broadly consistent with the predictions of the different functional territories.

Functional Territories in Human Basal Ganglia

The sensorimotor striatum was consistently activated for all types of movements performed using functional imaging, including complex or simple finger movements (Figure 7). Movement activation was somatotopically organized. Basic movement parameters, such as frequency, amplitude, and force, did not or weakly correlated with activational changes (regional cerebral blood flow using PET or percentage-signal increase using functional MRI). Therefore, the striatum is probably not a key structure in coding basic movement parameters.

Many studies have reported striatal activation during the preparation phase preceding movement (Figure 7(b)). Activation in the putamen during preparation was located anteriorly than during movement execution, similar to preparatory activity in cortical structures (SMA, motor cortex, etc.). This suggests that movement preparation and execution are represented within different corticostriatal circuits, in line with animal studies (Figure 7(b)). Simple, horizontal, saccadic eye movements elicit striatal activation in the caudate nucleus.

The associative striatum was activated during more complex types of motor acts. Complex movements require additional motor or cognitive demands. Numerous studies found that striatal activation depended upon the nature of the task and have shown activation in

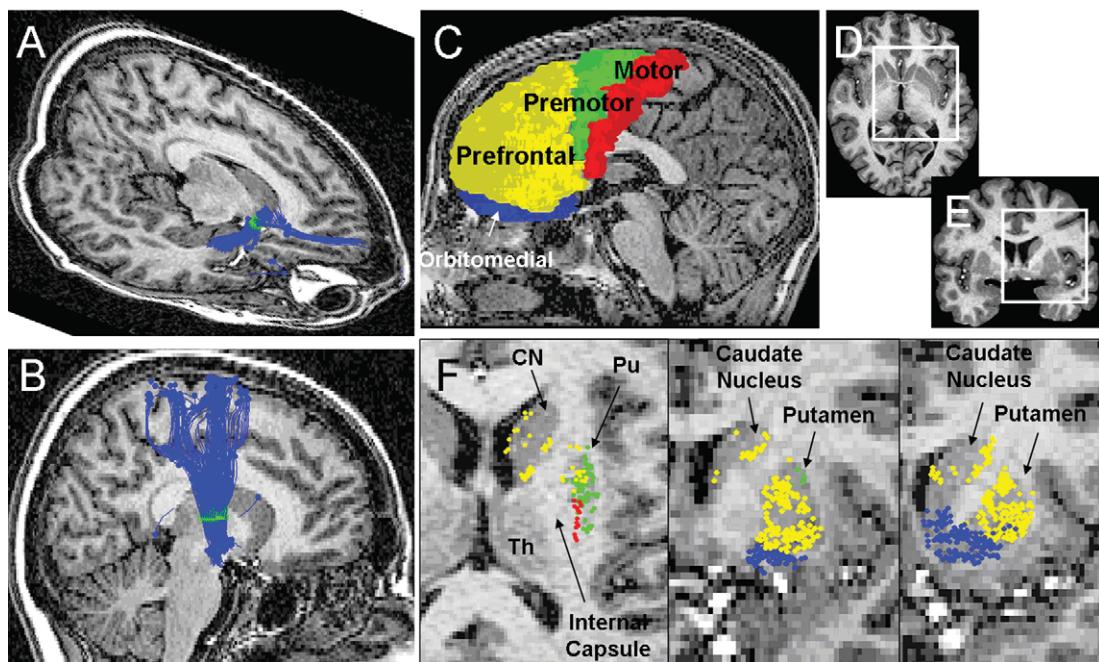


Figure 6 Diffusion tensor-imaging corticostriatal fiber tracts. (a) Fiber tracts from the ventral striatum (the ROI is represented in green). The blue fibers represent the tract in three-dimensional (3D) view as if viewed from the right and projected on a parasagittal slice (up) or from the anterior-right of the brain and projected on a 3D view (down). Fiber tracts connect to the ventral striatum and the medial orbitofrontal cortex, hippocampus, and temporal pole. (b) Fiber tracts from the posterior putamen. The blue fibers represent the tract in 3D as if viewed from the right and projected on a parasagittal slice (up) or from the top of the brain and projected on an axial slice (down). Fiber tracts ended in the primary sensorimotor cortex and adjacent premotor cortex, and the SMA. Fiber tracts are represented on T1-weighted sections. (c) Tractography in F was initiated from four ROIs located in the frontal lobes: the motor (red), premotor (green), prefrontal (yellow), and orbitomedial frontal cortex (blue). (d) Axial and coronal (e) planes presented in (f). (f) Fibers originating from the motor (red), premotor (green), prefrontal (yellow), and orbitomedial frontal cortex. Th = thalamus. Adapted from Lehericy S, Ducros M, Van De Moortele P-F, et al. (2004) Diffusion tensor fiber tracking shows distinct corticostriatal circuits in humans. *Annals of Neurology* 55: 522–529.

the associative compartment during complex movement, including movement selection/decision, mental representation including mental simulation of grasping and hand-movement simulation, complex finger-movement sequences (Figure 7(c)), working memory, and planning tasks. These tasks required the subject to attain a goal through a series of intermediate steps which do not necessarily lead toward that goal.

Activation in the anterior striatum was more specifically involved when subjects had to prepare a sequential action based on information stored in working memory than during simple maintenance of information in line with the hypothesis that striatal neurons convey information that are useful for behavioral acts. These results suggest that the dorsolateral prefrontal cortex (DLPFC)–caudate nucleus loop is activated during higher-order aspects of motor control, such as movement selection and planning.

The limbic circuit is implicated in the motivational aspects of behavior. Several functional magnetic resonance imaging (fMRI) studies have reported activation in the ventral striatum and orbitomedial frontal regions

during monetary reward or punishment, and expectation of monetary reward. Activation in the ventral striatum was more closely associated with anticipation of reward and reward-prediction errors than to reward *per se*, whereas reward outcome was more closely related to activation in the orbitomedial frontal cortex. Signal novelty or unpredictability were also determinants of ventral striatal activation.

In primates, the behavior of the DA neurons has been modeled using the temporal difference algorithm. In the human, the temporal difference algorithm successfully predicted responses in the ventral striatum during tasks that require learning from positive or negative feedback and more recently the ventral tegmental area. Moreover, drugs enhancing or reducing DA function modulated the magnitude of blood oxygen level dependent (BOLD) signal associated with reward-prediction error expressed in the human striatum. Dopamine release in the ventral striatum has also been demonstrated during a goal-directed behavior (a video game) using PET and D2 agonist raclopride binding.

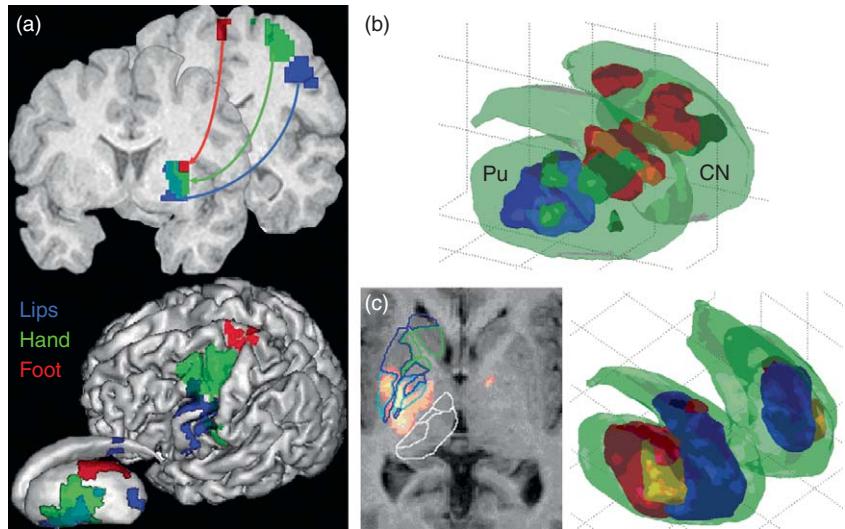


Figure 7 (a) Activation in the putamen and the primary sensorimotor cortex during foot (red), hand (green) and lip (blue) movements superimposed on a coronal T₁-weighted images (up) and a 3D surface render of the brain and striatum (down). (b) Activation observed in the striatum during the selection (red), preparation (green) and execution (blue) of a button press with the left or the right hand. Activation during movement selection was observed in the anterior striatum including the caudate nucleus. Activation during movement preparation was observed in the anterior putamen. Activation during movement execution was observed in the posterior putamen. Abbreviations: CN = caudate nucleus; Pu = putamen. (c) Left: axial view of activation maps obtained during a simple index tapping task was observed in the posterior putamen. The sensorimotor (light blue), associative (dark blue) and limbic (green) territories are territory in the striatum and globus pallidus. Thalamic nuclei are represented in white. Right: 3D reconstructions of the striatum showing activation maps obtained during performance of a complex sequence finger movements (in blue in the anterior striatum including the caudate nucleus), a simple sequence of finger movements (in yellow in the anterior putamen), and a simple index tapping task (in red in the posterior putamen). (a) Adapted from Lehericy S, van de Moortele P-F, Lobel E, et al. (1998) Somatotopical organization of striatal activation during finger and toe movement: A 3-T functional magnetic resonance imaging study. *Annals of Neurology* 44: 398–404. (b) Adapted from Gerardin E, Pochon JB, Poline JB, et al. (2004) Distinct striatal regions support movement selection, preparation and execution. *NeuroReport* 15: 2327–2331. (c) Adapted from Lehericy S, Bardinet E, Tremblay L, et al. (2006) Motor control in basal ganglia circuits using fMRI and brain atlas approaches. (*Cerebral Cortex* 16: 149–161.).

DA neurons are believed to have a preferential role in encoding positive rather than negative outcomes. High levels of dopamine improved learning rate to gains in pathological gamblers and Parkinsonian patients on medication, whereas parkinsonian patients off medication were better at learning to avoid choices that led to negative outcomes than they were at learning from positive outcomes. In congruence with these findings, subjects with high baseline dopamine synthesis, measured using PET in the striatum, showed relatively better reversal learning from unexpected rewards than from unexpected punishments, whereas subjects with low baseline dopamine synthesis in the striatum showed the reverse pattern.

The ventral striatum may thus function as a structure capable of using prediction-error signal to update successive predictions of future reward-associated events, whereas the dorsal striatum encodes stimulus-response-reward associations so that actions associated with greater reward are chosen more frequently (Figure 8). This model has been referred to as the actor-critic model of reinforcement learning, in which the ventral striatum acts as the critic and the posterior putamen as the actor (Figure 8).

Motor Learning

Imaging studies have demonstrated that the BG plays a critical role in the learning and execution of new motor programs. Motor learning has been studied using tasks measuring the acquisition of sequences of movements. These tasks require subjects to produce a sequence of movements that they either know explicitly before training (explicit learning), or learn implicitly through repeated practice (without explicit knowledge). Activation in rostral striatal areas is typically observed during the early planning phase of explicit motor learning, when higher-order selection, working memory, and planning aspects of motor control are needed. In contrast, posterior sensorimotor areas were activated during the execution of learned motor sequences as well as during implicit learning (e.g., in the absence of any explicit knowledge). In some studies although not in others (Figure 9), putamen activity increased with learning, suggesting that it may be implicated in the long-term storage of motor programs.

Thus, despite some conflicting observations, these results suggest that anterior associative striatal regions are implicated during the acquisition of new motor skills,

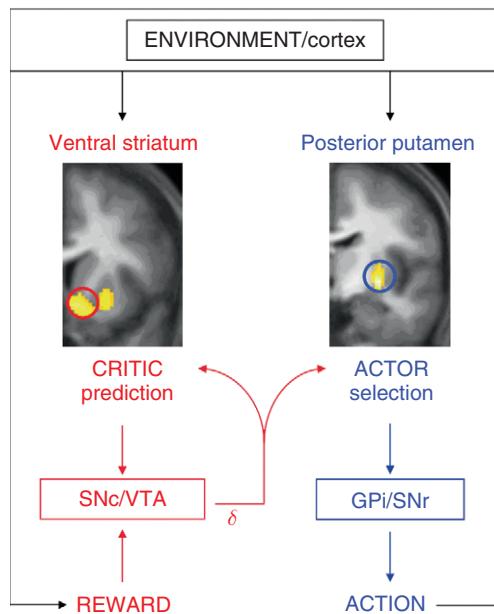


Figure 8 Model of reinforcement learning. The ventral striatum acts as a critic and uses a temporal difference prediction error signal (δ) to update successive predictions of future reward (determined by the arrangement of stimuli). The posterior putamen acts as an actor and uses a similar signal to modify stimulus-response associations, so that actions associated with greater long-term reward are chosen more frequently. Reward-prediction-error signal was observed in the ventral striatum whereas action-related signal was observed in the posterior putamen. Adapted from Pessiglione M, Seymour B, Flandin G, Dolan RJ, and Frith CD (2006) Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans. *Nature* 442: 1042–1045. In this model, the human uses dopamine-dependent prediction errors to guide their decisions, and dopamine modulates the apparent value of rewards as represented in the striatum (courtesy M. Pessiglione, Paris, France).

while posterior sensorimotor regions may be critical for the long-term storage of those skilled behaviors, in congruence with previous models. According to this model, early and advanced learning of motor skills depend upon two independent BG circuits, respectively, one involving the anterior associative/premotor loop, and the other implicating the posterior sensorimotor–BG loop.

The striatum has also been implicated in the consolidation of procedural memory – a process that characterizes the spontaneous performance gains that occur in the immediate posttraining period or during sleep. During a sequence-learning task, responses observed in hippocampus and striatum were linearly related to the gain in performance observed overnight (and related to consolidation), but not over the day, suggesting that both structures interact during motor-sequence memory-consolidation to optimize behavior. Learning performances have also been linked to dopamine release in the human BG and pre-SMA.

Movement Inhibition/Selection

One role commonly allocated to the BG in motor control is to coordinate decision-making processes by facilitating adaptive frontal motor commands while suppressing others. In the BG circuitry, the STN is critically located to reduce premature responding and, therefore, may have substantial effects on response selection. This hypothesis is supported by functional imaging studies. A network including the inferior frontal cortex, the STN, and the pre-SMA in the right hemisphere could either brake or completely stop motor responses during a stop-reaction time paradigm. The STN may act by modulating when a response is executed, reducing premature responding, and, therefore,

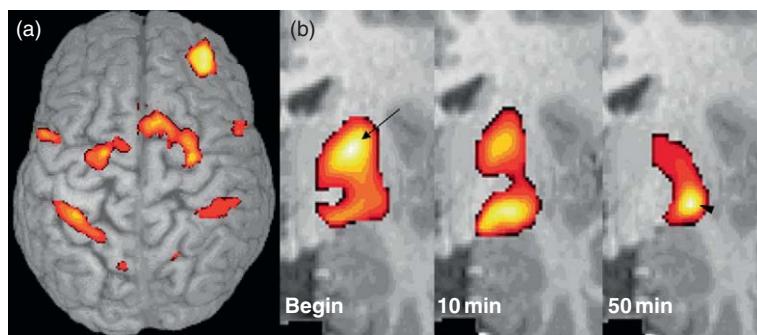


Figure 9 (a) Cortical areas activated during the early learning phase of a complex sequence of finger movement superimposed on a 3D surface render showing activation in bilateral pre-SMA, lateral premotor areas, ventral prefrontal cortex, associative parietal areas (BA 40), and the right dorsolateral prefrontal cortex. (b) Activation maps obtained in the putamen during the same task in the first run, and after 10 and 50 min of training superimposed on a coronal T_1 -weighted image. There was a progressive activation decrease in the dorsal part of the putamen (arrows) and an increase in a more ventrolateral areas (arrowheads) bilaterally. Adapted from Lehericy S, Benali H, Van de Moortele PF, et al. (2005) Distinct basal ganglia territories are engaged in early and advanced motor sequence learning. *Proceedings of the National Academy of Sciences of the United States of America* 102: 12566–12571.

may contribute to select the appropriate response, particularly when there are multiple competing responses.

Conclusion, Role of the Basal Ganglia

In summary, the BG are organized in distinct functional territories processing different types of information. Therefore, the BG have not only motor but also behavioral functions. These different functions are represented within distinct cortico-BG circuits, that is, the sensorimotor putamen in movement execution, the rostral striatum in higher-order aspects of motor control, and the ventral striatum in reward-based learning. Evidence also supports the notion that there exist some degree of overlap among corticostriatal circuits.

Within circuits, information is processed in three distinct pathways: direct, indirect, and hyperdirect pathways. A center-surround organization of information processing allows a temporally organized tuning of information by combining a large, divergent excitation and a centered focalized inhibition of BG output nuclei. This Go/No-Go organization of the BG would help coordinate decision-making processes by facilitating adaptive frontal motor commands while suppressing others. The Go pathway is activated by dopamine and helps facilitative responding, while the No-Go pathway is inhibited by dopamine and suppresses responding.

Therefore, a complex integration takes place in each of the BG nuclei that results in the elaboration of an output message that will be used for movement selection and planning, and for reward-based learning.

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Behavioral Planning: Neurophysiological Approach of the Frontal Lobe Function in Primates

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Glossary

Deep brain stimulation – Using electrodes

stereotactically implanted, current is delivered to precise targets the electrical activity of which is disturbed by a given pathology (Parkinson' disease, dystonia, dyskinesia, psychiatric disorders such as OCD or depression, epilepsy, pain, etc.) The purpose of the stimulation is to counteract these so-called bioelectrical abnormalities and thereby restore a situation near to normal in the underlying networks.

Error detection – A comparison process between the predicted and actual state of the planning process. It can be related either to the movement parameters or to the outcome of an action itself.

Goal-directed behavior – According to Brown and Pluck, a goal-directed behavior is construed as a set of related processes by which an internal state is translated, through action, into the attainment of a goal. The 'goal' object can be immediate and physical, such as relieving thirst, or long-term and abstract, such as being successful in one's job or the pursuit of happiness. By 'directed,' it is meant that the action is mediated by knowledge of the contingency between the action and the outcome.

Perseverative behavior – Inappropriate maintenance or repetition of a previous response.

Planning – Neuronal processes involved prior to the initiation of action and directly related to the achievement of a goal (see goal-directed behavior).

Representation – Global activity implemented within brain networks that encode features of the internal or external world. The question of the neuronal basis of representation is a key issue in neuroscience.

Temporal relation or timing – Temporal relation or timing, a method initiated by E.V. Evarts and his school to study the relationship between the neuronal activity of a central motor area and the structure of a precise movement recorded via mechanographic or electromyographic techniques. It was researched how a neuronal pattern of discharge is time-locked to the different cinematic and kinetic parameters of a motor act. The timing method has supported the concept of a hierarchical organization of the motor system with the naive quest of a putative conductor.

Introduction

Historical Considerations

One of the most important questions in science remains the origin of voluntary movement. While the sensorimotor organization of the spinal cord in movement generation was established in the beginning of the nineteenth century by the pioneering neurophysiologists François Magendie (1783–1855) and Charles Bell (1774–1842), the question of central (cortical) movement generation really arose in the last third of the nineteenth century. Paul Broca (1824–80), in France, and Hughlings Jackson (1835–1911), in England, proposed a cortical substrate for motor functions. First, Broca's description of a left frontal lobe lesion in a patient suffering from aphasia (or rather anarthria) showed that this region is essential for articulate speech. Second, Jackson noticed that there was a systematic spread of convulsions from one body part to the next during epileptic seizures, and proposed a 'somatotopically' organized cerebral motor mechanism. The experimental proof of such remarkable intuition arose experimentally from electrical stimulation of the cortex. By using a galvanic current, Fritsch and Hitzig succeeded in eliciting contralateral movements in the dog – providing evidence both for excitability of the cerebral cortex and its role in motor function (see **Figure 1**). Only 5 years later, Ferrier used faradic current to provide a very detailed motor map of the monkey brain spreading over the parietal and temporal lobe. However, the motor cortex in the precentral gyrus was located very precisely by Sherrington using low-intensity stimulation of the cortex in great apes. Finally, a complete functional map of the human primary motor cortex (precentral motor area) was provided by Penfield with his famous representation of body movement as a homunculus obtained by stimulation of the precentral area of conscious patients undergoing surgical procedures.

Paradigm Shift

Until the mid-twentieth century, most central nervous system (CNS) studies were based on stimulation, ablation, or cytoarchitectonia (the precentral gyrus is defined in Brodmann's nomenclature – area 4 – by the large pyramidal cells described by Betz) studies of region of interest. This led to the powerful, but somehow reductionist and

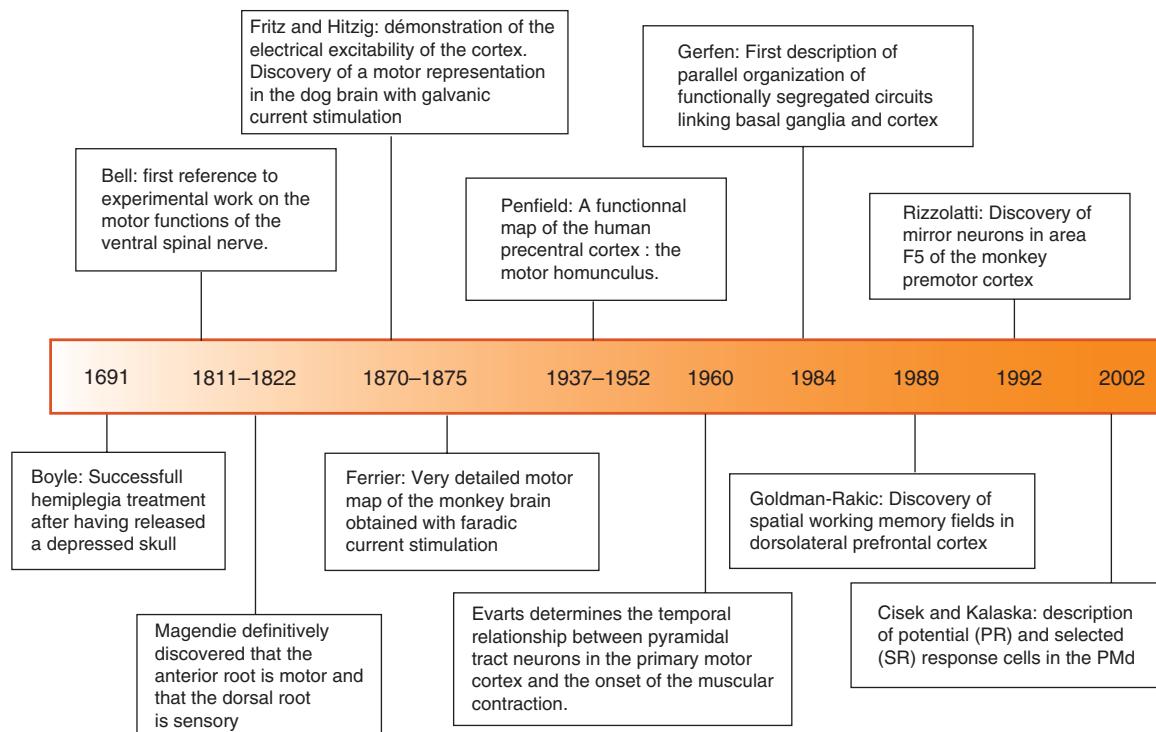


Figure 1 Timeline of selected key events contributing to an understanding of the frontal lobe function in motor/behavioral planning.

static, concept of localization. While in Jackson's view, the motor system is organized in a hierarchical manner, these tools did not allow the timing of brain activity in relation to movement to be studied.

As often occurs in science, a quantum leap came from a new technique – the development of extracellular recording of the spike activity of single neurons in behaving monkeys. This opened up the new field of 'behavioral neurophysiology.'

Thanks to the extracellular recording of neurons during movement performance, neurophysiologists have direct access to the output function of the cerebral cortex, thereby going beyond indirect methods like lesion and stimulation. Moreover, they provide access to the 'temporal relation' between neuronal discharge and movement. In order to determine when cortical motor neurons discharge in association with a voluntary movement, Evarts trained a monkey to depress a modified telegraph key (making a contact) until a light came on, and to release the key following light-onset (resulting in alternate flexion and extension movements). The simultaneous recording of neuronal and electromyographic (EMG) activity made it possible, for the first time, to determine the temporal relationship between them. Evarts found that corticospinal neurons discharge approximately 60–80 ms before movement onset. One of Evarts' pupils, W. Thach, found that the Purkinje cells of the cerebellum change their firing pattern well in advance of primary motor-cortex neurons. This provided the first proof that neuronal activity is involved

in internal generation of goal-directed movements or self-paced behavior. These studies greatly helped to demonstrate that motor-cortex neurons encode a variety of cinematic and kinetic parameters such as force, direction, and velocity. A question then arose as to the respective roles of central versus peripheral signals in the edification of cortical discharge patterns of the primary motor cortex (M1, area 4) and sensory cortex (S1, areas 3, 1, and 2) before and during movement. In monkeys, deafferentation by dorsal rhizotomy (C2–T7) of the trained limb produced major changes in activity of these two areas. S1 cells became silent while M1 neurons exhibited a drastic disorganization. Such clear-cut modifications demonstrated the powerful influence exerted by the peripheral feedback loop. Indeed, the latter affords the peripheral inputs linked to the actual motor act and allows their simultaneous computations with the wholly central activations originating from the cerebellum, basal ganglia, and prefrontal areas. Therefore, disruption of the peripheral afferents induces a severe disturbance in M1 and S1 during the final buildup, which normally encodes for harmonious motor messages. While some of these data led largely to the notion of the hierarchical organization of the motor system, others tended to show that the motor system is organized in a rather parallel manner.

Anatomical Substrates

Besides the primary motor cortex, additional motor regions were also defined on the basis of their response

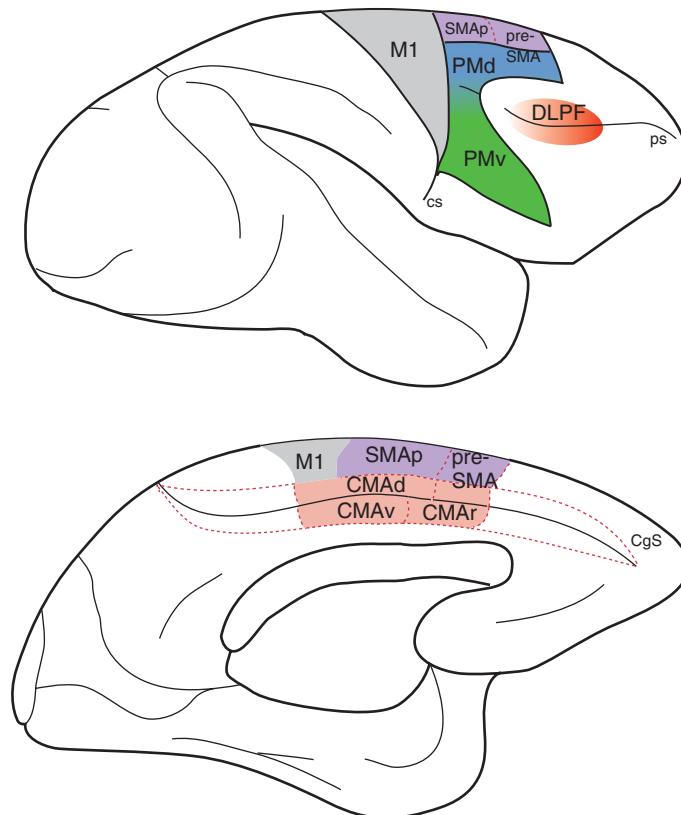


Figure 2 Motor areas in the frontal lobe of the macaque. Top: Lateral view of the left hemisphere. Bottom: Mediolateral view of the left hemisphere to show the location of the mesial motor areas. Abbreviations: CgS = cingulate sulcus; ps = principal sulcus; cs = central sulcus; M1 = primary motor cortex; PMd = dorsal pre-motor cortex ; PMv = ventral pre-motor cortex; SMAp = supplementary motor area proper; pre-SMA = pre-supplementary motor area; DLPF = dorsolateral prefrontal cortex; CMAd = dorsal cingulate motor area; CMAv = ventral cingulate motor area; CMAr = rostral cingulate motor area.

to microstimulation or their anatomical relation to the spinal cord. These premotor regions are classically subdivided into at least six independent subregions, namely: the ventral (PMv) and dorsal (PMd) premotor area, the supplementary motor area (SMA), and the rostral (CMAr), ventral (CMAv), and caudal (CMAc) portion of the anterior cingulate motor area (**Figure 2**). The output of these premotor areas has been shown to send direct connections to the spinal motoneurons. This suggests that the premotor areas are, in some respects, situated at the same level of hierarchical organization as the M1. This is well summarized by R. Dum and P. Strick who stated that “[...] each premotor area, along with the M1, may represent a separate source of ‘central command signals’ for the generation and control of movement.” Functionally, these premotor areas which are reciprocally interconnected with the dorsolateral prefrontal cortex (area 46) encode (in addition to simple activity in relation to movement execution) activity relative to a more abstract level such as reward-related activity, associative learning, mental imagery, or processing of perceived or memorized information.

In the following section, we describe some relatively recent developments in primate neurophysiology involving these areas that have greatly changed our approach to behavioral planning. We review the evidence and discuss new findings that throw light on how the cognitive processes preceding (self- or externally triggered) action are based on a mechanism transforming internal and external ‘representations’ into an action. We also demonstrate the complexity of such interactive processes through the prism of neurological and mental diseases.

Multiple Representational Systems in the Monkey Frontal Lobe

Representation of Potential Actions

A number of recent neurophysiological studies have shown that when decisions are made between overt actions, this process appears to involve many of the same brain regions subserving the planning and execution of movement. This challenges the classical cognitive view that splits brain function into three distinct serial stages:

(1) perceptual mechanisms are held to generate an internal representation of the world followed by (2) a cognitive process that evaluates this neuronal ‘world image’ in order to select (3) a plan of action. This decision is then finally relayed to motor systems for execution. In this perspective, the motor systems are viewed as devices that only implement the plans made by higher-level cognitive processes. This model assumes that decisions about action occur in an abstract decision-space independent of the details of the motor output itself, before engaging the motor system in planning and executing the chosen action. However, in 2005, Paul Cisek and John Kalaska, in Montreal, showed that when multiple reaching targets are presented, they involve the simultaneous activation of separate populations of neurons in PMd that encode parameters of the different reaching options before a final choice is made. They designed a task in which monkeys had to first memorize the locations of two color-coded potential movement targets (spatial cues (SCs)) and then use a subsequent nonspatial color cue (CC) to choose the correct movement target. They found PMd (mostly in the rostral part) neuronal activity representing the two potential motor outputs during the initial ambiguous spatial-cue delay period, and then a rapid change in activity after the presentation of the color cue that represented the selection of one action and the rejection of the other. This study was the first to show clear evidence for separate coexisting representations of competing reaching movement options during the period of evaluation of different choices prior to a final decision in populations of neurons that also encode the parameters (e.g., direction) of motor outputs. These theories unify the processes of movement selection and parametrization – contrary to the serial order of choosing an action and then encoding its parameters in standard ‘cognitive’ models.

Shared Representation

Another example of the intrinsic relationship between action and perception was provided with the discovery by Giacomo Rizzolatti and colleagues of mirror neurons in area F5 (within the PMv) and later in the rostral part of the inferior parietal lobule. These neurons became active before a monkey performed a movement directed to a reward, but they also responded when a monkey happened to see a researcher get the reward, even if the monkey did not move. In other words, these neurons provide a representation of a certain type of action irrespective of the agent performing it. Based on neuroimaging studies in the human, the hypothesis has arisen that such neurons play a role in learning by imitation and that, depending on the context, the mirror neurons can predict the next action in a series of actions. Ultimately these results suggest the role of mirror neurons in empathy, based on the idea that one strategy for

empathizing with others is by simulating aspects of their presumed emotional state within ourselves. It is likely that the impairment of the ability to interpret the actions and emotions of others in patients suffering from autism is linked to a dysfunctioning of these mirror neurons, which are much more widely distributed in the brain than suggested by the original F5 localization.

Rules Representation

In order to plan an action, subjects must maintain a clear internal representation of the instructions (rules) required to reach a goal or of the goal itself. Following studies providing evidence that prefrontal lesions in monkeys induced specific deficits in the delayed-response test, the concept of working memory was developed and extended to lesion studies in the human. In 1989, Patricia Goldman-Rakic definitively proved the neuronal basis of this working memory function in the dorsolateral prefrontal area (area 46). In her pioneering experiment, she recorded neurons in the dorsolateral prefrontal cortex of the monkey while the monkey performed an oculomotor delayed-response task. In the absence of available sensory information, she found that prefrontal neurons displayed elevated spike discharges throughout the delay period. Moreover, she found a clear directional tuning of this delay period activity, which led to the concept of ‘memory field.’ As stated in the 1989 seminal article: “Memory fields may be the cellular expression of a working memory process that allows mnemonic information to guide behavior.” The similarities existing between patients with prefrontal lobe lesions and those with attention-deficit/hyperactivity disorder (ADHD) exhibiting working memory impairment led to the suspicion of prefrontal impairment in such patients. Symptoms of inattention, distractibility, and restlessness fit particularly well with this hypothesis.

Outcome Representation

The planning process in goal-directed behavior relies on the ability to monitor the congruence between the actual outcome and the initial goal. In a recent study, Michelet *et al.*, using a Stroop-like visuomotor task in monkeys, studied the unitary neuronal activity of the anterior cingulate cortex (ACC; CMar: 24c) during performance evaluation. They found a high percentage of cingulate neurons modifying their firing frequency both during the attention and evaluation periods. During the latter period, however, changes in discharge rates were always much more pronounced for erroneous responses than for successful movements that induced reward delivery (**Figure 3**). Interestingly, firing activity during the evaluation period increased more in erroneously completed trials than in incomplete ones and when the reward was

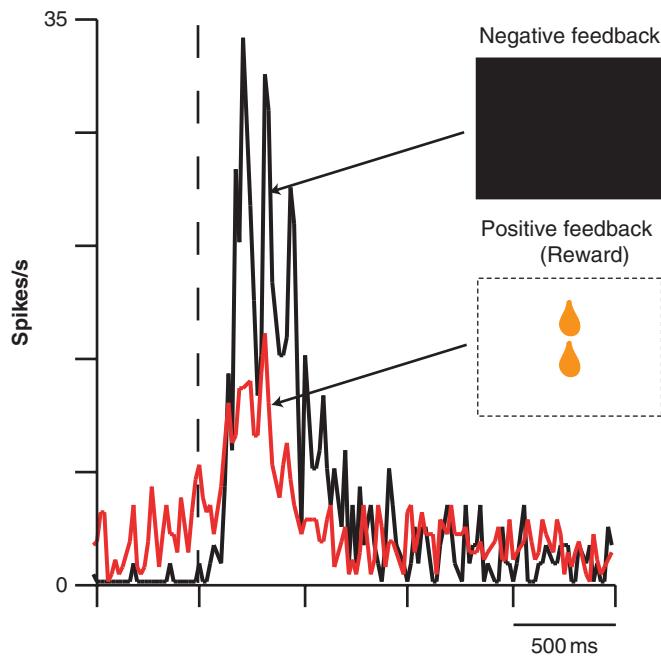


Figure 3 Histogram comparing firing activity of an individual CMAr neuron during the evaluation period following the negative and positive feedbacks. This neuron responded to both outcomes. However, firing rate increased more after negative (in black: error) than after positive (in red: success) feedback. This result confirms that CMAr plays an important role in performance evaluation and more particularly in error detection. Figure from Michelet T, Bioulac B, Guehl D, Escola L, and Burbaud P (2007) Impact of commitment on performance evaluation in the rostral cingulate motor area. *Journal of Neuroscience* 27(28): 7482–7489 (with permission from the *Journal of Neuroscience*).

delivered in an active rather than passive context, indicating that performance evaluation was conditioned by the degree of the animal's commitment to the task. It would thus seem that CMAr neurons could constitute a system for the evaluation of behavioral performance contingent on the subject's commitment to the task. Moreover, some neurons responded more markedly to the warning stimulus when a previous trial failed. In this case, the probability of a subsequent correct response increased. Such a neuronal apparatus could represent an error-compensation system that serves to adapt a subject's behavioral response following an unfavorable and unexpected outcome. Thus, ACC neurons encode a central representation of the congruence between expected and actual performance outcome (comparison process). The abnormal activity of these neuronal apparatus, which appear to take advantage of past errors, could contribute to the genesis of obsessive-compulsive disorders (OCD). Indeed, the heart of the obsessional process is the subject's underlying impression that 'something is wrong.' In other words, obsessions may be thought of as the permanent perception of a mistake and/or error in certain behavioral situations. Compulsions occur as behavioral responses that aim to relieve the tensions or anxiety generated by the situation. If obtained, this relief may be felt to be a form of reward. Nevertheless, it is only transient, thereby creating a feeling of considerable anxiety. This leads to

the immediate reproduction of the behavior in a cyclic manner on the basis of an internal motivational state through the expectation of a reward. Although it is undoubtedly simplistic, this phenomenological view is of interest because it suggests that the mechanism of error recognition malfunctioning is testable and that it could be used to study the physiopathology of the OCD.

Body Scheme Representation

Parkinsonian akinesia is a symptom in which patients suffering from a loss of dopaminergic neurons are unable to initiate any movement, while the corticospinal motor apparatus remains intact. It has been suggested that the underactivity of mesial frontal structures induced by dopamine depletion could constitute the functional substrate underlying akinesia in the Parkinsonian state. In order to test this hypothesis, Escola *et al.* trained rhesus monkeys to perform a delayed Go/No-Go motor task and recorded neuronal activity in the presupplementary motor area (pre-SMA) and the supplementary motor area (SMAp). Recordings were collected at different phases of treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that causes the progressive loss of dopaminergic neurons in the mesencephalic region of the brain. Neuronal activity was diminished and disorganized both during the instruction and the behavioral stages of the

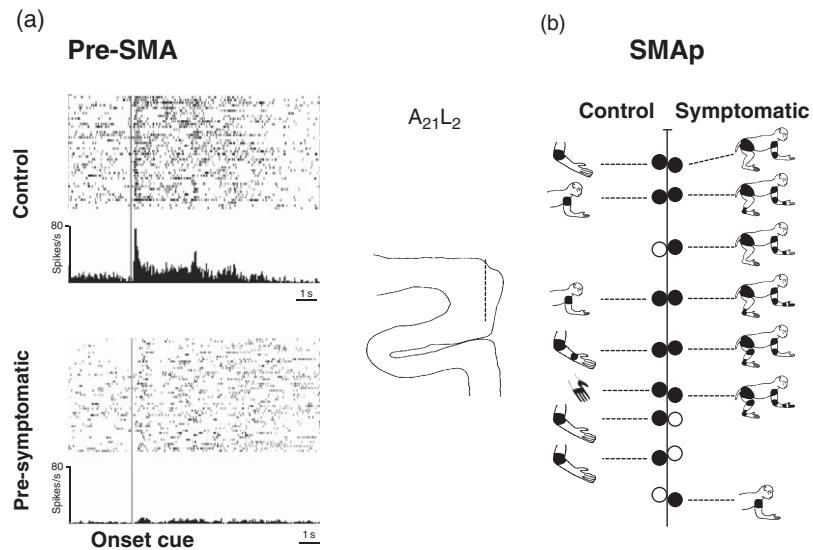


Figure 4 Input/output properties of the SMA before and after MPTP treatment: (a) Example of a Pre-SMA neuron which modifies its activity in response to the onset cue. Neuronal activity is represented in the form of a raster display (top, each point is an action potential, each line a different trial) and a histogram of firing frequency over time (bottom, bin-width 50 ms). (b) Somatosensory receptive fields of the arm regions of the SMAp in control versus symptomatic situation. Left: frontal section. The dotted line corresponds to the track (A₂₁-L₂) followed by a microelectrode successively lowered in control and symptomatic situation. Right: Somatosensory receptive fields observed for the neurons recorded (filled circle: responsive neuron; empty circle: unresponsive neuron) along the microelectrode track (vertical line). One must observe that in the control situation, responsive neurons possess few and focal receptive fields. Alternatively, in symptomatic situations responsive neurons exhibit numerous and diffusely scattered receptive fields. Reproduced with permission from Escola L, Michelet T, Douillard G, Guehl D, Bioulac B, and Burbaud P (2002) Disruption of the proprioceptive mapping in the medial wall of Parkinsonian monkeys. *Annals of Neurology* 52(5): 581–587; Escola L, Michelet T, Macia F, Guehl D, Bioulac B, and Burbaud P (2003) Disruption of information processing in the supplementary motor area of the MPTP-treated monkey: A clue to the pathophysiology of akinesia? *Brain* 126(Pt. 1): 95–114.

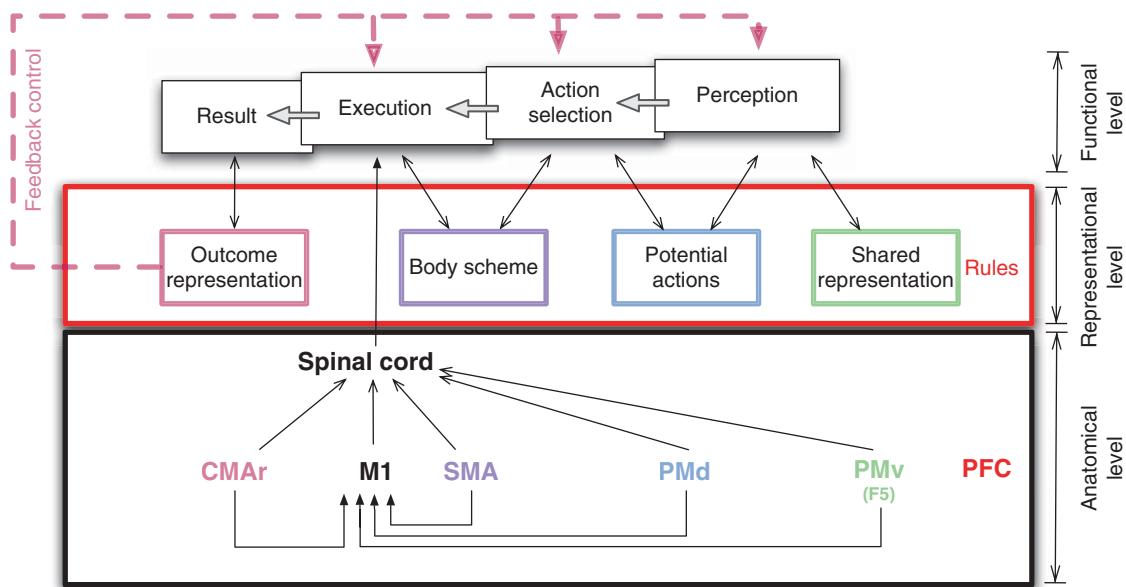


Figure 5 Hypothetical scheme of the planning process. This diagram illustrates the three levels of organization. Overlapping boxes at the functional level indicate that planning process is not a purely serial mechanism, while reciprocal arrows linking functional and representational levels illustrate the complex organization of behavioral planning. Colors and legends of anatomical levels as in Figure 2.

delayed motor task. All the different stages of the motor planning process are therefore likely to be affected: analysis of instructions, preparation, and initiation of movement. In both the pre-SMA and the SMAp, the progressive decrease in neuronal response paralleled the dopamine depletion (**Figure 4**). Firing frequency decreased sharply in both structures in the animal after the Go and No-Go signals at the moment when the monkey had to take the decision to initiate movement. These modifications in neuronal activity observed in Parkinsonian monkeys could reflect the inability of cortical neurons to trigger motor programs correctly in response to significant events. Furthermore, there is also a major impairment of the focused selection of proprioceptive inputs on the SMAp – a structure known to be involved in body schema representation (**Figure 4**). These two results are intimately linked because external information perception is constrained by motor schemas, that is, by the implicit knowledge within the CNS concerning the range of possible movements to be executed. The authors propose that the conjunction of these phenomena could impede motor planning and thus contribute to akinesia.

Conclusion

This article discusses the frontal areas which are interconnected by a complex network of projections that provide a series of internal representations involved in behavioral planning. These areas are organized both in a parallel and hierarchical way (see **Figure 5**). At the lowest level, the primary motor cortex can be viewed as the ‘cortical final common pathway’ in charge of the synthesis of all the information processes in the premotor areas. However, they send projections not only to M1 but also to the spinal cord. The exact role of these corticospinal pathways, which are as numerous as those in the classical pyramidal tract, remains to be elucidated.

Although discussion of the role of the basal ganglia is beyond the scope of this article, all the above-mentioned cortical structures are part of the so-called corticobasal ganglia loops (cortico–striato–pallido–thalamo–cortical loops). In this regard, they are intermixed in highly complex overlapping patterns, thereby contradicting the simple hierarchical view of the serial organization of motor systems. The divergence of cortical input to the striatum and reconvergence in the corticobasal ganglia pathway allows fusion between cognitive, motivational, and motor representations (binding). The intricateness of the (at least) three – sensorimotor, limbic, and cognitive – loops could explain the extensive overlap of cell-discharge patterns in these frontal areas, and the fact that it is almost impossible to attribute a single function to a unique area.

This could also explain not only the wide spectrum of pathologies related to the dysfunction of distinct parts of the circuits (from akinesia to OCD) but also their comorbidity. An absence of voluntary movement can be found in parkinsonian akinesia, akinetic mutism after bilateral anterior cingulate lesion, severe depression abulia (or ‘psychic akinesia’), and in catatonic schizophrenia. In contrast, both OCD- and PFDL-lesioned patients exhibit perseverative behaviors. It, therefore, remains difficult to make a clear distinction between neurological and psychiatric diseases. Most of these pathologies are thought to be related to an impairment of the midbrain dopaminergic system. The connection between both frontal (mesocortical) and striatal (nigrostriatal) areas strongly supports this hypothesis. Interestingly, these different neurological and psychiatric disorders related to dysfunctions of the corticobasal ganglia circuits may be alleviated by a recent and nonpharmacological therapy: deep brain stimulation (DBS). Even though the precise mechanisms of DBS (high-frequency stimulation (HFS)) remain unclear, it appears capable not only of thwarting local abnormal activities but also, and chiefly, restoring a dynamic near to normal in a given circuit. Thus, in Parkinson’s disease (PD), the HFS of the subthalamic nucleus (NST), a node of the sensorimotor loop, suppresses or strongly diminishes akinesia and rigidity. Furthermore, in OCD, HFS of the ventral striatum which is inserted in the limbic loop produces a clear-cut decrease in obsessive and compulsive symptoms.

Because frontal lobe function is mostly dedicated to allowing the planning of movement and deciding – or not – to move, it is not surprising that frontal lobe damage exhibits positive and negative symptoms of such function. Better understanding on this function will not only provide new avenues for treatments of brain diseases but will also provide insights into one of the most important questions in science since time immemorial: the concept of free will. This is particularly important in the twenty-first century during which new discoveries could even lead to the definition of new concepts in policy or economics as well as revision of laws, since man will have new insights into the moral intuitions concerning responsibility.

See also: Basal Ganglia; Cognition: Attention and Impulsivity; Emotion–Cognition Interactions; Incentive Motivation and Incentive Salience; Mirror Neuron Mechanism; Motivation; Neural Basis of Attention-Deficit/Hyperactivity Disorder; Neural Representations of Intended Movement; Parkinson’s Disease; Peripersonal Space and Body Schema; Primate Origins of Human Behavior; Voluntary Movement: Control, Learning and Memory.

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Relevant Websites

- <http://brainmaps.org> – Brain maps.
<http://thebrain.mcgill.ca> – The brain from top to bottom
 (Subchapter: Body movement and the brain).

Blocking, Neural Basis of

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Glossary

Classical conditioning – The simplest form of associative learning of two stimuli in which an initially neutral stimulus (conditional stimulus, CS) is paired in close temporal proximity with an innately significant stimulus (unconditional stimulus, US) that reflexively elicits unlearned behavior (unconditional response, UR). Through CS-US association formation, the animal acquires a learned behavior (conditional response, CR) to the CS that generally resembles the UR (but not always), precedes (or anticipates) the US in time, and reaches a maximum around the time of US onset.

Contiguity – The notion that the temporal relationship between the CS and the US is critical in classical conditioning.

Contingency – The notion that the informational (or predictive) relationship between the CS and the US is critical in classical conditioning.

Eyeblink conditioning – A type of motor classical conditioning task in which animals produce a discrete eyelid closure to an initially neutral CS (tone, light, or vibrations) by virtue of its association with a US (such as an airpuff to the eye). The cerebellum and its associated brainstem structures are necessary for the acquisition, maintenance, and performance of a conditional eyeblink response.

Fear conditioning – A type of emotional classical conditioning task in which animals become afraid of a previously innocuous CS (tone, light, or a distinctive environmental setting) by virtue of its association with an aversive US (such as an electric shock). The amygdala has been implicated in the formation of conditional fear memories.

Prediction errors – The difference between the actual US (or reinforcement) and predicted US. Large prediction error values indicate an unexpected (surprising) US occurrence, whereas small prediction error values signify an expected (unsurprising) US occurrence.

and memory. This simplest form of associative learning was characterized independently by Russian physiologist, Ivan P. Pavlov, and American psychologist, Edwin B. Twitmyer, in the early twentieth century. In general, classical conditioning takes place when an initially innocuous cue, the conditional stimulus (CS), is repeatedly followed in close temporal proximity by a biologically significant cue, the unconditional stimulus (US), which evokes a reflexive behavior known as an unconditional response (UR). From the formation of a CS-US association, the organism acquires a conditional response (CR) to the CS that generally (1) bears a behavioral resemblance to the UR (but not in all instances as some CRs are different or opposite from their URs), (2) occurs just prior to the US, and (3) reaches a maximum magnitude around the time of US onset. These characteristics of the CRs are thought to serve as adaptive functions in the organism's future encounters with the same US.

For nearly 70 years, the critical factor in classical conditioning was understood to be the temporal association between the CS and the US – the notion of contiguity. In other words, classical conditioning was thought to take place when a CS was simply followed by (or paired with) a US. However, three coincidentally independent studies by Leon J. Kamin, Robert A. Rescorla, and Allan R. Wagner in the late 1960s revealed that the informational relationship (the notion of contingency), and not contiguity, between the CS and US is the critical element in classical conditioning. This shift ushered in modern thinking of classical conditioning. This article focuses on Kamin's *Blocking*, though readers can refer to Rescorla's and Wagner's influential works on *Contingency* and *Relative Validity*, respectively.

What Is Blocking?

The phenomenon of blocking indicates that if a US is already accurately predicted by one CS and if the addition of a new CS offers no new information about the US, then conditioning will not develop to the new CS despite its contiguous pairing with a US. In a typical blocking experiment, the subject is first presented with a CS (denoted *A*) paired with a US (*A+*) repeatedly (see **Figure 1**; experimental group). In the second phase, a different CS (*B*) undergoes compound conditioning with *A* and the same US (*AB+*). Later, when *B* is tested, virtually no (or very little) conditioning has accrued to

Introduction

Classical or Pavlovian conditioning, in which an organism learns a predictive relationship between two (or more) stimuli, is considered the basic building block of learning

Kamin's blocking design

Procedure	Groups			
	Experimental	Control 1	Control 2	Control 3
Phase I	A+		C+	A+
Phase II	AB+	AB+	AB+	AB++
Test	B→no CR	B→CR	B→CR	B→CR

Figure 1 Experimental design of a blocking experiment. A blocking procedure typically consists of serial phase I and phase II training sessions followed by a test session. The animals in the experimental group undergo extensive A+ conditioning in phase I, until a stimulus A (CS) reliably predicts the US. Control 1 group animals undergo no conditioning experience in phase I; control 2 group animals undergo the same amount of C+ conditioning as the experimental animals receive to A+. In phase II, experimental, control 1 and control 2 animals all undergo AB+ compound conditioning (where B is a new added stimulus for experimental animals; both A and B are new stimuli for both control 1 and 2 animals). During compound conditioning, the magnitude of the US remains unchanged from phase I for experimental and control 2 animals. Later, when conditioning to B is assessed, the experimental animals typically exhibit no or very little CRs (demonstrating that A has blocked conditioning to B), whereas CRs are observed in the control 1 and 2 animals. Control 3 demonstrates that if the intensity of the US increases from phases I (+) to II (++) , blocking does not readily occur because B now provides new information about US (i.e., increased intensity) during compound conditioning.

B. However, if A had no history of conditioning with the US (control 1), or if a different CS was conditioned with the US (C+; control 2) in the first phase, then B (as well as A) will accrue substantial associative strength during the AB+ compound conditioning phase and will produce a CR when later tested. Thus, the associative strength accrued to B during the compound (AB+) conditioning is inversely proportional to the magnitude of associative strength amassed by A previously, and it is not simply B's contiguous relationship with the US that determines whether or not conditioning will develop to B.

In contrast, if the magnitude of the US is increased during phase II compound conditioning (control 3; AB++), then blocking will not occur; in this case, stimulus B provides new information about the US (i.e., increased intensity), that is not predicted by A alone. Kamin suggested that for conditioning to occur, some quality of the US must be unexpected (or surprising). Since its discovery 40 years ago, blocking has profoundly shaped all modern learning theories and has been considered a quintessential element of cognitive processing in classical conditioning.

Theoretical Models of Blocking

In Rescorla's and Wagner's classic formal learning theory, the necessity of contingency in classical conditioning is an emergent property of US processing represented as $\Delta V_n = \kappa(\lambda - \Sigma V_i)$, where κ is a learning rate parameter (a constant), λ is the asymptote of conditioning with a given US (a constant), ΣV_i is the sum of associative strengths of all CS elements present, and ΔV_n is the change in the associative strength to a CS on trial n (a variable).

This simple equation (with only one variable!) accurately models and predicts a number of key phenomena in

classical conditioning, including blocking. In essence, the Rescorla–Wagner equation posits that the associative strength between the CS and US (i.e., V_i) is driven by errors between the expected US (V_i) and the actual US (λ). Thus, large ($\lambda - V_i$) values indicate that the US is relatively unexpected and surprising, whereas small ($\lambda - V_i$) values denote that the US is relatively well predicted by the CS. When a CS fully predicts the occurrence of a US, then $\lambda - V_i = 0$. Accordingly, blocking will transpire when the associative strength initially acquired by a CS (A) that was paired with a US (A+) reaches the asymptotic λ value. Subsequently, in phase II, because stimulus A already fully predicts the US, by virtue of acquiring all of its associative strength ($V_A = \lambda$), when a new CS (B) is introduced during the compound conditioning (AB+), stimulus B will not accrue any associative strength, as $\Delta V_B = \kappa(\lambda - (V_A + V_B)) = 0$, where $V_A = \lambda$.

Figure 2 illustrates the simulations of blocking using constants $\lambda = 1$ and $\kappa = 0.2$ as examples in the Rescorla–Wagner equation. In this case, phase I conditioning to V_A starts from 0 (before conditioning) and accumulates values of 0.2 (after first CS-US pairing), 0.36 (after second pairing), 0.488 (after third pairing), 0.5904 (after fourth pairing), 0.67323 (after fifth pairing), and so on, until V_A approximates the λ value of 1 (**Figure 2(a)**). In phase II compound conditioning, a complete blocking to stimulus B will occur when V_A is already at a λ value (**Figure 2(b)**). However, if V_A acquired 0.5λ value during phase I, then during compound conditioning, stimulus B will gain some associative strength because stimulus A cannot fully predict the US (**Figure 2(c)**). On the other hand, if stimulus A had no prior conditioning with the US (control 1, **Figure 1**), then during compound conditioning, both stimuli A and B (assuming they are equally salient) will acquire equal associative strength (**Figure 2(d)**). One can also see why complete blocking will not occur when

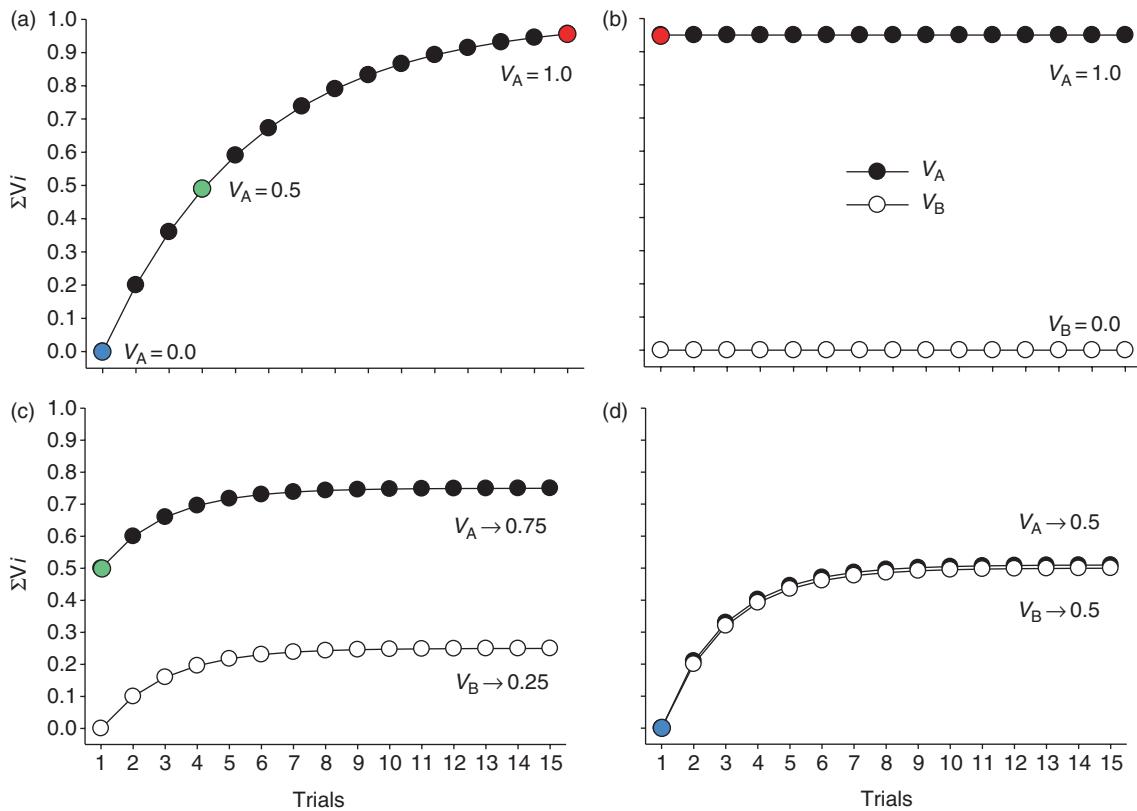


Figure 2 Simulation of the Rescorla–Wagner model of blocking as a function of stimulus A’s associative strength. (a) Stimulus A paired with a US acquires associative strength as a function of the number of CS–US pairings until V_A reaches the λ value of 1. (b) When V_A equals 1 at the start of AB+ compound conditioning, stimulus B will gain no associative strength. (c) When V_A equals 0.5 at the start of AB+ compound conditioning, stimulus B will gain some associative strength. (d) When V_A equals 0 during AB+ compound conditioning, stimuli B and A (of equal salience) will evenly share associative strength. (See the text for explanation.)

during phase II compound conditioning the magnitude of US was increased from λ to 2λ (control 3; AB++).

In addition to the Rescorla–Wagner’s US processing theory, there are other learning theories based on CS processing that can also account for blocking. For example, the CS processing theories of Nicholas J. Mackintosh and subsequently John M. Pearce and Geoffrey Hall postulate that the absence of surprising events (i.e., in the presence of a well-predicted US) reduces the associability of CSs to form new associations. By this view, blocking occurs during phase II because no surprising events occur after the compound AB+ presentation (in phase II; Figure 1), and consequently stimulus B rapidly loses associability, rather than any loss of US effectiveness. In certain situations, however, the US and CS processing theories make different predictions regarding blocking. For example, although the Rescorla–Wagner model predicts one-trial blocking, CS-processing-based theories do not. Conversely, CS processing models suggest unblocking (or disruption of blocking) when the magnitude of the US is decreased from phases I (A++) to II (AB+), although the Rescorla–Wagner model predicts blocking (i.e., B becomes a conditioned inhibitor).

With major progress in identifying neural circuits underlying particular types of learning, learning theorists have gained insight into the biological mechanisms of blocking. Below, we briefly present the involvement of the cerebellum, amygdala, and midbrain dopamine neurons in mediating blocking in different learning paradigms. For a neural circuit espousing a CS-processing account of blocking, readers are referred to the Baxter, Holland, and Gallagher study on the septohippocampal system’s role in an appetitive conditioning paradigm.

Eyeblink Conditioning and Blocking

The rabbit eyeblink conditioning paradigm, which was initially developed in humans, became a widely employed behavioral paradigm to investigate the general principles of learning theories and the neurobiological substrates of learning and memory. In typical eyeblink conditioning, a discrete CS (usually a tone or a light) is contingently paired with a discrete US (usually an airpuff to the eye or a mild electric stimulation to the paraorbital region) with particular temporal relationships between the CS and US.

Initially, the subject exhibits eyeblink URs only to the airpuff US. Over the course of repeated CS-US presentations, the animal develops eyeblink CRs to the CS that precedes the US in onset time, and peaks at about the time of US onset. Based on converging lines of evidence from lesion, recording, stimulation, pharmacological, genetic and brain-imaging studies, Richard F. Thompson and his colleagues identified the cerebellum and its associated structures as essential in eyeblink conditioning.

Notably, the US-processing view of the Rescorla-Wagner model is an emergent property of the anatomically based eyeblink conditioning circuit. The neural connections with particular relevance to blocking are: (1) the excitatory mossy fibers from the pontine nuclei to the cerebellum (CS information); (2) the excitatory climbing fibers from the inferior olive to the cerebellum (US information); and (3) the monosynaptic gamma-aminobutyric acid (GABA)-containing projections from the cerebellum to the inferior olive. Because GABA neurotransmitters exert inhibitory influences on their targets, it has been proposed that this cerebello-olivary pathway serves as negative feedback, specifically by gating the US information flow through the inferior olive en route to the cerebellum (the putative site of CS-US association formation). In support of this view, inferior olivary neurons show US-evoked neural activities during the initial stage of CS-US training (prior to the animal exhibiting any CRs), but not when the animal emits CRs during CS-US trials.

If the cerebello-olivary GABAergic projection serves as negative feedback by gating the US information from reaching the cerebellum, then the blocking effect in eyeblink conditioning can be understood as follows. According to **Figure 3**, blocking will occur when a CS (e.g., an

auditory CS_A) acquires sufficient associative strength to activate the cerebellum, which in turn inhibits the inferior olive (via the cerebello-olivary GABAergic pathway) from responding to a US. Because the input conveying the US information can no longer reach the cerebellum, it cannot support conditioning to a new CS (e.g., a visual CS_B) during the phase II compound conditioning. In validation of this view, Thompson and his colleagues demonstrated that preventing the cerebellum-to-inferior-olive inhibitory activity (by directly applying a GABA antagonist drug into the inferior olive) during the phase II compound tone CS and light CS pairing with the airpuff US – thereby releasing the inferior olive neurons from the CS_A-induced inhibitory control from the cerebellum – blocked blocking.

Fear Conditioning and Blocking

In typical fear-conditioning studies, initially neutral CSs (such as tones and lights) are paired with aversive USs (such as electric shock). After only a few CS-US pairings, the CS will rapidly become capable of evoking an ensemble of fear responses, including analgesia (the ability to inhibit/decrease sensitivity to nociceptive stimuli). The amygdala, the locus of fear conditioning, sends projections to the hypothalamus and brainstem nuclei that mediate the expression of various fear CRs. One conditioned fear response, analgesia, appears to play a role in mediating blocking in fear conditioning. The analgesia CR involves the conditioned fear-associated release of endogenous opioids (endorphins), due to amygdala's increased stimulation of the periaqueductal gray. It has been postulated that as fear conditioning progresses, the

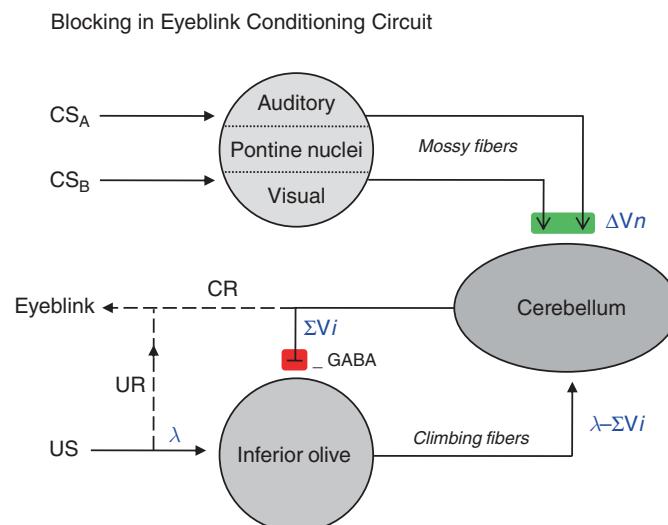


Figure 3 A simplified diagram of the essential neural circuit mediating eyeblink conditioning with the Rescorla-Wagner equation embedded. The conditioned stimulus (CS) and unconditioned stimulus (US) information are conveyed by the pontine nucleus and inferior olive, respectively, to the cerebellum where the CS-US association formation occurs. (See the text for blocking explanation.)

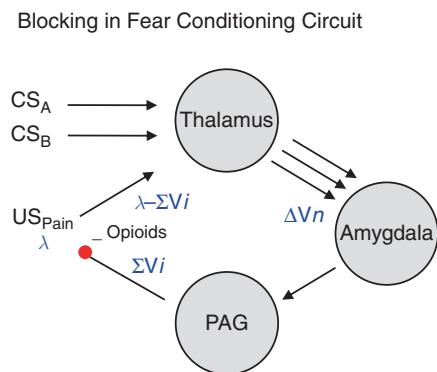


Figure 4 A simplified diagram of the fear conditioning circuit with the Rescorla–Wagner equation inserted. The conditioned stimulus (CS) and unconditioned stimulus (US) information is relayed from the thalamus to the amygdala, where fear conditioning takes place. (See the text for blocking explanation.)

effectiveness of the nociceptive US to support fear conditioning diminishes as the ability of CS to activate the amygdala increases, which in turn stimulates increased opioid release (Figure 4). Consistent with this notion, Michael S. Fanselow and Robert C. Bolles found that systemic injection of the opioid receptor antagonist naloxone during phase II compound conditioning can attenuate blocking. It is likely that naloxone, by opposing the analgesia CR to the first CS_A, prevents the CS-associated decline in the ability of the nociceptive US to support fear conditioning to CS_B during compound conditioning (*AB+*). However, the locus of naloxone's effects on blocking is unknown, and it remains to be established whether the US-evoked neural activities in the amygdala decrease as a function of fear conditioning.

Dopaminergic Midbrain Neurons and Blocking

Other brain structures appear to employ a similar negative feedback mechanism to those of the cerebellum and amygdala to regulate the US teaching input. For example, Schultz and his colleagues found that, in monkeys undergoing a cued delayed reward conditioning (an operant task), dopamine neurons in the substantia nigra (SN) and the ventral tegmental area (VTA) show phasic responses to the delivery of liquid reward US. However, once the animal learns that a light cue predicts the reward, the delivery of the reward no longer elicits phasic responses in dopaminergic neurons, but instead, the CS now elicits a dopaminergic response. Such negative feedback circuits in the midbrain may provide the neural instantiation of blocking.

Indeed, when dopamine neurons were examined in a blocking paradigm, the acquisition of shift in dopamine responses from the reward delivery to a second CS *B* (during *AB+* compound conditioning) did not occur

because the first CS *A* reduced the reward-evoked dopamine signal. The SN/VTA dopamine signals have been hypothesized to convey a prediction-error signal that enables the formation of cue–reward associations in forebrain structures. On a neural level, this system might be seen as analogous to that in the cerebellum (Figure 3), replacing the inferior olive with the SN/VTA and the cerebellum with the basal ganglia and neocortex (which receive dopaminergic innervations from the SN/VTA). These findings are also consistent with reports of disrupted blocking after manipulations of the dopamine system.

Summary

Evolutionarily, blocking (and other similar negative feedback processes) emerged because it serves an adaptive function in how animals process and attend to information in their environments. Because animals continually encounter a variety of stimuli in their environments, it is crucial for animals to respond selectively to those stimuli that reliably predict biologically significant events. Other stimuli that provide no new useful information should be filtered and discarded, otherwise the animal's nervous system will be constantly forming unnecessary associations with various stimuli in its surrounding, which may result in an information overload. This fact was succinctly stated by an American psychologist, William James, who stated: "Selection is the very keel on which our mental ship is built. And in this case of memory its utility is obvious. If we remembered everything, we should on most occasions be as ill off as if we remembered nothing."

Indeed, malfunctioning association mechanisms in the brain may contribute to psychopathological conditions such as schizophrenia. The behavioral phenomenon of blocking, by utilizing a heuristic negative feedback process, serves to circumvent redundant learning and conserve neural real estate.

See also: Analysis of Learning in Invertebrates; Cerebellum: Associative Learning; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Eyelid Classical Conditioning; Fear Conditioning.

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Brain Evolution in Vertebrates

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Glossary

Allometry – The relationship between the size and shape in morphology, often employed to evaluate relative growth rates or size of a specific structure. For example, the relationship between brain size and total body size (either during development or across individuals/species).

Cerebrotype – Comparative brain structure defined by the relative composition of different brain components (i.e., telencephalon, cerebellum, etc.).

Neocortex (also called isocortex and neopallium) – This consists of the outer layers of the cerebral hemispheres, and is divided into the frontal, parietal, temporal, and occipital lobes

Phylogenetic methods – Statistical methods used to remove the effect of autocorrelation, or biases due to similarity between closely related species, from an analysis.

Phylogeny – The pattern of relatedness between species, and/or taxonomic groups, representing the evolutionary branching pattern of speciation leading to extant (living) species.

Taxon – A systematically defined group of organisms. A taxon can refer to different ranks of classification (i.e., species, genus, family, order, etc.).

Telencephalon – The most anterior segment of the vertebrate brain. It consists of cerebral cortex (dorsal telencephalon or pallium), basal ganglia (ventral telencephalon or subpallium), and olfactory bulb.

In large-brained groups, there has been a general trend toward encephalization, or increase in brain size, over evolutionary time. Understanding the functional significance of the brain and its architecture can explain why some taxa have evolved brains that are metabolically costly to develop and maintain. This article will discuss patterns of evolutionary change in brain size in vertebrates, how to compare brains across species, and the adaptive explanations that have been proposed for the evolution of large brains.

Evolutionary Changes in Brain Architecture

Vertebrate Brains and Evolutionary Trends

A segmented brain (with fore-, mid-, and hindbrain regions) is an early vertebrate innovation, and vertebrates possess an expanded and compartmentalized forebrain relative to other chordates. Thus, the earliest vertebrates had segmented brains with identifiable regions that are homologous to those in more derived and complex brains. Reconstructing these brains from fossil evidence is very difficult; in fish, as well as in amphibians and reptiles, the brain does not occupy the entire cranial cavity. However, fossil crania suggest that early vertebrate brains were small rudimentary structures similar to those found in the agnathans, or jawless vertebrates. For later vertebrate classes, such as birds and mammals, a number of fossilized specimens provide a picture of brain size and architecture of these early groups. Jurassic flying reptiles (*Pterodactyls*) and early birds (*Archaeopteryx*) had brains that were considerably larger than other extant reptiles and dinosaurs, but their forebrains were considerably smaller than modern birds. Similarly, Jurassic mammal-like reptiles and Paleocene mammals had brains that were roughly equivalent to modern-day marsupials; they were proportionately larger than contemporary reptile species, but, like modern insectivores, they had relatively small neocortices and large olfactory bulbs. In most recent groups, especially the homeotherms, brain size has increased over evolutionary time.

Although brain structure varies widely over vertebrates as a whole, individual taxa tend to have distinct cerebrotypes, and the architecture of the brain is scalable with size. The between-taxa variation in brain architecture has been interpreted as evidence for mosaic (individual brain components evolving separately) rather

Introduction

Brain size and structure vary widely across vertebrate groups. At one end of the spectrum are the primitive jawless vertebrates such as lampreys and hagfish with small, relatively simple brains. At the other end are the homeothermic, or warm-blooded, vertebrates (i.e., birds and mammals), with large, complex, and metabolically expensive brains. The difference in size of the two ends of the vertebrate continuum is marked: jawless vertebrates have brains that are approximately 0.05% of their body weight, whereas a pocket mouse has a brain that is about 10% of its body weight. Cartilaginous and bony fish, amphibians, and reptiles respectively have increasing brain size along the vertebrate continuum. In the larger-

than concerted (brain structures increasing in a scalable fashion) brain evolution. However, on closer inspection, brain evolution is not random, and there are some very predictable changes in architecture with increasing brain size over taxonomic groups.

The Vertebrate Forebrain

The forebrain, or telencephalon, is one of the areas that varies most between groups. Not only has the overall size of the telencephalon increased with brain size relative to the rest of the brain, but so also has the level of folding and number of gyres. The disproportionate increase in the telencephalon, and particularly the neocortex in mammals, means that the overall proportion of the brain that is composed of the neocortex varies dramatically across taxa (**Figure 1**). For example, in insectivores the neocortex is about 20% of the overall brain size, whereas in hominoids (apes) the neocortex is nearly 80% of the total brain. In contrast to the telencephalon, the proportionate size of the cerebellum is remarkably static across most mammals (~ 0.13). The reason for the disproportionate increase in the forebrain may be either adaptive (i.e., the forebrain is associated with higher, or executive, cognition) or developmental; during ontogeny the hindbrain is the first to develop and the forebrain is the last. Ontogenetically, it is easier to change late developing structures through heterochrony; delaying the termination of brain development will lead to the latest developing structures being relatively larger. Therefore, although it appears that the relative size of different brain components varies across species, on closer inspection, much of this pattern is driven by relatively larger-brained species that predictably have proportionately larger neocortices. This indicates that developmental constraints are important and that the brain does not develop in a truly mosaic manner.

In addition to overall size changes, the brain structure has changed in more derived groups: there has been an increase in overall neural density, and the ratio of white matter to gray matter has increased disproportionately. Additionally, many structures such as the neocortex, dorsal lateral geniculate nucleus (LGN) in mammals, and the vagal lobe of teleost fish have become highly laminated, which may allow efficient partitioning of information.

How to Compare Brains

Brain Size versus Architecture

Given that overall brain size has increased across vertebrate taxa, and that the proportional size of the neocortex has driven this trend, it seems logical to conclude that the larger the brain, the more powerful the computer. However, assuming a direct relationship between size and power is problematic for several reasons. First, larger brains are not necessarily more efficient than smaller brains. A good analogy for how this assumption might be flawed is the development of computer technology over the second half of the twentieth century. The difference in power and efficiency between early and modern computers is not a factor of size, but of the efficiency and density of the circuitry. Brains similarly vary in the size and density of their neurons and the connectiveness between different brain components. Therefore, total brain size may not reflect cognitive ability accurately.

Thus, when we compare brains from different species, it is important to consider variation in the architecture as well as the size. In fact, comparing the absolute size across brains is likely to be meaningful only where they have similar structures and composition. Distantly related groups have potentially undergone very different selective pressures. For example, bats must be aerodynamic and therefore have strong constraints on maximum brain size; neural structure in bats should be expected to be

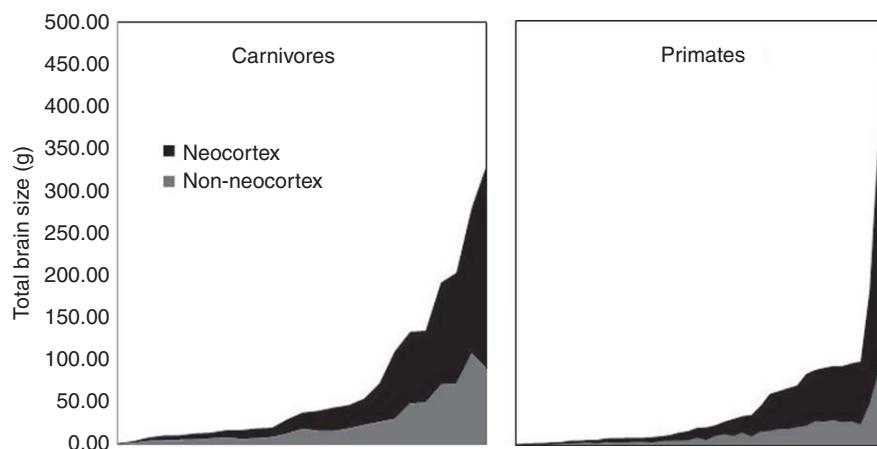


Figure 1 Proportional increase in neocortex volume with increasing brain size in carnivores and primates.

dense and compact. Relative to bats, a taxon such as cetaceans (dolphins and whales) has few constraints on maximum brain size. Marine mammals tend to have relatively exploded brains with very low neural density compared to small terrestrial mammal species. Therefore, it is reasonable to predict that in addition to overall size, network and neuronal density will be indicative of cognitive capacity. However, there is currently not enough comparative data on neuronal structure to look at the relative importance of density and neuron size versus overall brain size for cognitive ability.

A second issue is the allometric relationship between brain size and body size; brain size increases predictably by a factor between 0.67 and 0.75 with body size (**Figure 2**). Jerison proposed that the reason for this allometric relationship was that body volume to surface area increases at a $2/3$ exponential rate. Thus, the number of (neural) receptors and effectors should also increase at this rate. Alternative arguments have focused on metabolic scaling (also 0.75), which may allow larger animals to support more expensive cognitive architecture. If brain size is the result of metabolic or surface-to-volume ratios, then large brains may not have adaptive significance, but may be a result of released energetic constraints.

Given these issues, there have been a number of suggestions about how to measure relative cognitive capacity in animals. First, within closely related taxa, it may be appropriate to compare brain volume directly. If brains are structured in similar ways, then a direct comparison of volumes may be appropriate. Recent analyses of primates

have demonstrated that overall brain size is a better measure of cognition than relative brain sizes. However, across taxa, where body size and ecology vary widely, relative measures are probably more suitable.

Estimating Relative Brain Size

Jerison's solution to this problem was to calculate an encephalization quotient (EQ). An EQ is calculated by the ratio of the actual brain volume to the volume expected for its body size (i.e., $\text{bodysize}^{2/3}$). A similar measure is residual brain size, which is calculated by fitting a regression line through logged brain and body sizes. A residual value, or the distance between the regression line and each species point, gives a measure of relative brain size; those species with points above the line have larger brains than expected for their body size, whereas species that fall below the line have smaller brains than expected (**Figure 2**). Although this estimate is widely used, there are several criticisms. First, the species that are included will bias the regression line. Thus, a residual value is not absolute but relative to the species included in the analyses. Additionally, different taxonomic groups have different slopes and different intercepts (i.e., groups with large brains relative to body size will have a higher regression line than groups with small brains). For example, primates and carnivores have higher intercept values (larger relative brain sizes) than ungulates or insectivores. If a single line is fitted through multiple groups, the resulting residuals will be

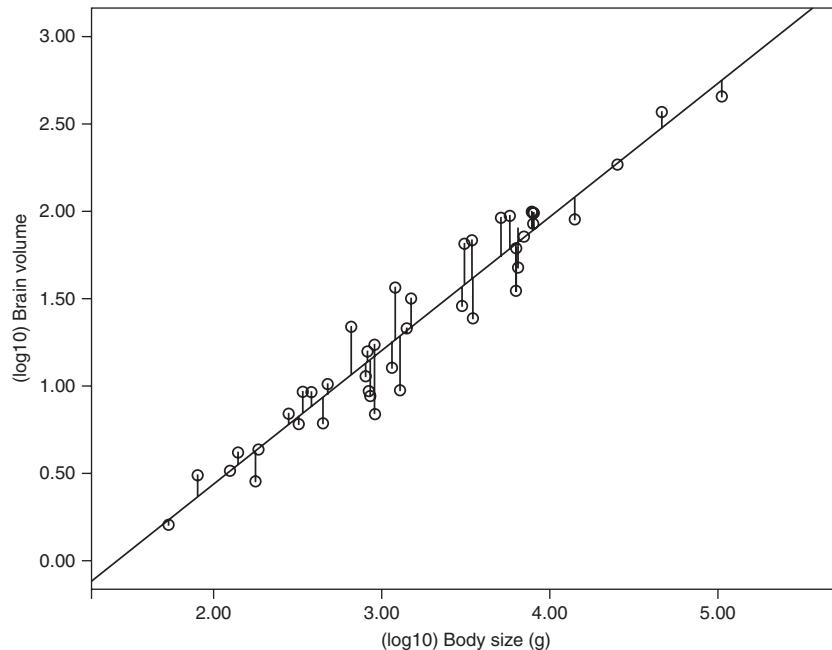


Figure 2 The relationship between brain size and body size in primates. The line represents a fitted regression, and the spikes, residual brain size of individual species.

nonnormally distributed; the group with larger brains will be clustered with high positive residuals, whereas the group with small brains will be clustered with negative residuals. A possible solution for these problems is to calculate regression lines for individual families rather than over mammals as a whole. Overrepresentation of closely related species, which are likely to be autocorrelated for both brain and body measures, can also bias the overall relationships between brain and body size. Phylogenetic methods can reduce the influence of autocorrelation between closely related species. Finally, the method used to calculate the regression line will affect the residual values. The conventional regression line, a least squares regression, underestimates the slope of the relationship and results in small-bodied species having negative residuals. Although using a reduced major axis eliminates this problem, most statistical packages use the least-squares regression methods (and thus, so do most comparative brain analyses). This is a particular issue when trying to control for phylogenetic relationships when estimating relative brain size.

The problem becomes more complicated when we wish to consider more fine-grained relationships between cognitive capacity and behavior. Not all parts of the brain are expected to be important in higher-level cognitive processing. For example, the medulla/brainstem is associated with controlling metabolic and maintenance functions of the body and the cerebellum is implicated in physical coordination. In contrast, the parts of the brain most commonly implicated in learning, decision making, discrimination, and memory are parts of the telencephalon, especially the hippocampus and neocortex. If specific areas of the brain are linked with higher cognition and behavioral complexity, then absolute or relative measures of these parts should be more enlightening than direct or relative measures of overall brain size. Thus, neocortex volume and neocortex ratio, the ratio of the volume of the neocortex to the rest of the brain, can be used as comparable measures of executive brain size. In summary, there are a multitude of methods to compare brain size using both absolute and relative measures. The best approach depends on both the question and the species used in the analysis.

Evolutionary Explanations for Brain Size Increase

Brains are incredibly costly to develop and maintain. Adaptationists, who seek functional (fitness benefits) explanations for the existence of traits, argue that costly traits will not be maintained unless their adaptive benefit outweighs their costs. Given that brains are so costly, it seems intuitive that growing a large brain must offer some kind of cognitive advantage. Additionally, some

vertebrate taxa have seen increases in brain size concurrent, or at least associated, with rapid speciation leading to adaptive radiations, such as within the passerines.

This section presents the major arguments for brain size evolution in vertebrates, starting with the nonadaptive developmental hypothesis, then the ecological, and finally the social hypotheses for encephalization. Despite the recognition that brain size is likely to have adaptive significance, there are surprisingly few attempts to relate measures of brain size with cognitive function or performance on cognitive tasks. One exception is the recent collation of various experimental cognitive paradigms administered across primate species during the twentieth century. Species performance on these tasks is positively associated with measures of brain size (especially total brain size). Thus, there is some evidence for there being a cognitive benefit for having large brains. However, this does not provide an adaptive explanation for brain size increases.

Developmental Hypotheses

Beginning in the 1980s, comparative studies of brain size in birds and mammals identified numerous life-history correlates with brain size. Species with larger brains tend to have delayed development, reach reproductive maturity later than small-brained species, have fewer offspring, and typically have longer life spans. Many of these associations may be primarily driven by body size, as larger-bodied animals have larger brains and slower life histories. However, for a given body size, species that have large brains have higher relative basal metabolic rates (BMRs) than species with smaller brains because brains are metabolically costly to maintain. It therefore appears that species with large brains need to be able to energetically support them. Martin extended this argument further by suggesting that the available maternal energy and gestation period exert the strongest constraints on fetal and, hence, adult brain weight. However, the question remains whether these life-history variables are causes or consequences of large brains. If large brains are an adaptive response to environmental challenges, then it follows that the life history and physiology of large-brained species will need to be able to support the demands of growing and maintaining a large brain.

Ecological Hypotheses

Behavioral flexibility should allow individuals to respond to unpredictable environments and to use novel resources. There have been a number of efforts to quantify behavioral flexibility. In 1997, Lefebvre and colleagues compiled a database of innovative or novel behaviors recorded in the literature for different bird

species. Their index of innovativeness is positively correlated with brain size in birds and has since been extended to primates. Wyles' behavioral drive hypothesis suggests that novel behaviors spreading through a population can change (or potentially release) selection pressures. Thus, Nicolakakis and colleagues argue that the correlation between species richness in birds and relative brain size is the result of behavioral drive leading to adaptive radiations in behaviorally flexible species.

There are a number of studies that have shown macro-ecological patterns that are associated with relative brain size. Shultz and colleagues identified relative brain size as a predictor of long-term population trends in UK farmland birds. Species with larger relative brain sizes have decreased less than species with small relative brain sizes. Sol and colleagues extended this logic demonstrating that both invasion success and migratory propensity are associated with relative brain size; large-brain species are more successful at invading novel habitats in both birds and mammals. Additionally, species that migrate, and hence avoid unpredictable environments, have smaller relative brain size than resident species that are able to survive extreme seasonal variation in resource availability and climate. Additionally, resident species are more innovative and have been documented as using more novel behaviors than migratory species. These studies suggest that larger-brained species are more behaviorally flexible and can adaptively change their foraging strategy in novel and unpredictable environments.

For primates, ecological hypotheses have centered on the need to track resources that vary over time and space. Frugivorous species have larger relative brain size than folivorous species; larger-brained species also tend to have larger home ranges than smaller-brained species. This has been interpreted as an indication that the variable nature of fruit resources (both over space and time) has imposed a cognitive demand on individuals which need to track resource availability. However, there are several inconsistencies with this logic. First, there are a number of relatively small-brained frugivores that are sympatric with primates, and thus experience the same ecological pressures (e.g., forest duikers, flying lemurs, squirrels, and viverrids). Thus, it does not seem essential for frugivorous species to be large brained. Second, Dunbar and Shultz have partialled out the relative contribution of different traits to brain size and have shown that high-quality fruit diets are more likely necessary to support the high metabolic demands of large brains rather than be the driving selective pressure increasing brain size.

Social Hypotheses

In the late 1970s, several primatologists suggested that the apparent intelligence of monkeys could be the result of their complex social environment. This concept was

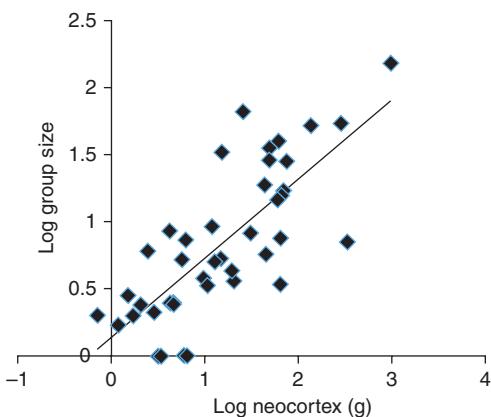


Figure 3 The relationship between neocortex size and group size in primates.

developed in the social brain hypothesis, which proposes that the evolution of large brains characteristic of primates has been a response to complex sociality. There is a strong correlation between primate relative brain (and neocortex) size and the size of social groups (**Figure 3**) and grooming cliques. The relationship has been interpreted to indicate that individuals in larger groups need to monitor, maintain, and remember more relationships.

Byrne and Whiten suggested that the cognitive demands of living in a social group may be more linked to cheating and detecting cheating than just monitoring relationships; they termed this Machiavellian intelligence. As a test of the hypothesis, Byrne and Corp collated data on deception rates in primates and demonstrated a positive relationship with relative neocortex size. Although Byrne and Whiten's hypothesis is not in opposition to Dunbar's social brain, it does put a slightly more malevolent twist on primate intelligence.

Primates are not the only social animals and the group size relationship with brain size should generalize to other taxa; however, several studies have shown that it does not. In fact, in nonprimates, large brains appear to be associated with stable and enduring relationships, such as between pair-bonded partners or between members of small cohesive groups, rather than overall group size. A potential explanation for this pattern is that many social species occur in large, unstable aggregations rather than bonded groups. It is only individuals in stable and bonded groups that need to coordinate activities and monitor relationships. Although brain size does not appear to be linked to cooperative breeding in birds, relative brain size is associated with stable pair bonding. Pair-bonded individuals need to monitor their reproductive partners to maintain territories, avoid cuckoldry, and synchronize vigilance and parental care.

A related, but slightly different take on the social intelligence hypotheses is the potential benefit of social learning. Individuals that can effectively exploit and

utilize the knowledge of others can avoid a costly trial-and-error learning process, and adaptive behavior can spread quickly through populations. Populations of many animals show cultural behaviors which, on the surface, do not appear to be adaptive, but are consistently maintained in a population. A lot of interest has been focused on cultural differences in chimpanzees; as culture is seen a distinctive feature of human societies, the logic that it is seen as cognitively advanced appears to be logical. However, some of the best examples of cultural behavior come from fish and birds. Many of the cultural differences between chimpanzee populations can be interpreted as adaptations to local resources and environmental conditions. Although it is not reasonable to state that there are no cultures in primates, interpreting population differences in a cultural context does not really highlight the cognitive demands or adaptive significance of these behaviors.

Reconciling Social and Ecological Hypotheses

In essence, all of these hypotheses focus on an individual's ability to make flexible adaptive decisions in the face of unpredictability. One approach to reconciling the ecological and social hypotheses may be to look at an animal's environment as hierarchically structured, with environmental variability, resource availability and distribution forming the first layer of complexity and inter- and intraspecific interactions forming higher tiers of complexity, which interact and compound the initial ecological problems that all individuals need to solve. A solitary animal needs to maintain its energy balance by finding enough food of appropriate nutritional quality. Thus, the animal needs to be able to locate appropriate food, decide which items to include in its diet, how to optimally use patches, and how to allocate its time to various maintenance activities. However, individuals do not live in isolation, and have to avoid predators, locate mates, minimize competition over resources, and they need to make strategic decisions about how to balance predation risk, reproduction time, and resource allocation. However, when social groups become more stable (why would they), then all resource-acquisition decisions need to be made in the context of what other group members are doing.

Human Evolution and Brain Size

Although humans have brains that are much bigger than those of other primates, early hominids had brains that were little bigger than modern apes. Therefore, there has been much speculation about what has driven the increase in brain size in later species. Hominids such as *Australopithecus afarensis* evolved bipedal locomotion, with no concurrent increase in brain size. In fact, it was

over a million years later that there was a marked increase in brain size. So what factors could have driven the brain size jump seen in the genus *Homo*? It is unlikely to have been driven by tool use, as evidence for manufactured tools appears later in the fossil record. Ecological arguments also do not seem to hold; the seasonality of environments where hominids were found does not tightly correlate with brain size changes. Dunbar and Aiello suggested that increases in group size, similar to the relationship shown in primates, could explain the increased demand on cognitive ability. Moving from a forest or woodland environment into one where predation risks are high could provide an initial pressure to increase group size. Savanna-living primate species are found in larger groups than their forest counterparts. Coordination, relationship maintenance, and information transfer in such large groups would not only have been cognitively demanding, but may have also been the impetus driving the evolution of language.

Where to Next?

One of the prevailing criticisms of comparative brain studies is that we are yet to identify strong links between relative brain structure size and cognitive function. Thus, it is essential to relate cognitive performance across taxa to brain architecture. Additionally, the relative importance of size versus internal network structure (connectivity) and neuronal density needs to be compared across species. There is evidence from humans that intelligence and brain structure are associated and heritable. However, little is known about the relative effects of the environment on brain size and structure. Hippocampus size has been shown to be sensitive to environment, with spatial learning leading to increased size. Whether the flexibility seen in the hippocampus is a general phenomenon across the telecephalon is unknown. Thus, although there are a number of associations between brain size and behavior, the causal nature of these relationships needs further investigation.

See also: Behavior Adaptation and Selection; Behavioral Development and Socialization; Cholinergic Systems in Aging and Alzheimer's Disease: Neurotrophic Molecular Analysis; Cooperation; Evolutionary and Developmental Issues in Cognitive Neuroscience; Mating Behavior; Primate Origins of Human Behavior; Social Bonding and Attachment; Social Cognition: From Behavior-Reading to Mind-Reading; Social Communication; Social Competition and Conflict Resolution; Social Learning and Behavior Transmission; Social Relationships and Social Knowledge.

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Brain Imaging

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Glossary

Apical dendrite – Branched projections located at the apex of a neural cell that receive and conduct signals from other neural cells.

Conductive coil – A series of one or more loops of conductive wire that compose a critical hardware component in MRI machines; it is used to create a magnetic field and/or to detect a changing magnetic field by the voltage induced in the wire.

Dipole – A pair of electric charges or magnetic poles of equal magnitude but of opposite sign or polarity and separated by a distance. Electric dipoles contribute to the generation of a current, which contributes to the generation of a magnetic field (c.f., electromagnetic, conductive coil).

Dura mater – The outermost, toughest and most fibrous of the three membranes covering the brain. It lies beneath the skull and skin, and contributes obstruction to signals generated in neuronal cells.

Electromagnetic – The property by which a change in an electric field induces a magnetic field, and vice versa. This property is what underlies the ability of a conductive coil to produce or detect changes in a magnetic field (c.f., conductive coil), and of a neuron to produce a measurable magnetic field (e.g., by MEG, c.f., dipole).

Gray matter – Tissue in the brain that consists mostly of neuronal cell bodies and capillaries, appears gray in color and lies primarily at the outer surface of the brain (Figure 1(a)).

Hemoglobin – Iron-containing oxygen-transport protein in red blood cells. Hemoglobin oxygenation increases when neuronal cells are active, contributing to the blood-oxygen level dependent (BOLD) signal measured by fMRI.

Nuclear – Relating to the properties of an atomic nucleus. Nuclear properties, such as resonance or stability, are critical to imaging technology (e.g., MRI and PET respectively).

Perfusion – The delivery of arterial blood to capillaries in biological tissue such gray matter of the brain.

Pyramidal neurons – A type of neuronal cell that serves as the primary excitation unit in the human brain.

Radioactive isotope – An atom with an unstable nucleus, characterized by excess energy. In PET, radioactive isotopes that emit positrons are utilized, whereas in SPECT radioactive isotopes that emit a single unit of energy (or photon) are employed.

Resonance – The tendency of a system to oscillate at larger amplitude at some frequency more than at others. Changes in frequency change the oscillation, or spin, of atoms in brain tissue, which produces a measurable signal that is captured in MRI.

Radiofrequency – Frequency or rate of oscillation within the range of about 3 Hz to 300 GHz (used to produce and detect radio waves).

White matter – Tissue in the brain that consists mostly of myelinated (lipid or “fatty” covered) axons, that appear white in color, comprising connecting tracts between regions of gray matter (Figure 1(a)).

Introduction

Brain imaging refers to techniques that employ an interaction between brain tissue and various forms of energy (e.g., electromagnetic or particle radiation), rather than physical incision, to capture positional data about the structure and function of the brain. Such data are used to create corresponding brain maps. Structural images delineate brain tissues such as white versus gray matter, vasculature, and bone, based on their physical properties (tissue density or nuclear resonance characteristics). Functional images capture physiological activities in the brain (metabolism, blood flow, chemical composition, absorption) typically coupled to neuronal firing. Functional imaging has two possible aims. In clinical applications the goal is typically to differentiate normal physiological activities in a healthy brain from those in perturbed states (e.g., stroke, Alzheimer’s disease). In cognitive neuroscience the goal is to understand how brain function mediates human cognition and behavior (e.g., memory, language, and vision). Attaining these goals depends on the nature of the measured signal, spatial and temporal resolution, and practical constraints such as invasiveness and cost of each technique.

Brain Imaging Techniques

Brain Structure

Magnetic resonance imaging

The most powerful method of structural imaging today, largely replacing X-ray-dependent computed tomography (CT) in research applications, is magnetic resonance

imaging (MRI). MRI is based on nuclear magnetic resonance (NMR), the tendency of certain nuclei to resonate when placed in a magnetic field, independently discovered by Felix Bloch and Edward Purcell in the 1940s. In MRI a strong electromagnet, typically 1.5–4.0 tesla (T) for human imaging, is first used to produce net nuclear magnetization in hydrogen atoms in the body. Radiofrequency pulses are then applied at the resonant frequency of the hydrogen atoms, which displaces them into a higher-energy state (i.e., out of alignment with the net magnetization). As the protons then return to their original state they release energy, creating an oscillating magnetic field that can be picked up (via electromagnetic induction) by a conductive coil placed in the field. This signal is localized spatially by using a combination of magnetic field gradients in different planes to produce unique spin properties across the brain that can be used to reconstruct the spatial location of the signal source. The contrast in MR images is obtained by

modifying the timing of both radiofrequency pulses and signal acquisition to take advantage of natural differences in physical properties of the different tissues, such as the time needed for the tissue to return to the net magnetic field after excitation. Typical MR images (**Figure 1(a)**) capture detailed three-dimensional (3D) structure of the brain distinguishing between tissues such as gray and white matter, cerebrospinal fluid, bone, fat and air, as well as being able to detect the presence of abnormal tissues such as tumors or cysts.

One advantage of MRI is its extreme flexibility in the types of signals that it can measure. Diffusion-weighted MRI (e.g., Diffusion Tensor Imaging or DTI) is sensitive to the movement of water molecules over time. The measurement of directional diffusion signals allows characterization of white matter structure, which has allowed the development of MRI-based tractography methods for imaging white matter connectivity. When

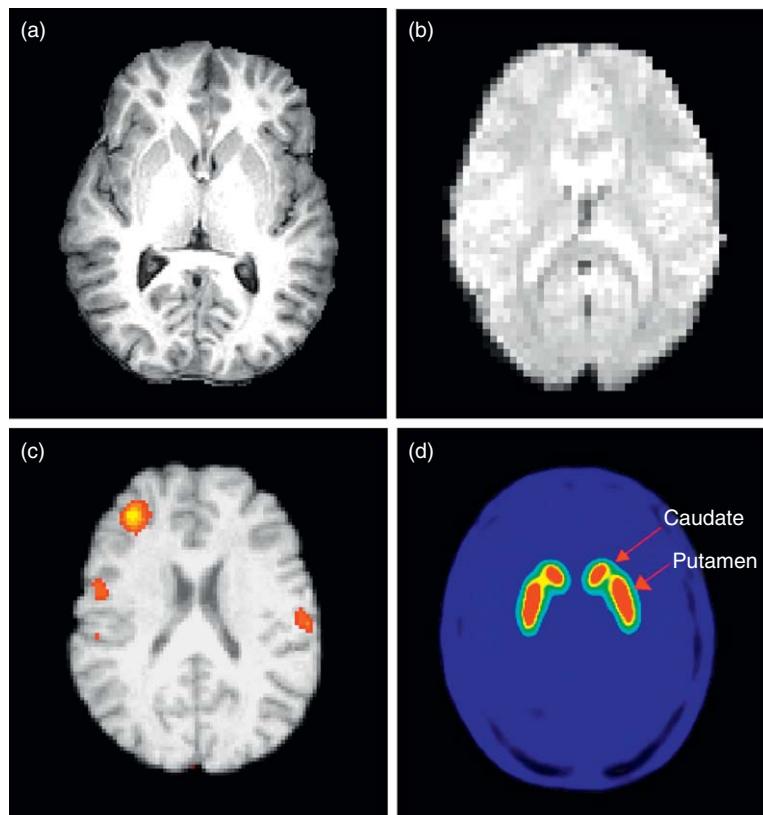


Figure 1 Maps of the brain vary with the type of signal detected. In magnetic resonance imaging (MRI) (a) the signal typically arises from variation in magnetic properties of hydrogen atoms across different brain tissues, producing high-resolution (~1 mm) structural images that delineate structures such as white vs. gray matter, deep nuclei, bone and ventricles. MRI can also be used to detect brain function by measuring the oxygenation level of blood hemoglobin (b). The resulting images are of lower resolution (~3 mm) at the gain of higher temporal resolution. Comparisons of the resulting images across tasks or states reveal the locations of task-specific changes in brain metabolism. Localization of these changes is accomplished by overlaying the activation maps onto a structural image (c). An alternate measure of brain function is shown in figure (d). Here the binding potential of D2-like dopamine receptors for the radioactive tracer [¹⁸F]fallypride is revealed using positron emission tomography (PET). The density of the receptors increases (hot colors like red and yellow) in caudate and putamen structures of the basal ganglia compared to other parts of the brain (blue). Figure (d) has been provided courtesy of E. London, UCLA.

used to measure overall diffusion, it is very sensitive to changes that occur early in stroke and other neurological disorders. MRI spectroscopy measures regional concentration of specific metabolites, which can signal neural cell integrity and energy metabolism. MRI can also be used to measure blood flow and characterize brain perfusion, which is a sensitive measure for stroke and other cerebrovascular disorders. Finally, one of the most powerful applications of MRI is to measure brain function by measuring changes in oxygenation of hemoglobin in the blood.

Brain Function

Electro- and magnetoencephalography

Electroencephalography (EEG), first described by Hans Berger in 1924, and more recent magnetoencephalography (MEG), first introduced by David Cohen in 1968, measure electrical signals resulting directly from post-synaptic potentials in the apical dendrites of pyramidal neurons of the cortex. Such potentials produce currents running along the length of the neuron that summate across neuronal assemblies due to the parallel configuration of such neurons (i.e., creating electrical dipoles). This summation produces a measurable voltage (V) potential at the scalp surface, measured by EEG. Perpendicular to these currents arises a magnetic field that also summates across neurons and is measured by MEG. In EEG changes in scalp voltage potential are measured by electrodes attached to the scalp. Changes in voltage (approximate range of 10–100 μ V) can then be recorded across time at each of the electrodes. In MEG, magnetic fields at the scalp are measured using a superconducting quantum interference device (SQUID) containing highly sensitive detectors that translate the magnetic field back into current values. SQUIDS are necessary because the summed magnetic fields produced by pyramidal neurons are small, \sim 10 fT (femtotesla), while noise in daily urban environments (i.e., cars, power lines) averages between 10^6 and 10^9 fT. In both modalities the resulting signals can be used to monitor brain activity across time. Distinct frequency profiles are associated with states of sleep, alertness, and awareness during anesthesia. These signals can also be observed during a time window associated with a cognitive function of interest. For instance, in EEG, low-frequency negativity can be observed immediately preceding a motor response. Subsequent properties of this potential, such as laterality differences across the scalp, can further differentiate between the cognitive expectation of an event and preparation of a motor response. In both EEG and MEG, additional information may also be obtained from the frequency domain of the temporal signal.

Positron emission and single photon emission computed tomography

The advent of nuclear medicine in the 1940s and 1950s and subsequent developments in production of radioactive isotopes and gamma ray detectors have contributed to emission computed tomography modes of brain imaging, including positron emission tomography (PET) and single photon emission computed tomography (SPECT). They are used to image blood flow and/or metabolic processes in the brain by measuring radiation energy emitted from radioactive isotopes that are intravenously injected, and subsequently distributed by the circulatory system of the brain (Figure 1(d)). In PET, isotopes can be created to measure blood flow (e.g., $^{15}\text{O}_2$), blood volume (e.g., ^{11}CO), or metabolic operations such as glucose metabolism or dopamine synthesis (e.g., ^{18}F). As these isotopes are circulated in the brain, they release positrons that collide with an electron, annihilate and release two gamma rays traveling in opposite directions. Coincidence detectors determine the source of the collision and the number of collisions occurring, providing information about location and concentration of that compound in the brain. From this information, a 3D image of the metabolic effects on the radioactive isotope in the brain can be reconstructed. For instance, glucose labeled with ^{18}F has been effectively used to measure increased glucose metabolism in visual and auditory sensory cortices in response to visual versus auditory stimuli respectively, and in motor cortex during finger tapping movements. Changes in metabolism across various areas of the cortex can also be tracked, for instance, as a function of metabolic maturation during development or with the progression of disease. A similar process applies to SPECT; however, single photon emission isotopes (e.g., ^{123}I , ^{111}In) are used instead of positron-emitting isotopes and the detectors are gamma cameras designed to measure single photon emission rather than coincidences. Unlike PET, SPECT isotopes have been developed primarily for measurement of cerebral blood flow changes, not metabolic processes. Thus while this technique can detect the effects of a stroke on blood flow patterns, it cannot detect functional changes in which blood flow is less affected than metabolism, such as altered synthesis of the neurotransmitter dopamine in Parkinson's disease.

Functional magnetic resonance imaging

Although MRI has been in use since the 1970s and has many applications in structural imaging, its application to measuring brain function was not possible until 1990 when Seiji Ogawa and colleagues demonstrated that the oxygenation state of blood hemoglobin modulates MRI signal around large veins. This finding resulted in development of MRI sequences sensitive to changes in blood flow in the brain (Figure 1(b)), without the need

for any exogenous contrast agent to be injected (e.g., PET, SPECT). In fMRI, the contrast arises from the effects of deoxygenated hemoglobin on the magnetic field. As demonstrated by Ogawa, when blood hemoglobin lacks oxygen it has magnetic properties that disturb the effects of magnetization in its vicinity and thus lowers the measured signal. In contrast, when hemoglobin is oxygenated, it has less effect on neighboring molecules and thus produces a net larger signal during an MRI scan. The utility of this finding to measuring brain function is that both blood flow and local hemoglobin oxygenation increase in response to neuronal activity, thus increasing the local MRI signal. This is referred to as the blood-oxygen-level-dependent (BOLD) signal; while there are other ways to measure functional activity using fMRI, BOLD fMRI is by far the most popular method. The BOLD response provides a robust but indirect measure of brain activity and, like PET, can detect active regions during perceptual and cognitive tasks in both healthy and diseased brains (Figure 1(c)).

Applications: Determining Factors

Spatiotemporal Resolution

Imaging techniques vary in both spatial and temporal resolution, which constrains the types of questions that they can answer within their imaging domain. For structural imaging, spatial resolution is the primary concern (since structures change over the course of months to years), whereas functional imaging requires high spatial and temporal resolution (since neuronal function occurs over milliseconds at a spatial scale of microns). Functional imaging modalities fall into two categories: those that directly reflect neuronal activity (EEG/MEG) and those that measure physiological processes associated with neuronal activity (fMRI/PET). These categories are also defined by tradeoffs between spatial and temporal resolution. The former (EEG/MEG) provide excellent temporal resolution (0.01 s) but low spatial resolution (\sim 10 mm), whereas the latter provide better spatial resolution (fMRI: 1–5 mm, PET: 4–8 mm) at the cost of temporal resolution (fMRI: 1–6 s, PET: 60–1000 s). These differences differentiate how the two categories of methods are applied. EEG and MEG are well suited to examine the dynamics of brain function because they provide continuous and temporally precise measures of neuronal activity. One application of such data is for monitoring of brain states. For instance, the EEG signal is useful in diagnosing the quality and type of sleep that an individual engages in during the course of a night, or in monitoring the alertness of an individual in an anaesthetized state. Both EEG and MEG can also be used to analyze the dynamics of brain function. For instance, the processing sequence

of an audiovisual stimulus can be obtained by comparing the timing of signal over visual versus auditory cortices following stimulus onset. The questions that EEG and MEG can answer are thus tied to the idea of when processes happen and only coarsely related to where they happen. In contrast, PET and fMRI can answer the question of “where?” better than the question of “when?” neuronal activity occurs. Both techniques provide spatial resolution under 10 mm, with fMRI potentially providing resolution down to 1–2 mm. This implies that these techniques are more accurate in localizing where within the visual and/or auditory cortex the audiovisual stimulus is processed. If the visual stimulus involves motion, for instance, it will produce the strongest activity in region V5/MT of the visual cortex relative to other visual processing regions such as V1 or V4. Unlike EEG or MEG, these techniques do not dissociate which perceptual modality was processed first, visual or auditory, because such processing occurs at the time scale of milliseconds, well below that of either PET or fMRI.

Practical Considerations

The utility of imaging methods, particularly in the functional imaging domain, is also influenced by two practical considerations: invasiveness and cost. The invasiveness of a technique has the effect of distinguishing between clinical and cognitive neuroscience applications. This is apparent in the comparison of PET and SPECT with EEG, MEG, and fMRI. Whereas the former use radioactive isotopes, and thus are more invasive, the latter use intrinsic signals to identify function. Reliance on radioactive isotopes has two effects, both of which make the method unsuitable for cognitive neuroscience applications. First it increases health risk, which is less easily justifiable by the benefits of basic research relative to clinical applications. Second, invasiveness has the effect of constraining the amount of data that can be acquired. This is undesirable because the neural signals related to cognitive processes are often relatively small, and many repeated observations are required to maximize the power and interpretability of the experimental results. In contrast, sampling is restricted in both PET and SPECT because safety concerns about side effects of radiation limit the number of times that an individual can be tested and second because the number of scans obtained within a session is constrained by the distribution time and half-life of the isotope. For instance, for ^{15}O with half-life of 2.03 min, approximately 60 s are required for the isotope to circulate throughout the brain and 10 min required between dosage injections (i.e., 5 half-lives) for most of the isotope to be eliminated from the body between scans. In a 60 min session no more than six scans can be administered with 60 s temporal resolution.

EEG, MEG, and fMRI are not limited in the amount of data that can be collected on an individual, and thus are more suitable to cognitive neuroscience applications.

Finally, cost also has a role in the utility of imaging methods. For instance, whereas PET is considerably more accurate than SPECT, the latter is cheaper and uses more easily accessible isotopes. Its availability makes it a more common tool in clinical settings. EEG and MEG are less disparate in quality, but the necessity for SQUIDS as detectors in MEG makes it much more costly in comparison to EEG. Consequently, EEG is more prevalent than MEG in both clinical and research settings. MRI, in contrast, is relatively common in both clinical and research settings, making it both a popular and accessible tool.

Challenges

Limits on Resolution

The preceding discussion raised the concern of an apparent tradeoff in spatial versus temporal resolution among brain imaging methods. Both temporal and spatial resolution are constrained by the nature of the signals that they measure. In EEG, the current generated by neuronal population spreads passively from its source, being distorted and diffused by the dura, scalp, and skull, such that exactly reconstructing the source of the signals is impossible. In fMRI changes in neuronal activity are measured indirectly through the BOLD response, which occurs primarily in larger veins that are downstream of the true neuronal response. Technological developments in the techniques themselves will not greatly improve the resolution of these techniques. Rather, new developments in the type of signal measured may offer an alternative approach. For example, recent advances in MRI suggest that it may be possible to measure the magnetic fields produced by neuronal activity, akin to the signal measured in MEG. This approach may present perhaps the best resolution to date: millisecond temporal resolution similar to EEG and MEG while maintaining the spatial resolution of fMRI. However, these signals are exceedingly small and to date they have not been measured reliably in the human brain.

An alternate approach to optimizing resolution is to combine technologies either through simultaneously recording from different modalities, or by using the data from one modality to improve the interpretation of another. MRI images can improve EEG spatial resolution by facilitating reconstruction of realistic head models to estimate conductivities throughout the scalp, skull, and dura. Such models can then be integrated into deblurring algorithms designed to decrease the attenuating effects of these structures on the EEG signal. Due to the complementary nature of the signals recorded, simultaneous recording from EEG and MEG can improve the sampling and thus spatial resolution of the underlying neuronal activity. It is also possible

to acquire EEG data simultaneously with fMRI data, but it is very technically challenging. An important caveat to these strategies is that the techniques be compatible in measured signal. For instance, although an apparently useful approach would be to combine EEG and fMRI recordings to integrate over space and time, the signals measured by these methodologies correspond to different processes (neuronal current vs. changes in blood deoxyhemoglobin) and their co-occurrence is not easy to interpret. Integrating across different sources of information within a modality, such as structural and functional MRI images, is more straightforward; for example, high-resolution MRI structural images are often used to localize the sources of activity in low-resolution fMRI images (**Figure 1(c)**). Registration algorithms can align the two data sets in space and across time, allowing researchers to infer the location of a lower resolution (3–4 mm) BOLD signal on a higher resolution (1 mm) MRI structural image. Similarly, diffusion imaging and fMRI can be combined to define the bounds on functional regions by comparing the parameter of activation in a given region as measured by fMRI, with the connections that it makes to another region as measured by DTI.

Functional Localization and Inference

Another challenge in functional neuroimaging concerns functional localization (i.e., matching brain activity to cognitive function). This is complicated by the presence of interactions between regions; measurements of activity in a single region are potentially incomplete in describing how the brain performs a given function. To demonstrate, the fusiform face area (FFA) in the inferotemporal visual cortex shows a stronger activation in response to faces than other stimuli, supported by the presence of face-selective cells in this region, and shows decreases in activity in patients with prosopagnosia, an inability to recognize faces. The activation patterns of the FFA thus suggest that it is involved in face perception. Upon closer inspection however, it has become clear that the FFA also responds to stimuli other than faces (e.g., objects and birds) (**Figure 2**) and patients with prosopagnosia show abnormal activation patterns in regions other than the FFA (e.g., inferior occipital cortex), questioning the selectivity of this region for face perception. Moreover, face-selective cells have been found in other parts of the brain, including temporal and occipital cortex, prefrontal cortex, and amygdala, suggesting that other parts of the brain may also be critical to face perception. Thus, understanding activation in FFA alone may be insufficient to understanding this function.

This conclusion is supported by recent proposals that accommodate the above inconsistencies by considering the FFA as one contributor to a network of regions that together result in face perception. For instance, one recent

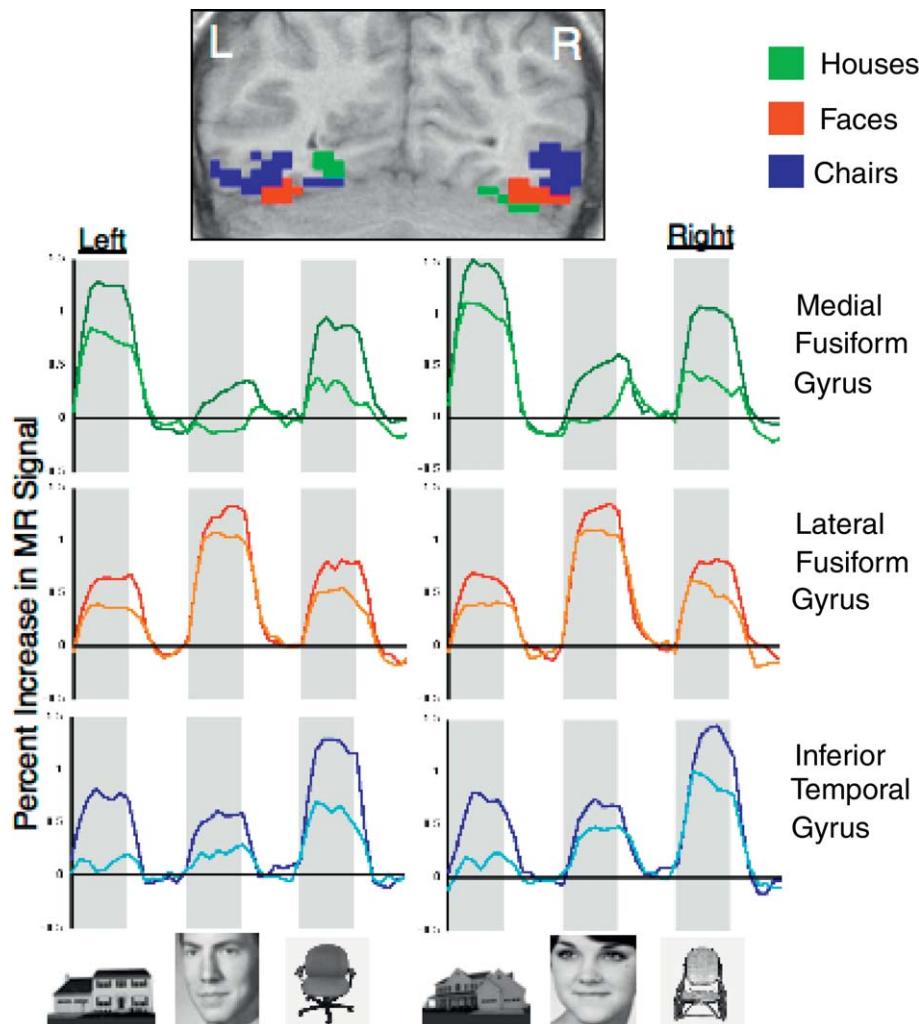


Figure 2 The lateral fusiform gyrus (red/orange in top figure and middle row graph) shows a greater response to faces than to other stimuli, indicating its involvement in face processing. However, the selectivity of this region for faces is not exclusive as the region also responds to other stimuli such as chairs and houses. Similarly, nearby medial fusiform gyrus and inferior temporal gyrus respond to faces as well as to other stimuli. These data suggest that face processing includes multiple regions in the brain, not only the fusiform gyrus. Reproduced with permission from Ishai A, Ungerleider LG, Martin A, Schouten JL, and Haxby JV (1999) Distributed representation of objects in the human ventral visual pathway. *Proceedings of the National Academy of Sciences of the United States of America* 96: 9379–9384. Copyright (1999) National Academy of Sciences, USA.

model suggests that the role of the FFA in face perception is to distinguish between faces. In addition, the role of cells in the temporal and occipital cortices may be to identify speech patterns and gaze associated with faces, in the prefrontal cortex to identify the semantic goal guiding the need for face perception, and in the amygdala to facilitate identification of facial expressions. The integration of activity across multiple regions, rather than only in FFA, captures the function of face perception. Not only does such a scheme accommodate the presence of face selective cells in regions other than the FFA, but it also explains variability in selectivity of FFA for faces. The role that FFA plays in distinguishing unique faces may be applicable to distinguishing between other stimuli (e.g., objects) given, for instance, a different set of

interactions with other regions in the brain. The example of FFA and face perception demonstrates that understanding brain function may be better served by considering activity across a network of regions rather than within a single region and thus implies that cognitive functions likely correspond to integrated functional networks of regions rather than to activity within individual regions.

Understanding brain function is therefore contingent on understanding functional networks, which requires the integration of experimental and analytic techniques. Recent advances in multivariate analytic approaches have made possible the identification of distributed networks of regions that show similar activity patterns and changes in activity patterns across tasks, participants, or time. Interactions between regions and their direction of

influence can be assessed through causal models. This is beneficial in distinguishing which regions modulate activity in another region by determining, for instance, the strength of interactions between other sets of regions. As an example, activity in the prefrontal cortex may increase the connection strength between FFA and amygdala when the individual's goal is to determine which of their colleagues is in a bad mood, but between FFA and temporal cortex when locating a colleague with a distinguishing accent in their speech. In addition, networks can be assessed by evaluating the types of activity in various regions (e.g., sustained activation after viewing a face or a short burst of activity at the onset of the face) that can determine the individual roles of regions (e.g., remembering a face or detecting a face, respectively), by correlating network activity to behavior and by evaluating the effects of disruptions to a network on both activity in other regions and behavior. Finally, a complementary approach to localizing function may be to better categorize the types of underlying cognitive processes that are involved in cognition. This goal requires the development of an ontology of the basic units of cognition; it could then be used to interpret network brain activity by the degree to which individual units are engaged in a task. Ultimately, how the brain produces mental activity is determined not only by which regions are active but also by their interactions across space and time, and understanding these interactions remains challenging for brain imaging.

Acknowledgments

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See also: Brain Aging: Structural Imaging Biomarkers of Risk of Alzheimer's Disease; Brain Imaging and Addiction; Brain Mapping of Language and Memory in Epilepsy; Contribution of Split-Brain Studies to the Evolution of the Concept of Hemispheric Specialization; Declarative Memory; Development and Language; Episodic and Autobiographical Memory: Psychological and Neural Aspects; Evolutionary and Developmental Issues in Cognitive Neuroscience; Implicit Learning and Memory: Psychological and Neural Aspects; Language and Communication – Brain Substrate; Neural Basis of Attention-Deficit/Hyperactivity Disorder; Neural Basis of

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- <http://www.med.harvard.edu> – Harvard Medicine, Whole Brain Atlas.
- <http://www.nitric.org> – Neuroimaging Informatics Tools and Resources Clearinghouse.
- <http://www.humanbrainmapping.org> – Organization for Human Brain Mapping.

Brain-Machine Interfaces

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Glossary

Directional tuning – A preferred movement direction that results in maximum discharge of a primary motor cortex cell.

Electrocorticography (ECoG) – The measurement of electrical activity produced by the brain as recorded from electrodes placed on the epidural or subdural surface of the cerebral cortex.

Electroencephalography (EEG) – The measurement of electrical activity produced by the brain as recorded from electrodes placed on the scalp.

Microelectrode arrays – These arrays contain multiple recording sites through which intra-cortical neural signals, namely action potentials and local field potentials, can be acquired. They can also be used to deliver electrical stimulation to the brain.

Volitional control of neural activity – A term introduced by E. Fetz in the 1970s, referring to the capacity for a subject to voluntarily control the firing rate of a given neuron (e.g., bring it to a designated target value) provided the feedback is delivered.

A brain-machine interface (BMI, also referred to as brain-computer interface, BCI) is a direct communication pathway between the brain and an external artificial actuator, such as a computer or a robot. This novel experimental paradigm contends that a user can perceive sensory information and enact voluntary motor actions through a direct interface between the brain and an artificial actuator in virtually the same way that we see, walk, or grab an object with our own natural limbs. Specifically, BMIs are meant to play a major role in the near future as they could lead to significant advances in restoring the sensorimotor functions for patients suffering from spinal cord injuries, stroke, and other neurological disorders. Instead of reconstructing the connectivity and functionality of damaged nerve fibers by repairing the damaged tissues, BMIs will allow to bypass the lesion, creating an artificial link between healthy brain structures and the system to be controlled. These could be the natural limbs of the patient under functional electrical stimulation (FES), a prosthetic device, or any virtual object that is part of a computer interface.

A BMI is generally comprised of four parts: first, a recording device captures neurophysiological signals

from the brain. Then, a decoding algorithm converts these signals into a semantic variable representing an action to be performed. These commands are streamed to the artificial actuator that will execute them. Finally, feedback is provided to the user in order to close the control loop (see [Figure 1](#)). It is usually provided as visual feedback, but other schemes can be implemented, which are discussed later.

Research in BMIs has flourished in the last decade, with impressive demonstrations of rodents, non-human primates, and humans controlling robots or cursors in real time through single-unit, multiunit, and field-potential signals collected from the brain. Next, we review the main aspects of the four building blocks of a BMI system, namely, recording technologies, decoding algorithms for estimation and control, and feedback mechanisms. We focus more on cortical BMIs that employ neuronal ensemble activity. A discussion on the future directions concludes the article.

Recording Technologies

Three main recording technologies dominate the BMI spectrum: electroencephalography (EEG), electrocorticography (ECoG), and cortically implanted microelectrodes arrays.

EEG is the measurement of electrical activity produced by the brain as recorded from electrodes placed on the scalp. EEG is a very popular recording method because it is relatively inexpensive, fast (~ 15 min to set up), and noninvasive. This technique allows rapid application of BMIs in human subjects, leading to the creation of an online cooperative database of EEG data for BMI. The low spatial resolution and signal-to-noise ratio (SNR) are main drawbacks of EEG measurements since the sources of the recorded electrical signal are far away from the recording site.

Low spatial resolution and SNR result in limited performance and make online single-trial performance difficult. Most of the published works using EEG are offline studies in which a large number of trials must be averaged for the SNR to be sufficient for use in a BMI system. Few groups are succeeding at online control studies with single trials, such as the wheelchair control work of Millan and colleagues.

Two classes of EEG-based BMIs can be distinguished: asynchronous and synchronous BMIs. Asynchronous

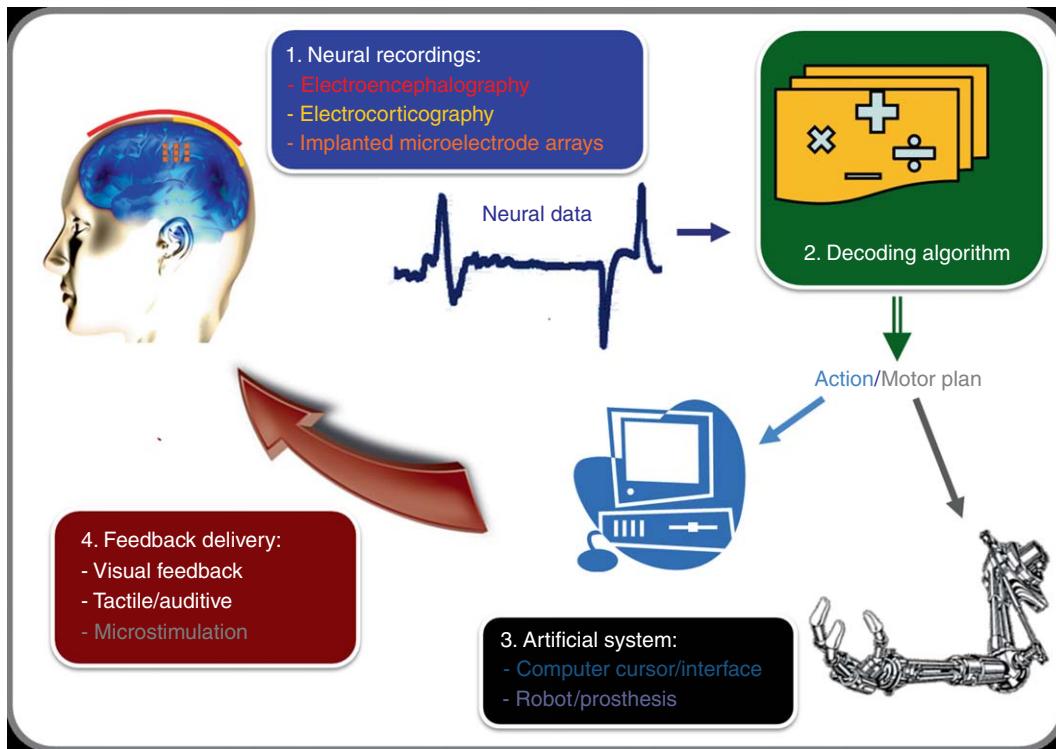


Figure 1 The BMI loop. The four main building blocks of a closed-loop BMI system: recording system, decoding algorithm, device to be controlled, and feedback delivered to the user.

systems will look for changes in amplitude, frequency, or phase of natural rhythmic signals of the brain, linked to the volitional activity of the subject. For example, the amplitude of μ -rhythm (8–12 Hz) is modified not only when a subject performs a movement, but also when he or she imagines it. After proper training, subjects learn how to control this rhythm to perform a specific action; a recent study showed an impressive demonstration of a brain-actuated wheelchair. Synchronous systems aim at detecting specific events in the EEG signals, which are caused by stimuli perceived by the subject (ERPs: event-related potentials). Specifically, P300-type ERPs (meaning that the measured EEG potential occurs 300 ms after the stimulus) caused by visual, tactile, or auditory stimuli have been used to control computer-based tasks. One advantage of synchronous systems is that they do not require any training period from the user, since ERPs are an innate feature of the brain. However, several repetitions of the stimulus are needed in order to extract the information from noisy signals. Improvements may come from source localization methods, which allow better discrimination among events using spatial information.

The nature of the signals recorded through electrocorticography (ECoG) is similar to EEG, since it measures electrical potentials resulting from the spatial average of a large area of the brain, and hence utilizes a large group of neurons. However, the fact that the

recording electrodes are placed under the dura leads to higher spatial resolution in ECoG than EEG (respectively, tenths of millimeters vs. centimeters), broader bandwidth (0–500 Hz vs. 0–50 Hz), higher characteristic amplitude (50–100 μ V vs. 10–20 μ V), and far less vulnerability to artifacts, such as EMG or ambient noise. The main drawback with respect to EEG is the invasiveness of the procedure as it requires opening the skull, and in the case of subdural implants, also opening the dura mater. In addition, similar to EEG, ECoG can be used either in an asynchronous or synchronous manner. Schalk and colleagues showed recently an application of asynchronous ECoG to two-dimensional cursor control with good success rates. In this study, ECoG features, such as spectrum amplitude at specific frequencies and electrode locations, were used to drive the cursor in one or the other direction.

Microelectrode arrays form a much more invasive physical interface, but they permit collection of spiking data from neural ensembles. To date, this is the only recording technique with the ability to reconstruct the intended movements of the subject with high accuracy. Microelectrode arrays are chronically implanted in frontoparietal areas of the brain, such as the primary motor cortex (M1), the dorsal premotor cortex (PMd), or the posterior parietal cortex (PPC). Single-unit and multiunit activities are recorded on each electrode. The activity of

each recording site must be sorted in real time for use in a BMI paradigm; units with a clearly distinguishable waveform are isolated. An instantaneous spiking frequency, or spike count for a given time bin, is derived for each cell, and then used as input by the decoding algorithm. Of great concern with implanted microelectrode arrays is their stability, both in short and long periods of time. In the short term, one must confirm, from one day to another, that the same cells are used by the decoder. This is not granted, since microelectrodes can move into the brain, leading to changes in the observed waveforms, or to a below-threshold SNR. In the long term, the brain tends to protect itself by creating a layer of scar tissue around the electrodes, leading to a slow decrease of the SNR. Thus far, studies have reported high-quality recordings up to 18 months after surgery.

Estimation and Control of Movement Parameters from Neural Data

The role of the decoding algorithm is to transform the neural activity into a control signal for the prosthetic device. The usual procedure to build a decoder is to first have the subject perform the task manually while spike activity and motor parameters are being recorded (also referred to as ‘manual control’), followed by training of the decoder with the chosen algorithm. The trained model is then used to generate predictions of motor parameters from neural data recorded online (also referred to as ‘brain control’). Various types of modeling approaches have been tested in previous studies. Among them, three different types of linear models have been widely used to predict the movement based on spike activity: the population vector algorithm, multidimensional linear regression methods such as the Wiener filter, and dynamic filters such as the Kalman filter.

The population vector algorithm used in several BMI studies was proposed by Georgopoulos and colleagues in the 1980s. They originally observed that M1 neurons were broadly tuned to hand-movement direction, noting that each neuron had a preferred direction for which it exhibited maximal firing rate. In this algorithm, the preferred directions of individual neurons are independently determined during a training phase. Then, in the online phase, the preferred directions of individual neurons are weighted by their instantaneous firing rate. The sum of the weighted preferred directions defines the population vector which estimates the current movement direction. The movement speed can be inferred from the magnitude of the population vector.

Regression models use behavioral variables, such as hand position or velocity, as a weighted linear combination of neuronal activity. The filter weights are determined using a multidimensional linear regression.

The spike count for each neuron is typically binned and regressed at several time lags, that is, for a movement prediction at time t , neural data from time t , $t-1, \dots, t-l$ will be used, where l is the number of time lags. This allows the model to capture time-delayed dependencies between neural activity and movement that exist across different cortical areas. Similar approaches using non-linear elements, such as artificial neural networks, have also proved to be efficient.

The models described thus far, can be viewed as attempts to directly approximate the mapping from neural firing rates to movement. In contrast, most models of neural encoding express the neural activity as function of a stimulus. This motivated other groups to use dynamic filtering models, where an explicit generative model of neural firing rate is used. In the Kalman filter framework, one can model the hand behavior as the system state, and the firing rate as the observation (measurement). The observation is a linear function of the state at time t , assumed to be a linear function of state at time $t-1$, plus Gaussian noise. The linear relationships between consecutive states and between state and observation are estimated from training data using least-squares estimation, thus building the Kalman model. The Kalman filter is derived from the model, allowing online estimation of the state (behavior) based on the observation (neural data). Recently, more sophisticated decoding schemes have been proposed based on hidden Markov models and Bayesian inference approaches.

Data acquired during the manual task are generally divided into two sets: a training set and a test set. The test set is used for evaluating the prediction performance of the decoding algorithms. The metric commonly used to evaluate the performance of a decoder is the squared correlation coefficient between the predicted movement and the actual movement. This performance is often referred to as the ‘offline performance’ of the decoding algorithm because it does not imply any real-time prediction of the movement, nor any feedback to the user.

Once the motor parameters are estimated, the next step in the BMI loop is to control the prosthetic device. Cortical BMI demonstrations can be divided largely into two categories: continuous control of position or velocity, or discrete control of more abstract information, such as intended targets, intended actions, and the onset of movements. The former are typically referred to as motor prosthetics, whereas the latter are referred to as communication prosthetics.

Feedback Delivery

A crucial part of a BMI system is the feedback signal that is sent back to the user so that the brain can adapt the firing rate of the recorded neurons and reduce the error in

the control signal. This is also known as ‘biofeedback’, demonstrated by Fetz and colleagues in the late 1960s. Previous BMI studies have mostly relied on visual feedback to close the loop. Whether using a synchronous or asynchronous BMI, controlling a cursor or a robot, visual feedback provides excellent information about the prosthetic device to the user, at virtually no cost. However, visual feedback may not be enough for proficient control of a prosthetic device in the long term. It is well known that peripheral tactile and proprioceptive information are fundamental for controlling our own limbs. For a neuroprosthesis to feel as a natural extension of the body, it presumably must be instrumented with sensors that can provide such information to the brain. These sensory signals could also take advantage of the BMI paradigm, and be directly delivered to the brain. Intracortical micro-stimulation (ICMS) is a method for delivering sensory stimuli to the central nervous system. Previously, ICMS work has sought to deliver artificial sensory stimuli to the somatosensory, visual, and auditory cortices. In the long term, when it comes to motor prostheses, ICMS aims to reproduce a wide range of sensory functions performed by the human limbs, such as proprioception and touch.

Discussion

BMI research has implications for both neuroengineering and systems neuroscience. In the former, neuroprosthetic systems will play a major role in restoring communication and sensorimotor function for patients suffering from spinal cord injuries and other neurological disorders. On the other hand, BMIs are also a powerful tool for studying sensorimotor learning and control, as well as cortical plasticity. In the BMI paradigm, the experimenter has full control of the motor transformation linking the neural activity to the behavior, or the sensory transformation linking a behavioral or external event to neural activity. For example, sensorimotor maps can be arbitrarily changed by the experimenter, allowing the neural adaptations to environmental changes to be studied in a controlled way.

When it comes to comparing decoder performance using different modeling approaches, it is important to realize the fundamental difference between open-loop and closed-loop BMIs. In an open-loop BMI, neural data and behavioral data are recorded, and the goal is to build a decoder that maximizes the prediction power (e.g., correlation between predicted and actual movement). The problem can be seen as a pure statistical learning issue. However, a good predictive power in an open-loop BMI does not guarantee good performance of the BMI system in closed loop. This is because the recorded neurons may behave differently when performing the BMI task as opposed to when performing the manual task. In

fact, it has been shown that the directional tuning of neurons is subject to change when switching from the manual task to the BMI task. One possible explanation for these changes in the firing properties of the cells is learning; as the subject adapts to the BMI task, the behavior of the neurons involved in the BMI task also changes. Nevertheless, a good performance of the decoder in open loop is still important because it will provide a ‘first guess’ during the closed-loop operation, where performance increases with practice.

The fact that neurons behave differently during BMI control as compared to manual control suggests that there is a ‘BMI space’ of motor control. During a closed-loop BMI experiment, the activity of the cells entirely determines the motor behavior. And conversely, to perform a given motor action, neurons must behave in a specific way. Thus, the subject must volitionally modulate the activity of the neurons to achieve the desired task. This fundamental principle for BMI was proposed by Eberhard Fetz in the 1970s.

Future Directions

Several questions concerning BMI research are currently being addressed by the scientific community. For instance, understanding the learning process during closed-loop BMI is crucial. Building a computational model of this process could help determine which type of decoder would enhance the learning process: for instance, decreasing the duration of a subject’s adaptation to the BMI task, or increasing final performance of the BMI.

Another challenge for BMI research will be to show the ability to finely control high-dimensional robotic actuators. To date, BMIs have focused on extracting kinematic data from neural signals during reaching or grasping tasks. Pure motion control, however, lacks the information required for interaction with a real environment, where the dynamic interaction of forces and torques between the robotic system and the environment play crucial roles. For instance, in the human body, the neuromuscular system naturally modulates mechanical impedance (i.e., intended inertia, damping, and stiffness) to achieve proper interaction with the environment. Ultimately, and particularly when dealing with rehabilitation prostheses, BMI systems must provide a similar degree of control when interacting with the environment. To achieve this goal, decoding algorithms may need to include modules that account for the viscoelastic properties of muscles. For example, instead of directly predicting end-point kinematics of the hand, the BMI could predict the muscle activity based on neural recordings, and then use these predictions to drive a musculoskeletal model of the arm. This will provide access to other types of variables concerning the

movement, such end-point force or stiffness. Indeed, such biomimetic algorithms constitute an elegant way to solve the decoder problem; ideally, every component of the natural motor-control loop would be modeled and implemented in the BMI system. However, while the biomimetic approach is appealing, computational issues may arise: for example, the human arm is driven by more than 30 muscles, each of which exhibits complex, non-linear behavior. Building such a large musculoskeletal model, estimating its parameters, and running it online is not a straightforward task. Software tools that specifically target such applications will be crucial for progress in the field.

As more recording technologies, decoding methods, robotic devices, and feedback schemes become available, it is important to consider BMIs as modular systems. Each one of the four essential components can be seen as a module that could potentially be replaced by another one that performs the same function (e.g., computing kinematics from neural data) but in a different way. Each module by itself can be divided into submodules to be inserted or removed as desired. This modular approach will address each issue separately, in hopes of leading to greater improvements in the field.

See also: Neural Representations of Intended Movement; Voluntary Movement: Control, Learning and Memory.

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Relevant Websites

- <http://www.bci2000.org> – BCI2000.

Brain Mapping of Language and Memory in Epilepsy

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Glossary

Cortical mapping – Invasive mapping technique that consists of the application of electrical pulses to the cortical surface during or after open surgery to reveal eloquent functional regions.

DTI (diffusion tensor imaging) – Brain mapping technique that allows noninvasive imaging of white matter tracts' organization through the depiction of microscopic movements of water molecules in the brain tissue.

fMRI (functional magnetic resonance imaging) – Brain mapping technique that highlights noninvasively the cortical areas related to specific cognitive processes during a task of interest, through the local hemodynamic response to neuronal activity.

Hemispherotomy – Surgical procedure that consists of disconnecting a whole diseased hemisphere, while leaving intact the blood supply, leading to a situation of isolated contralateral functional hemisphere.

Hemisphereectomy – Surgical procedure consisting of removing a whole diseased hemisphere.

IAT-Wada test – The intracarotid amobarbital test (IAT) was proposed by Wada and Rasmussen in the 1960s. This invasive test relies on anesthetizing one cerebral hemisphere by injecting a short-acting barbiturate while proposing specific language and memory tasks to assess hemispheric dominance in patients.

aetiology of epilepsy. Only in the early twentieth century did Jackson define a seizure as "an occasional, an excessive, and a disorderly discharge of nerve tissue on muscles," also recognizing that seizures could alter consciousness, sensation, and behavior. One century later, many brain mapping techniques have become available to explore the cognitive symptoms that epileptic adults and children encounter. Strongly settled on neuropsychology, recent studies have brought major findings to understand language and memory impairments in epilepsy. This article aims at summarizing important contribution of brain imaging in adulthood epilepsy on these two cognitive fields. Recently, similar research in childhood epilepsy has already drawn promising data to better understand abnormal cognitive development in epileptic children.

Epilepsy and Language

Scientific and medical interest for language networks in epilepsy has emerged from the challenges of surgery of pharmacological intractable epilepsy in adulthood. Meanwhile, these researches have been very useful to understand neural substrates of normal language functioning. Both electrical cortical language mapping and the intracarotid amobarbital test have been widely used to map language cortical organization in patients. They revealed that, contrary to traditional assumption, language efficiency requires integrated neural network within and beyond Broca's and Wernicke's areas. For instance, the left inferior frontal gyrus sustains both phonological skills and semantic processes, while temporal and parietal regions contribute to speech sound processing and word recognition. However, as these techniques remain invasive, magnetic resonance imaging (MRI) appears as an alternative noninvasive, reliable, and complementary technique to explore language networks organization.

It is thus with regard to the disease called sacred: it appears

to me to be in no way more divine nor more sacred than other diseases, but has a natural cause from the originates like other affections. Men regard its nature and cause as divine from ignorance and wonder, because it is not at all like to other diseases. [...] The brain is the cause of this affliction. (On the Sacred Disease, Hippocrates, 400 BCE).

It has been a long way until medical and scientific communities established the neurophysiologic

Clinical Rationale for Language Mapping in Epilepsy

While 60% of epileptic adults positively respond to anti-epileptic drugs, some are considered for surgical treatment (resection of the epileptic zone) to minimize putative risks on cognition of repeated seizures and/or life-long medications. In adulthood, early onset of seizures is associated with less pronounced left hemispheric dominance. Indeed, a larger number of epileptic patients showed bilateral activations or right dominance suggesting that repeated insults lead to substantial reorganization. As no anatomical landmark is reliably associated with potential functional reorganization, an extensive preoperative functional assessment of language is necessary to identify: (1) hemispheric dominance for language and (2) intra-hemisphere organization of specific language skills. The main clinical point is the postsurgical outcome and therefore to find reliable preoperative prognostic factors. We propose an overview of recent work that has explored these issues.

Methodological Considerations

First, it is noteworthy that language functional magnetic resonance imaging (fMRI) protocols are multiple according to different goals and contexts (e.g., cognitive question to be tested in a group of healthy subjects versus individual analysis in cognitively disabled epileptic patient to discuss the surgical procedure and prevent postoperative deficit), making generalizations inappropriate. In clinical settings, the most commonly used ‘verb generation’ paradigm requires the subject to find and generate a corresponding verb for each presented noun (usually printed in the center of a black screen). Verbal fluency has also been reported in many early studies. Of simple technical implementation, these tasks however need the patient to perform substantially. In addition, these studies require the patient to perform the task silently, to avoid head motion artifacts, but this results in increased difficulty and possible cognitive confounds. Herein, several clinical teams prefer to involve epileptic patients in less-demanding tasks. Passive listening, naming, silent repetition, and many kinds of semantic decision tasks have been proposed, with or without controlling for performance during imaging sessions. Moreover, fMRI protocols require the choice of an adequate reference condition to be contrasted with recorded fMRI signal during language functioning. Reported works alternatively used rest or control tasks involving viewed symbols or tones. These varying parameters do contribute to apparent inconsistent findings among studies. In addition, strength of the scanner magnetic field (i.e., 1.5 or 3 T), voxel and cluster sizes, preprocessing techniques (i.e., smoothing, image normalisation, and motion

correction), and statistical thresholds are all to be considered with attention. Finally, in epilepsy context, ongoing medication has to be remembered not only when looking at individual maps but also when comparing groups (patients and/or controls).

Hemispheric Dominance for Language

Early language fMRI studies have focused on ‘language laterality,’ as surgeons need to know whether the left hemisphere they have to operate on is indeed dominant for language, like in almost healthy right-handed people. As IAT-Wada test has long been the ‘gold standard’ to test for language dominance (despite strong intrinsic limitations: rough assessment over a few minutes of numerous language subprocesses), several fMRI studies have compared both approaches to validate fMRI. Calculating a laterality index (LI) (with $LI = [V_L - V_R]/[V_L + V_R] * 100$, where V_L and V_R represent the volumes of activations in left and right hemispheres), authors have demonstrated a strong concordance between fMRI and Wada test (from 67% to 100% depending on the studies). For instance, one study reported that 78% of their 50 right-handed patients were left dominant for language, whereas 16% showed bilateral language organization and 6% were right lateralized. Another work has investigated covert word generation in 100 epileptic patients. Instead of calculating LIs, the study visually inspected patterns of activations of each individual and rated the images as typically (left) or atypically (right or bilateral) lateralized. According to fMRI, 71 were left dominant and 29 showed atypical lateralization. Data suggested good concordance between the techniques since 91% of scans gave conclusions similar to that of the Wada test. It is noteworthy that most of the patients with clear lateralization (82%) showed bilateral activations, demonstrating that language substrates are bilateral even if there is greater activation in one hemisphere. Importantly, LI values differ according to the language task. Expressive tasks such as overt fluency and word generation tasks are best lateralized and usually give better concordance between fMRI and IAT-Wada test. However, these tasks predominantly involve frontal regions while the tissue to be resected is frequently located in the temporal lobe (at least the temporal pole in temporal lobe epilepsy). To elicit temporal lobe activations, one team used a reading ‘responsive’ naming task requiring the subject to name an object described by a single written phrase. In addition to inferior and middle frontal activations, the authors reported posterior temporal lobe implication in this task. Nonetheless, the lateralization power of the temporal activation during perceptive tasks is lower than that of the frontal activation during expressive tasks.

Rather than considering the whole brain for LI calculation, several authors have focused on regions of interest

(ROIs) and showed that concordance rates were higher for frontal ROIs when compared with temporoparietal ones, independently of task designs. Interestingly, an effect of the presentation modality has been suggested in one study. Indeed, the results suggested that semantic visual decision tasks gave more lateralized patterns in frontal regions than auditory tasks.

Altogether, these findings strengthen the need for multitasking fMRI protocols in the preoperative work-up of epilepsy patients in order to ascertain language dominance at the individual level. When rigorously performed and analyzed by well-trained teams, fMRI appears now as a reliable noninvasive replacement of the Wada test for the assessment of language dominance in patients.

Intra-Hemispheric Cortical Organization of Specific Language Skills

Beyond the issue of language dominance in surgical candidates is the possibility of intra-hemispheric reorganization due to epileptic seizures and/or structural lesions. Thus, the question becomes: if language dominance has not shifted from the left to the right hemisphere, has there been any functional reorganization in the surrounding tissue or in remote ipsilateral regions? Cortical language mapping is considered the ‘gold standard’ to answer that question, leading to the localization of critical areas underlying specific skills. The stimulation of a critical language area in an awake subject leads to a disruption of the corresponding function (speech arrest, paraphasias, anomia, etc.). Thus, visual naming has been demonstrated to involve posterior inferior temporal cortex, posterior superior temporal gyrus, or mid- to posterior superior temporal cortex. However, this invasive approach remains technically challenging, and the extent of the cortical surface tested is limited by operative field constraints and length of testing. When studying the putative intra-hemispheric reorganization of language neuronal substrates in adulthood epilepsy, authors found intra-hemispheric differences between patients and controls, with patients activating the basal ganglia and cerebellum more than controls. A few studies have compared intra-operative stimulations and fMRI in short series of adults, revealing that the sensitivity of fMRI varied from 38% to 100%, and the specificity from 65% to 97%. However, one must remember that the underlying mechanisms of both techniques are drastically different. While fMRI shows all neuronal networks involved in a specific task, it does not take into account the hierarchy between participating regions. An activated region, therefore, may not be functionally critical for the language task under study (i.e., it could be part of the resection, without inducing permanent language deficit). Conversely, invasive cortical stimulation shows small

areas critical to language, but does not highlight the whole underlying network. To date, those techniques thus appear complementary, with fMRI used in a first step to highlight the lateralized network, and help localize the cortical regions to be subsequently tested by stimulation.

Predicting Postsurgical Outcome

fMRI constitutes a challenging opportunity to predict postoperative outcome after seizures foci resection. Indeed, while naming is frequently impaired after surgery of the dominant hemisphere, left-temporal lobe epilepsy patients with early age at onset appear to be at lower risk to experience postoperative language decline when compared with patient with later onset. Researchers have assumed that it was a consequence of early language network reorganization (shift) in the former group. In addition, presurgical good performance in naming has been found to predict poorer outcome, suggesting that the resected tissue remained functional. Recently, scientists explored the prognostic value of fMRI in patients undergoing left anterior temporal lobectomy. Patients performed a semantic decision fMRI paradigm both before and after surgery. The authors found that the language lateralization in the temporal lobe was predictive of naming postoperative decline, that is, the stronger the left lateralization, the worst the postoperative naming performance. In a similar approach, another team recently emphasized the utility of looking at anatomical connectivity lateralization in addition to functional asymmetry. Using diffusion tensor imaging (DTI), they observed that dominant anterior temporal lobe epilepsy patients had less dominant and greater nondominant intra-hemispheric connections when compared with controls. Moreover, they found a negative correlation between the lateralization of connections and the postoperative naming performance. That is, the patients with more extensive connections on the resected dominant side had the greatest language decline. Even though encouraging, more imaging studies are needed to understand postsurgical risks on language abilities in adulthood epilepsy.

Neurodevelopmental Plasticity

Nowadays, surgery is more frequently discussed in cases of young patients with drug-resistant epilepsies, because epileptic insults have a strong negative impact on neurological and neuropsychological development. On the other hand, the peculiar plasticity of the immature brain may limit these alterations. Several studies have been conducted in pediatric patients with severe epilepsy using pre- and/or postoperative language fMRI. In 1997, Hertz-Pannier and colleagues reported a first-language

fMRI study involving 11 children and adolescents with intractable complex partial seizures. The functional paradigm consisted of silent word generation beginning by a given letter or belonging to a given semantic category. Beyond the great concordance between IAT and fMRI in this population, the authors showed that functional MRI could be used in pediatric patients with good compliance in order to assess language dominance noninvasively.

Several surgical teams have proposed either hemispherotomy or hemispherectomy techniques to control for severe infantile epilepsy syndromes that involved a whole hemisphere. However, one may wonder how people could speak with a single cerebral hemisphere. Using a similar fMRI approach, Hertz-Pannier and colleagues examined the postsurgical mechanisms of neurodevelopmental plasticity in a young boy with intractable left seizures in the context of Rasmussen's encephalitis. This boy had developed normal language abilities before the onset of seizures. The presurgical fMRI investigation (using semantic verbal fluency) when he was 6 years and 10 months old revealed clear left lateralization of language functions. After left hemispherotomy at the age of 9 years, the boy experienced profound aphasia and alexia. Language outcome was spectacular with good recovery of receptive language and slower but almost complete recovery of expressive language and reading at 18 months postoperatively. Postoperative fMRI conducted at the age of 10 years 6 months revealed a shift of language-related network to the contralateral healthy hemisphere for both receptive and expressive tasks (word and sentence generation, story listening), in right regions homologous to the previously left activated areas (inferior frontal, temporal, and parietal cortex). Importantly, these right regions had not been found activated preoperatively. This unique study demonstrates the possible reorganization within preexisting bilateral but asymmetric language network in childhood. Furthermore, these findings argue in favor of the possible relatively late disconnection of the dominant hemisphere in cases of intractable epilepsies (earlier studies had proposed the age of 6 years as the limit). Recently, scientists reported the importance of specific regional activations for language recovery. Studying six hemispherectomy adolescents and young adults, they demonstrated that activations within Broca's area and its right homologous differed from those of normal controls. Patients with best outcome showed activation of both pars triangularis and orbitalis of Broca's area, whereas pars opercularis alone seemed unable to sustain efficient language. The authors suggested that distinct subregions within Broca's area and their right homologs subserve language, and that their variable activation may determine functional outcome.

Further fMRI studies are however needed to extend such findings to larger samples through early and late childhood.

Epilepsy and Memory

Historically, research on memory impairments in epilepsy has marked an important step with the famous case of patient HM, in whom bilateral amygdalo-hippocampectomy for intractable epilepsy had led to profound amnesia. Indeed, bilateral temporal damage may disrupt memory function and more specifically episodic memory. Fortunately, both improved patient selection and understanding of memory organization with the help of advances in functional imaging nowadays contribute to avoid such clinical outcome. Henceforth, it is well established that the hippocampus is involved in both novelty detection and multimodal information association. It is supposed to differentially sustain episodic encoding and retrieval according to its parts (anterior for encoding and posterior for retrieval). Several studies of healthy subjects demonstrated that hemispheric memory dominance differed with the type of encoded information: words preferentially activate the left hippocampus, faces and objects the right hippocampus and visual complex scenes activate both left and right hippocampi. Otherwise, frontal lobes also have an important implication in episodic memory processes and hemispheric specialization has been suggested, with the left frontal lobe rather sustaining episodic encoding, and the right one rather involved in retrieval.

In this article, we first report functional imaging studies that outlined hemispheric dominance for episodic memory in adulthood epilepsy. To date, published research has mainly been conducted in temporal lobe epilepsy. Subsequently, increasing literature has revealed possible alterations in specific skills within episodic memory functions. Therefore, we summarize those up-to-date studies that rely on structural mapping and neuropsychological investigations. Finally, we mention future directions for fMRI episodic memory research in childhood epilepsy.

Hemispheric Lateralization of Memory in Adulthood Epilepsy

Increasing literature is currently published concerning episodic memory substrates in adulthood epilepsy. Similarly to language functions, these studies principally aim at demonstrating whether fMRI could reliably replace the IAT-Wada test in assessing patient's hemispheric dominance for memory. During encoding of a verbal memory task, researchers found a lower activation in the left parahippocampal gyrus contrasting with a stronger left prefrontal activation in left temporal epilepsy patients when compared with healthy controls. A few years later, scientists designed an fMRI paradigm with encoding of complex visual scenes, faces, and

abstract drawings. They found asymmetrical hippocampal activations with good concordance with the IAT-Wada test. However, one team found more discordant results with a good agreement between both techniques only in patients with right temporal epilepsy. Recently, this team has examined the differential effects on memory lateralization of left versus right seizures and studied the duration of epilepsy as a possible factor of cerebral plasticity. Using a scene-encoding paradigm, the team found right-lateralized activation in the left onset epilepsy group. By contrast, right temporal epilepsy patients did not show significant increase in the degree of lateralization, suggesting a differential influence of left and right insults on memory neural reorganization. The authors also demonstrated a strong relationship between the degree of memory lateralization and mnemonic performances. Indeed, left epileptic patients performed more poorly than right ones, but greater left lateralization was associated with higher scores in both groups. Their findings strengthened previous results showing that left hippocampal lateralization was associated with better scores of verbal memory, whereas right lateralization was correlated to better nonverbal memory performance. However, neither age at onset nor duration of epilepsy was found related to the laterality indices.

Recently, researchers examined the relationship between the lateralization of language and memory-related activations. Left and right onset epilepsy patients performed an fMRI encoding and retrieval of word-pair associates, and the authors analyzed memory-related activation patterns of the hippocampi, as well as inferior frontal and temporolateral areas, which are linked to language functions. Similarly to the study mentioned above, they found more pronounced right lateralization of hippocampal activation in left epilepsy patients than in right ones. No such group differences were detected in frontal and temporolateral areas, suggesting that patients can show a shift of memory functions without substantial reorganization of language functions. However, this work did not use a specific fMRI paradigm to investigate language dominance. Future research may benefit from combining memory and language functional mapping.

Predicting Postsurgical Outcome

Even though the risk of memory decline after surgery on the temporal lobe is of major concern, few studies have been published. One study, which used presurgical activations during scene encoding to predict patients' postoperative performance, found that asymmetrical activation in the hippocampal, parahippocampal, and fusiform gyri was correlated to postsurgical outcome, that is, lower activation in the epileptic temporal lobe was associated with a smaller change (either decline or improvement) in memory performance postoperatively. Recently, researchers assessed the

preoperative value of fMRI to predict modality-related memory impairments following either left or right anterior temporal lobe resection. Main data revealed that patients with greater activation ipsilateral to the seizure focus compared to the contralateral side experienced greater memory decline after surgery, regardless of modality. That is, patients with more left lateralized presurgical activations had more difficulties in the verbal modality. Reciprocal results were obtained regarding the nonverbal modality. Interestingly, the authors did not detect significant correlations between hippocampal volume and memory decline (while controlling for side and modality). Such findings question both the relationship between functional and structural aspects of the neural substrates of episodic memory and the reliability of structural analyses to predict surgical outcome.

Recent Studies on Episodic Memory in Epilepsy

Several architectural models are now available to understand human memory functioning in adulthood, and more specifically, encoding, long-term consolidation, and retrieval of episodic information. Among available theories of episodic memory is Tulving's conception, in which episodic memory refers to "memory for personally experienced events" and especially includes the "autonoetic awareness of one's experiences in the continuity of subjectively apprehended time that extends both backward into the past [...] and forward into the future." The studies mentioned above did not integrate these crucial aspects in their episodic memory assessments. To date, only two brain mapping studies have been conducted in adulthood epilepsy to understand specific memory impairments concerning autobiographical memory or recollection as opposed to familiarity retrieval. The first study has measured the volumes of medial temporal lobe structures (e.g., hippocampus and parahippocampal cortices) and correlated them to memory performance. The authors showed that, when compared with controls, patients with left and right medial temporal lobe resection were impaired in retrieving personal past events and justifying them with spatial and temporal phenomenological details. These impairments concerned all periods of patients' life. The correlations between medial temporal lobe structures volumes and memory scores suggested that these structures are involved in reliving the episodic context of personal past events (i.e., recollection). The second study reported a specific memory retrieval profile in a woman who underwent surgical resection of left anterior temporal lobe structures for the relief of epileptic seizures. The resection spared the hippocampus, but included the parahippocampal cortex. Four different experiments comprising recollection-based versus familiarity-based retrieval showed that she performed normally in recognition tasks and provided much recollection-based answers

but that she was impaired when her answers were based on familiarity. Altogether, these findings outline the functional specialization of medial temporal lobe structures (i.e., hippocampus for recollection and context retrieval; parahippocampal gyrus for familiarity). Furthermore, the findings emphasize the need for developing such approaches using fMRI in order to better understand the everyday-life memory troubles of patients with various epilepsies and mnemonic complaints.

Future Directions to Assess Episodic Memory Impairments in Childhood Epilepsy

Due to ongoing deleterious processes of epileptic seizures on the immature brain, it has long been argued that epilepsy leads to global or undifferentiated cognitive deterioration. In the past 15 years however, it has been recognized that childhood epilepsy, and more peculiarly temporal lobe epilepsy, can cause specific memory impairment in otherwise normally developing children. Therefore, the putative reliability and validity of fMRI to assess neural memory networks in drug-resistant epileptic children is a recent preoccupation. This may partially explain nonexistent fMRI reports on episodic memory in childhood epilepsy in the scientific literature to date. However, several neuropsychological and methodological limitations also have to be considered. First, little is currently known about normally developing neural networks of episodic memory and the scarce recent findings need to be replicated. Second, involving children in episodic memory fMRI paradigms requires the child to understand complex and effortful task demands. Moreover, fMRI investigation of episodic memory requires good compliance, and minimal head motion, which can be difficult to obtain from epileptic children, notably if they experience additional slowness, attention, or intellectual deficiency. Furthermore, both number of stimuli and time duration in the magnet remain an important problem, as appropriate detection of local hemodynamic response constrains the experimenters to multiply stimulus presentation to reach statistical significance of activations (especially in medial temporal lobes).

Consequently, it is necessary to develop playful and motivating paradigms (i.e., no lists of long words but rather colored images), in concordance with theoretical knowledge about behavioral memory development (in terms of quantity of information, time processing, and attention for instance) and global intellectual efficiency at a given age in order to better understand episodic memory development from early childhood to late adolescence. Then, researchers would be able to determine specific memory reorganization in childhood epilepsy, taking cerebral development and onset and frequency of epileptic seizures and medication into account.

Conclusion

Brain mapping in epilepsy constitutes a robust fashion both to study complex cognitive skills, such as language and memory (and better understand their normal functioning), and to grasp the dynamic time course of epileptic seizures' impact on neural plasticity. Strongly related to the clinical issue of pre-/postsurgical outcomes of patients suffering from drug-resistant syndromes, these techniques bring complementary data to map the cortical networks in individuals (cortical mapping and fMRI; fMRI of language and memory). From now onwards, coupling imaging techniques and sophisticated neuropsychological designs may improve our knowledge concerning brain–behavior relationships during brain development and in adulthood.

See also: Brain Imaging; Development and Language; Hemispheric Specialization: Language, Space, and Sexual Differentiation; Memory Consolidation; Temporal Lobe and Object Recognition.

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C

Cardiovascular Conditioning: Neural Substrates

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Glossary

Arterial blood pressure – The arterial blood pressure maintained in the circulatory system. Mean arterial blood pressure ranges from 80 to 110 mmHg in C57BL/6J and 6N mice when recorded throughout the circadian cycle under stress-free conditions at constant room temperature (21°C) in the home cage. Peak values during fear memory recall reach ~140 mmHg in mice.

Autonomic nervous system (ANS) – Part of the peripheral (visceral) nervous system that is subdivided into the parasympathetic (PNS) and the sympathetic nervous system (SNS).

Bradycardia – The decrease of heart rate with regard to reference (baseline) values.

Conditioned contextual fear – Fear elicited by an array of various specific sensory stimuli (e.g., background noise, illumination, smell, and surface texture such as the shock grid) that remind of a particular environment, where fear was previously experienced.

Conditioned cued fear – Fear elicited by an explicit sensory cue such as light or a tone stimulus.

Conditioned stimulus (CS) – The initially neutral stimulus that elicits the conditioned response after the association of the CS with the unconditioned stimulus (US) has been made.

Heart rate – The rate (frequency) at which the heart beats as generally determined as average value across a defined time interval or assessed instantaneously, for example, based on the time interval between successive RR waves of the ECG complex. The maximum physiological range of heart rate is 400–800 beats per min (bpm) at constant room temperature (21°C) in C57BL/6J and 6N mice.

Radio-telemetry – Technique for remote (wireless) recording of various measures and used to monitor arterial blood pressure and ECG in man and mouse.

Tachycardia – The increase of heart rate with regard to reference (baseline) values.

Unconditioned stimulus (US) – The sensory stimulus that serves as either negative or positive reinforcer to form the association with the future conditioned stimulus (CS).

Introduction

General Introduction

Since the fundamental studies by Ivan Petrovich Pavlov on classical conditioning, a form of associative learning, especially eyeblink and fear conditioning, is extensively used to investigate the neural and molecular substrates of learning and memory. Fear conditioning results in the establishment of nonspecific emotional responses acquired after brief training. These emotional responses are also elicited by innate fear. By contrast, eyeblink responses are specific somatic motor responses that require extensive training, because they are not easily acquired. The gradual development of these eyeblink responses along many trials is preceded by cardiovascular adjustments.

Fear conditioning is a behavior test in which the conditioned stimulus (CS), an initially neutral sensory stimulus such as tone, light, or a specific environment (context), comes to evoke a fear response (conditioned response; CR) because it was previously paired with a noxious unconditioned stimulus (US), commonly an electric foot shock, that elicited the unconditioned response (UR). Based on associative learning, the CR will be elicited by the CS in the absence of any adverse stimulation as predictor of the US. Thus, the CS serves as psychological stressor evoking fear. Since dysregulation of fear pathways is thought to underlie affective disorders, the role of brain areas in the control of emotional responses, ranging from fear learning via its expression to reconsolidation and extinction of fearful memories, has recently gained enormous interest. Experiments are predominantly performed using behavioral readouts with a focus

on freezing, a species-specific defensive response in rodents that occurs as active suppression of ongoing behavior, an evolutionary adaptation to avoid visual attraction of potential predators. As independent concomitant measure to behavioral readouts, cardiovascular readouts (heart rate and blood pressure) have gained considerable interest to complement and improve our understanding of the neural circuitry involved in different phases of emotional learning and memory across different species, and to identify mechanisms by which affective disorders contribute to elevated cardiovascular risk. Comorbidity of cardiovascular and affective disorders is well characterized and cardiac risk is increased under conditions of emotional challenges such as psychological stress exploited by fear conditioning. Thus, cardiovascular responses, a subset of autonomic adjustments, serve as important physiological parameters with high temporal dynamics. The understanding of the brain–heart interaction with regard to basal cardiovascular function and stimulus-induced adjustments has become an area of research generally referred to as *Neurocardiology* with investigations employing CNS manipulations and its effects on autonomic function (neurovisceral integration), including learning-induced plastic changes. This research heavily depends on animal models for specific pharmacological and/or genetic interventions. Recent noninvasive human studies have focused on imaging techniques in combination with cardiovascular measurements and emotional tests, including fear conditioning.

Emotion and the Cardiovascular System

The cardiovascular system is crucially involved in the preparation for fight and flight – a concept introduced by Walter Cannon. Based on environmental challenges such as predatory threats, organisms need to adjust their physiological state by increasing metabolic supplies to prepare for the necessary anticipatory response to promote survival. This response is under high evolutionary selection pressure, and thus can be investigated across a wide range of animal species. In mammals, physiological adjustments, mediated by the autonomic nervous system, provide for valuable readouts indicative of changes of the emotional state in the absence of, for example, properly quantifiable behavioral measures. In general, an increased cardiac output is achieved by elevating heart rate and blood pressure via reduction of tonic parasympathetic (PNS) and activation of sympathetic nervous system (SNS) activity. Elevated cardiac output is achieved not only through heart rate increase but also through concomitant decrease in atrioventricular (AV) node conduction delay of cardiac excitation and increased contractile force. In addition, the peripheral blood flow is altered with reduced supply to intestinal organs and increased blood flow to the musculature and brain for an

optimal defense reaction. In view of the strong effect of aversive stimuli on cardiovascular adjustments, it is not surprising that the focus of research has been on aversive forms of URs (learning-independent; innate fear or anxiety responses) and CRs (associative learning-induced; based on fear conditioning). Experiments on appetitive conditioning have been performed in rats, monkeys, and dogs, and, if at all, the magnitude of generally observed tachycardia is relatively small when compared with that observed in aversive conditioning. In general, the cardiovascular effects are blocked by peripherally acting β -adrenergic antagonists, thereby indicating regulatory effects mediated by the activation of the SNS. For that reason the focus of this article will be on neural substrates underlying aversive conditioning predominantly based on research in rodents.

Methods and Limitations

While straightforward methods exist to measure cardiovascular parameters in humans, the outcome of animal studies crucially depends on the experimental conditions. Unspecific stressors influence experiments in animals even more than in humans. Examples of stressors are restraining, recording conditions using externally placed electrodes, and interference by the experimenter. Blood pressure methods under semi-restrained conditions using tail-cuff systems in rodents are essentially inadequate to determine physiologically relevant cardiovascular adjustments in a defined behavioral setting. With the development of implantable miniaturized radio-telemetry techniques (Figure 1), remote recording of cardiovascular parameters in freely moving mice and rats has become a standard method. A major disadvantage of radio-telemetry is the considerable surgical demand followed by sometimes long-lasting recovery periods that are necessary for full recovery of small animal such as mice, before actual experiments can be performed.

The invaluable advantage of the radio-telemetry approach is that physiological measures can be obtained even under experimental conditions with restricted possibilities for behavioral assessment such as experiments in the home cage in which all unspecific stress can be avoided. In this way, valid baseline data can be obtained that are needed for the assessment of autonomic adjustments under physiological conditions. Environmental challenges such as novelty exposure are emotionally demanding for mice, and easily elevate baseline heart rate. Based on elevated heart rate values, physiological response magnitudes may be diminished when elevations are expected because species-specific maximum physiological levels exist (~ 800 bpm in mice). Another complicating issue in cardiovascular assessment is that physical activity contributes to autonomic changes paralleling effects that are generally observed during aversive

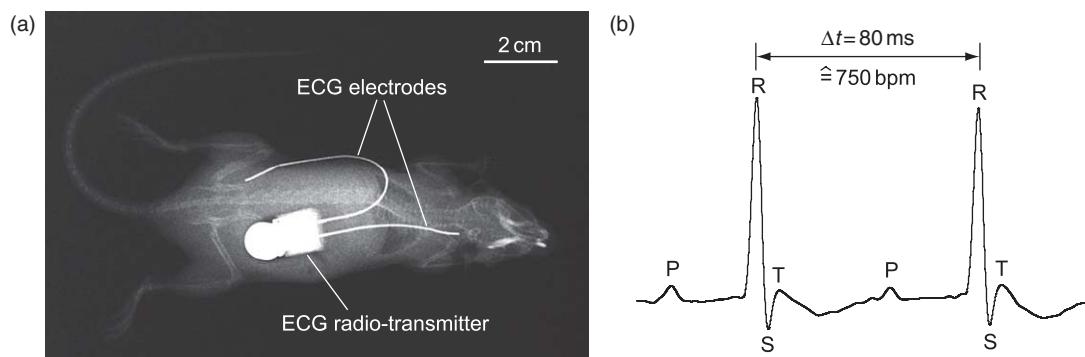


Figure 1 X-ray image (dorsal view) of a mouse with intraperitoneally implanted ECG radio-transmitter and subcutaneously placed electrodes positioned for a type II ECG recording (a) and optimal ECG signal of the same mouse (b) with the conversion of the RR interval of the ECG complex (with individual wave forms P-T) into heart rate (given in beats per min (bpm)).

emotional challenges. Therefore, it is crucial to determine whether autonomic effects are attributable to fear or anxiety, or simply to increased locomotor activity.

Cardiovascular measures are predominantly presented as time-averaged values reporting an increased or decreased heart rate and/or blood pressure without specific information on the underlying short-term dynamics. The dynamical features especially of heart rate variability (beat-by-beat variation) provide useful information on the interdependent activity, the sympathovagal balance of the sympathetic and parasympathetic subdivisions of the autonomic nervous system. Heart rate variability serves as important index of pathological changes and is used to diagnose cardiovascular risk in man. Studies that have focused on relative heart rate and/or blood pressure changes as opposed to absolute values often ignored the impact of interventions on basal physiological function. Thus, future research carefully needs to address the effects of local interventions on baseline function using sensitive and physiologically relevant (e.g., nonlinear) measures since small cardiovascular changes may have statistical significance, but their physiological relevance is limited. Nonlinear measures identified an important contribution of tonic parasympathetic activity to long-range correlation of heartbeats in their temporal sequence indicating the central role of the central nervous system to the dynamical features. Nonlinear (scale-invariant) measures have superior sensitivity in forecasting cardiac risk states in man in the absence of electrocardiogram (ECG) alterations and allow for translation of results across different mammalian species.

Conditioning-Specific Cardiovascular Responses in Different Species

Differences in cardiovascular fear responses exist in various species beyond the well-described allometric relation between body size and heart rate that are linked

to absolute heart rate values in mammals and birds, that is, the larger the animal the lower its basal heart rate. Ambient temperature has a strong impact on baseline heart rate in mammals, since ambient temperature affects the metabolic needs to maintain body temperature. These effects are especially prominent in small animals such as mice with a relatively high baseline heart rate due to higher metabolic needs to maintain body temperature as a consequence of their large surface area to volume ratio compared with larger animals. The closer the ambient temperature is to that of the thermoneutral zone of a species, the lower the baseline heart rate will be due to reduced metabolic activity to sustain body temperature, and the larger stimulus-induced adjustments may become.

In humans, rats, and mice, generally tachycardia and blood pressure increase are common responses to a conditioned or unconditioned stressor. Interestingly, the dynamical properties of heart rate and blood pressure adjustments in mice (fast and slow, respectively) are opposite to that reported in rats, where fast blood pressure increase and slower increase of heart rate occur. The conditioned fear-induced tachycardia in mice occurs after a single tone-shock presentation when CS (tone) and US (foot shock) are presented during training in the delay mode, that is, CS offset coincides with US onset. In mice the beat-by-beat fluctuation of heart rate illustrates the highly dynamical properties of this physiological function: extremely fast heart rate changes (half-time $t_{1/2} \sim 3 \text{ s}$) and relatively large response magnitudes occur, while the concomitant blood pressure increase is considerably slower (half-time $t_{1/2} \sim 52 \text{ s}$) when mice are re-exposed to the tone-CS in the home cage 24 h after training (Figure 2). The heart rate response is thus characterized by high heart rate variability under baseline stress-free conditions (pre-CS phase). When heart rate is increased to maximum level during CS (tone) presentation, the variability is substantially reduced (Figure 2(a)). Thus, heart rate and its

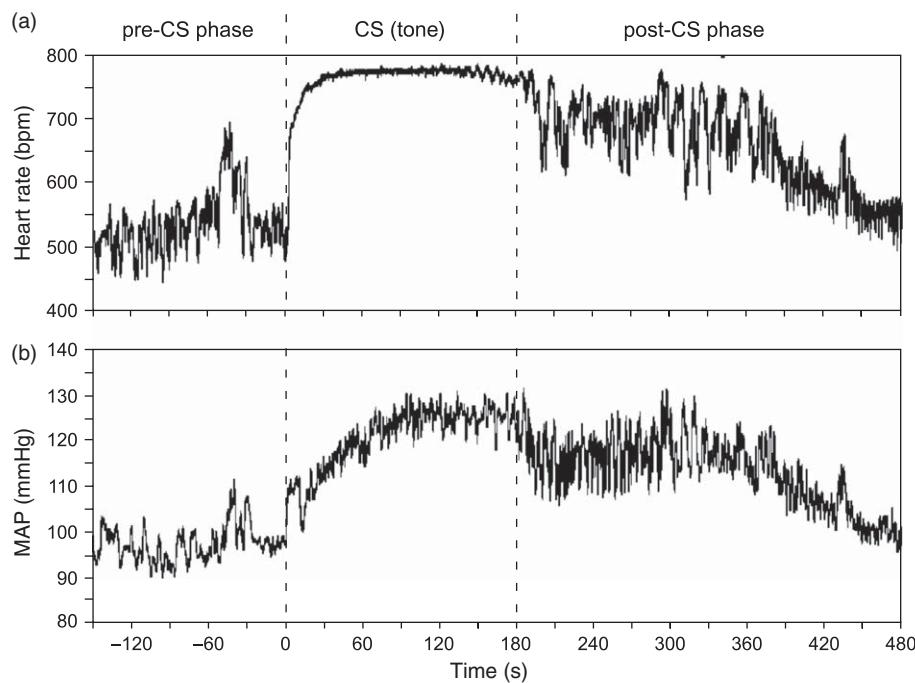


Figure 2 Typical heart rate (a) and mean arterial blood pressure (MAP) responses (b) during expression of fear conditioned to an auditory cue in a C57BL/6N mouse. Heart rate is characterized by high variability under baseline stress-free conditions (pre-CS phase) that is substantially reduced during CS (tone) presentation, since heart rate and its variability are inversely related. The temporal dynamics of conditioned fear-induced adjustments are fast for heart rate and relatively slow for blood pressure. The apparently increased noise in the MAP values during the first part of the post-CS phase is due to moving artifacts that have an impact on the blood pressure waveform recorded by a blood pressure radio-transmitter with the pressure catheter placed in the abdominal aorta. The tone-CS (180 s duration) was presented in the home cage 24 h after training with six paired tone-CS/US presentations.

variability are inversely related. The conditioned tachycardia occurs only under specific training conditions demonstrating its dependency of the association of the CS with the US on its contingency relation (Figure 3). The relatively mild and transient heart rate increase (~ 60 bpm) at tone onset in mice that did not form an aversive association has been interpreted to reflect attention processes (Figure 3).

The conditioned tachycardia acquired after a single-paired CS-US presentation (delay conditioning) during training is prevented by systemic injection of the protein synthesis inhibitor cycloheximide (Figure 4(a)) indicating that memory consolidation, needed to establish long-term memory 24 h after training, is impaired. Furthermore, nonreinforced re-exposure to the CS (tone) once daily in the home cage leads to a gradual extinction of the conditioned tachycardia (Figures 4(b) and 4(c)).

Rabbits display fear-induced heart rate decrease (bradycardia, as an adaptive strategy when playing dead (Thanatosis) during ultimate predatory threat) and blood pressure fall, indicating species-specific differences in the dynamical properties of various conditioned cardiovascular responses. Similarly, a tone-CS-induced profound bradycardia is observed in bats. The bradycardia response

in rabbits is relatively low in magnitude but allows one to record dynamical changes with regard to transient adjustments to repeated CS presentation at high rate.

Central Autonomic Network

The central autonomic network (CAN) has been characterized in considerable detail using various anatomical/histological techniques. It is not surprising that the CAN has considerable overlap with the central fear pathways. Various labeling techniques identified the CAN from brainstem to forebrain areas that are involved in direct and indirect regulation of the autorhythmicity of the primary and secondary pacemaker centers in the heart, the sinus node, and the AV node, respectively. A simplified schematic overview of the CAN and its peripheral action on the cardiovascular system is presented in Figure 5. In the following sections the effects of local interventions on the function of individual brain areas of the CAN will be described. The CAN will be followed from cortical areas via efferent connections to areas in the lower brainstem and their effects on conditioned cardiovascular responses will be described. Effects on unconditioned (innate) cardiovascular responses will be

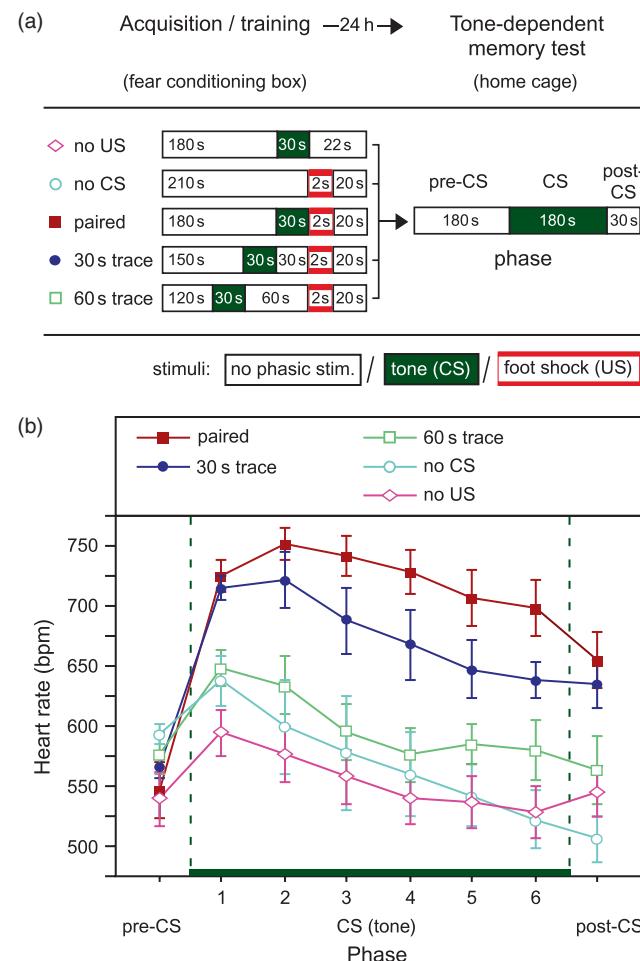


Figure 3 Training sequences (a) and averaged heart rate responses of C57BL/6N mice in the retention test (b) during re-exposure to the tone as a function of the training sequence. Mice were subjected to five different training sequences (no US, no CS, paired, 30 s trace, and 60 s trace) and 24 h later re-exposed to the tone in the home cage (a). A strong tachycardia was observed in mice only if they were trained with CS and US presented in a paired or at 30 s trace interval, whereas the three other groups responded with a mild and transient tachycardia that is interpreted to reflect an attention response (b). Mice that show the strong tachycardia also exhibit conditioned freezing responses to the tone-CS (data not shown). Modified with permission from a publication of Neuroscience and Biobehavioral with reviews from Elsevier.

included to differentiate the specific contribution of these brain areas to innate versus learned cardiovascular adjustments.

Forebrain Areas Involved in Conditioned Cardiovascular Adjustments

Higher cognitive centers are involved in the processing of environmental information such as conditioned stimuli that trigger cardiovascular adjustments via efferent outputs. These adjustments alter the tonus of both the sympathetic and parasympathetic branches of the autonomic nervous system. In the following section we discuss the specific brain areas involved in the adjustments of cardiovascular responses elicited by conditioned fear by altering basal function in preparation to cope with a potential threat.

Prefrontal cortex

The prefrontal cortex (PFC) plays an important role in executive functions, including decision making and stress controllability. Thus, the PFC is involved in behavioral flexibility and in extinction of fear responses. Of particular interest (from the viewpoint of autonomic involvement) is the ventromedial portion of the PFC in humans and the medial PFC in rodents. The medial PFC is comprised of the anterior cingulate, prelimbic, and infralimbic cortices. Anterior cingulate lesions attenuated conditioned heart rate decelerations in comparison to posterior cingulate or sham lesions, but enhanced the magnitude of the bradycardiac component of the orienting reflex in rabbits. Electrical stimulation revealed effective sites for eliciting heart rate and blood pressure changes in rabbits only in the anterior cingulate cortex. Here, relatively large (70–100 bpm) heart rate decelerations were accompanied by relatively small (1–5 mmHg)

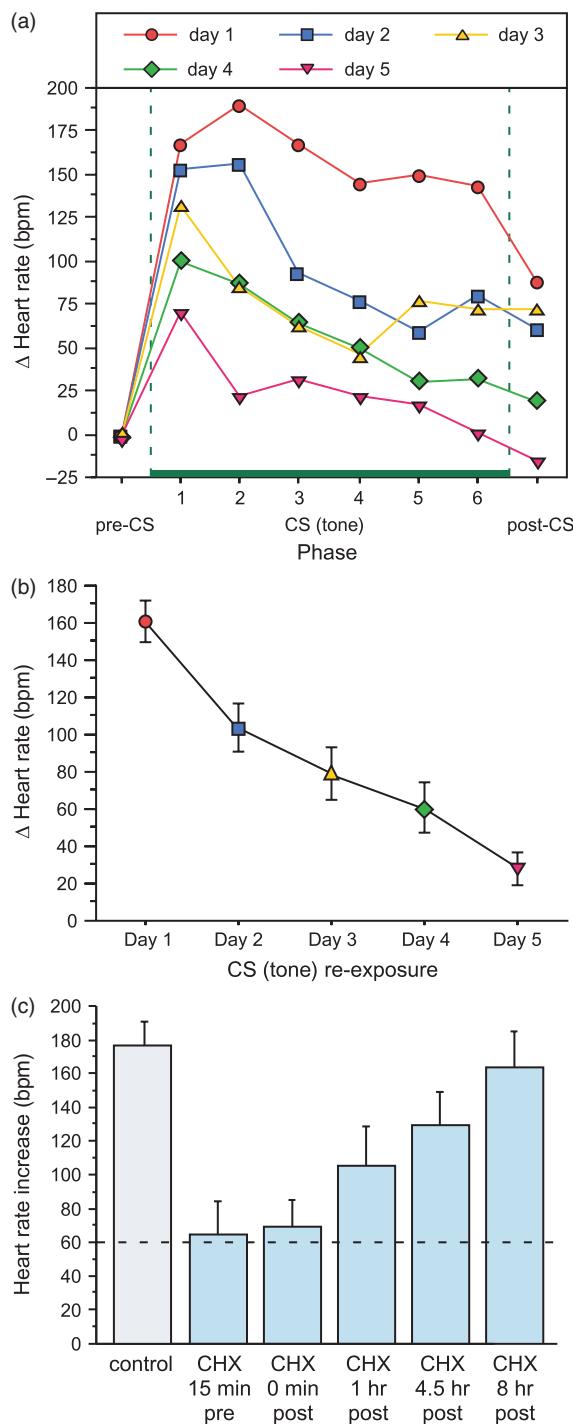


Figure 4 Effects of extinction and protein synthesis inhibition on heart rate responses as index of fear conditioned to an auditory cue. Repeated (daily) nonreinforced re-exposure to the 180-s tone serving as CS in C57BL/6N mice causes a decrease of the conditioned tachycardia along six consecutive 30-s intervals (a) and the grand means of the 180-s tone-CS period shown in (b). Impairment of consolidation processes by the protein synthesis inhibitor cycloheximide (CHX) attenuates the tone-CS induced tachycardia to the heart rate increase (~60 bpm) observed in nonconditioned mice (c). Pre and post refer to the timing of drug injection with regard to training. Modified with permission from a publication of Neuroscience and Biobehavioral with reviews from Elsevier.

blood pressure changes. Increase of regional blood flow occurs in the right anterior cingulate cortex during emotional challenge and covaries with mean arterial blood pressure in human subjects. The infralimbic part of the PFC projects to various brain areas that are implicated in autonomic regulation. Lesions and transient inactivation of the medial PFC before training reduce conditioned freezing, tachycardia, and blood pressure in various species from rats to rabbits when they are re-exposed to both conditioned explicit (tone) and contextual cues. Transient inactivation studies suggest that the medial PFC plays a role in encoding and/or consolidation of contextual fear conditioning. Moreover, experiments with pretest inactivation of the PFC indicated a role of the ventral (infralimbic) portion of the medial PFC in expression of cardiovascular fear response. However, the effects were not specific to CRs because reduced cardiovascular responses were also observed to unconditioned emotional stimuli suggesting a more general role in the expression of aversive emotional responses. Stimulation of the medial PFC neurons elicits cardiovascular effects as observed in conditioning experiments. Pharmacological interventions investigating the role of sympathetic and parasympathetic outflow showed that the medial PFC predominantly controls the sympathetic nervous system. This view is consistent with an increased selective attention and elevated sympathetic activity upon magnetic stimulation of the PFC in humans presented with emotionally challenging pictures such as angry faces.

Insular cortex

The insular cortex is part of the CAN. When stimulated by implanted electrodes in the anterior and posterior insular cortex, bradycardia was observed in rabbits. Lesioning of these areas, however, did not abolish but only mildly attenuated the conditioned bradycardia in rabbits. The role of this brain in conditioned fear responses in other species requires further investigation.

Hippocampus

Evidence exists for a role of the hippocampus in conditioned cardiovascular responses indicative of conditioned contextual fear. CA1 neurons of the hippocampus project predominantly to prelimbic and infralimbic areas of the PFC. The ventral hippocampus projects to the amygdala and is involved in emotional memory and anxiety. Post-training and pretest inhibition of dorsal hippocampal function decreased cardiovascular fear responses. Additionally, dorsohippocampal lesions abolished bradycardic responses in rabbits during conditioning only when CS and US were presented in a trace but not delay mode. This finding is consistent with the role of the dorsal hippocampus for the encoding of fear memory to explicit cues in trace conditioning experiments.

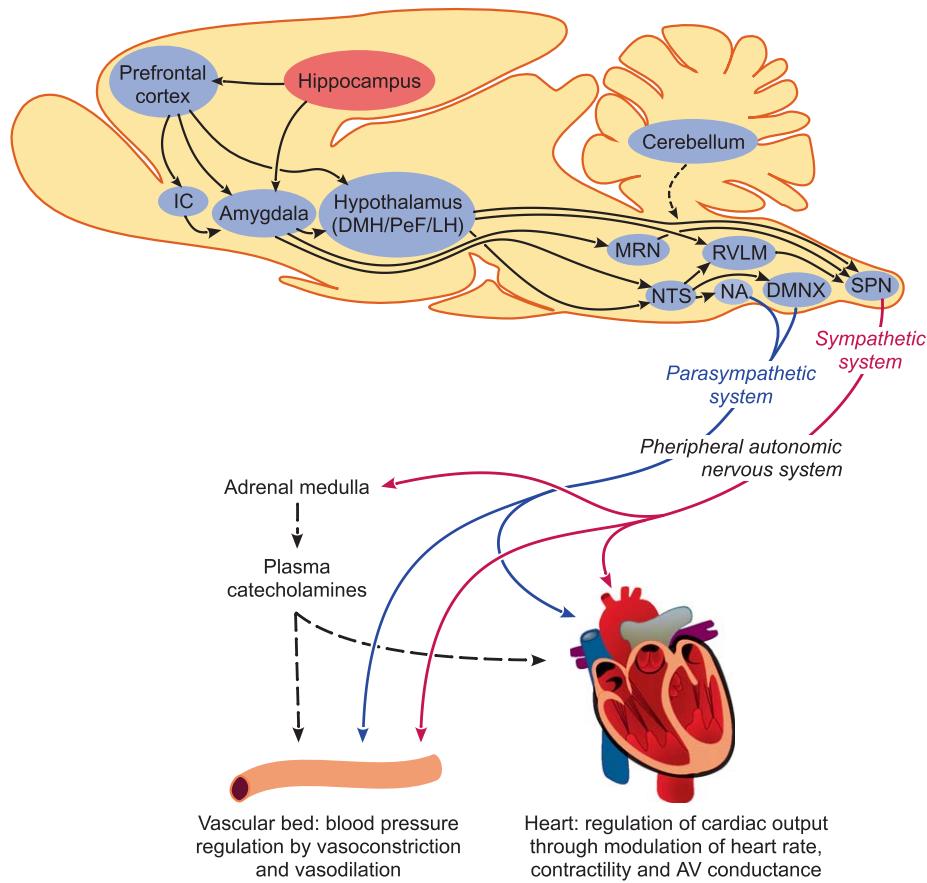


Figure 5 Simplified schematic diagram of prominent central autonomic pathways involved in conditioned cardiovascular adjustments. Blue ellipses denote brain areas that are part of the central autonomic network, whereas the hippocampus (red ellipse) is implicated in cognitive modulation of the central autonomic network depicted on the sagittal section of a rodent brain. The thin blue line from the parasympathetic system to the vascular bed indicates its low contribution to vasoconstriction and -dilation. Besides fast neural regulation via central and peripheral autonomic pathways, slower stress hormone release (catecholamines from the adrenal medulla) also contributes to cardiovascular regulation. AV: atrioventricular; DMNX: dorsal motor nucleus of the nervus vagus; DMH/PeF/LH: dorsomedial/perifornical lateral hypothalamus; IC: insular cortex; MRN: median raphe nucleus; NA: nucleus ambiguus; NTS: nucleus tractus solitarius; RVLM: rostral ventrolateral medulla; SPN: sympathetic preganglionic neurons of the spinal cord.

Amygdala and extended amygdala

The amygdala is an assembly of functionally different subnuclei. Information processing of an auditory cue such as tone that serves as CS requires auditory afferents projecting to the thalamus and, depending on the complexity of e.g. discriminatory learning, also auditory cortex function. From the thalamus and the auditory cortex information is then relayed to the amygdala with the basolateral amygdala (BLA) important for CS-US association and thereafter to the central nucleus (CNA) for fear expression.

In rats with lesioned BLA the normally observed heart rate responses to a tone-CS were absent when tested 1 and 24 h after training, while CS-induced heart rate effects were still observed during training suggesting an important role of the BLA for learned rather than innate fear responses. However, the BLA is under tonic gamma aminobutyric acid (GABA)ergic inhibition. Removal of this inhibition by local antagonist injection (dysinhibition)

increases anxiety- or panic-like behavior and elicits cardiovascular activation in rats.

The medial nucleus of the amygdala receives input from the main and accessory olfactory bulb and is involved in cardiovascular responses to innate fear responses, for example, those triggered by olfactory cues such as the fox feces compound 2,5-dihydro-2,4,5-trimethyl-thiazolidin (TMT) in rats (sustained blood pressure increase). Similarly, alarm pheromones (detected in the Grüneberg ganglion) trigger innate freezing responses in mice and are also expected to elicit cardiovascular responses indicative of fear.

Electrolytic lesions of the CNA inhibit the conditioned bradycardia in eyeblink conditioning in rabbits and both the conditioned cardiovascular social stress responses and the conditioned fear-induced blood pressure increase in rats. Stimulation of CNA neurons through chronically implanted electrodes elicits cardiovascular responses in awake but not anesthetized rats. These responses resemble those observed in emotional

conditioning. CNA lesions blunt the cardiovascular responses to unconditioned stimuli. Different projections from the CNA to hypothalamic and brainstem areas (see below) mediate the expression of learned and innate behavioral and cardiovascular responses indicative of fear.

The role of the bed nucleus of the stria terminalis (BNST), a part of the extended amygdala, in conditioned behavioral and cardiovascular responses is controversial. No effects on blood pressure changes were observed in rats with neurochemical lesion of the BNST when using conditioned auditory fear cues. In contrast, attenuated tachycardia and blood pressure increase to conditioned contextual cues was observed in rats following pretest inactivation of the BNST. These results suggest an anxiety-like role of the BNST in the expression of conditioned cardiovascular fear responses. This difference may be related to a more general anxiety-like role of contextual versus explicit unisensory cues and warrants further investigation.

Hypothalamus (perifornical area, dorsomedial hypothalamus, and paraventricular nucleus)

The hypothalamic defense area, the dorsomedial/perifornical lateral hypothalamus (DMH/PeF/LH), has long been identified to be crucial for the expression of conditioned but also unconditioned cardiovascular responses indicative of fear. Projections from this area to the caudal part of the periaqueductal gray (PAG) are essential for the somatic component of the defense response such as freezing, the active suppression of ongoing behavior in fear conditioning, as a default response to avoid visual attraction of predators. Sympathetic regulation of the cardiovascular fear and anxiety response occurs directly through projections from the DMH/PeF/LH to sympathetic preganglionic neurons (SPNs), and indirectly via the rostral ventrolateral medulla (RVLM) and the median raphe nucleus (MRN).

Cerebellum

The cerebellar vermis is involved in cardiovascular regulation during movement and posture changes. Eyeblink conditioning experiments demonstrated that the cerebellar vermis is also involved in conditioned bradycardia in rabbits. Vermal lesions impair heart rate conditioning. During emotional challenge an increase of regional blood flow occurred in the cerebellar vermis that covaried with mean arterial blood pressure changes in human subjects. Patients with cerebellar lesions did not show the conditioned bradycardia by fear as observed in controls. The pathways mediating the conditioned bradycardia are not well understood. Different cardiovascular modules in the cerebellum have been described that project to the parabrachial nucleus but may include other brainstem areas.

Brain Areas Involved in Basal Cardiovascular Regulation

Basal cardiovascular regulation is a complex physiological process that depends on feedback systems, including the baroreflex from the periphery to the central nervous system. Forebrain areas provide modulatory inputs to various brain areas which in turn provide outputs to specific areas involved in the adjustment of cardiovascular functions to meet the demands in preparation for, for example, fight and flight. Maintenance of basal cardiovascular function is attributed to hypothalamic and lower brainstem areas. Interference with the function of brain areas will always have an impact on both conditioned and unconditioned (innate) autonomic adjustments indicative of fear responses.

Although ibotenic acid lesions of the ventrolateral PAG cause a strong reduction of the freezing response of rats to conditioned auditory and contextual cues, the concomitantly occurring blood pressure increase is unaffected. These findings demonstrate the dissociation of projections from the CNA via the hypothalamus to different brainstem areas for the control of conditioned (but also unconditioned) behavioral versus cardiovascular responses. The dorsal and median raphe nuclei (MRN) are the principal source of serotonin and project to virtually all brain areas. Descending MRN neurons are involved in cardiorespiratory regulation and descending projections to the spinal cord participate in blood pressure control with serotonin playing a critical role in vagal outflow. The rostral ventrolateral medulla (RVLM) is the primary regulator of the sympathetic nervous system, with projections to the sympathetic preganglionic neurons (SPNs) in the spinal cord that are essential for the blood pressure response. The nucleus tractus solitarius (NTS) is the primary input area for the baroreflex modulation to maintain blood pressure within the physiological range. The nucleus ambiguus (NA) is involved in heart rate control and also in central regulation of blood pressure. Vagal preganglionic neurons originate from the NA and the dorsal motor nucleus of the nervus vagus (DMNX).

Implications for Affective Disorders

The beat-by-beat fluctuation of the heart serves as an index of the brain state. The cardiac time series, the beat-by-beat variation of heart rate, collectively reflects the total efferent nerve traffic of the autonomic nervous system. Dysregulation of the fear circuitry is implicated in the pathology of affective disorders from anxiety disorder to posttraumatic stress disorder. An improved understanding of the role of individual brain areas in central autonomic regulation and its contribution to

dysregulation is of high relevance in view of the comorbidity of affective and cardiovascular disorders. Fear conditioning therefore is an extremely useful model to investigate the role of specific brain areas in different processes from encoding via consolidation to reconsolidation and extinction of learned cardiovascular responses. The comparison with URs allows the dissection of contributions of these brain areas for learned versus innate responses.

Berntson and colleagues drew three main conclusions on anxiety and cardiovascular reactivity of patients suffering from anxiety disorders. (1) Anxiety disorders are frequently associated with altered autonomic function, although these alterations may vary considerably across different categories of anxiety disorders, and among individuals within a given category (personality traits). (2) Autonomic reactions often mirror the pattern of exaggerated affective/behavioral response, rather than reflecting a primary abnormality in autonomic regulation. (3) Enhanced autonomic reactivity is most often apparent in phasic responses to specific stimuli or contexts, rather than in basal measures. In view of these conclusions, it is hypothesized that forebrain areas such as the PFC may contribute to exaggerated cardiovascular responses due to lack of inhibitory control of emotional centers such as the central nucleus of the amygdala. This control has been reported to occur via the intercalated nucleus of the amygdala. It remains to be determined whether this pathway, for which behavioral and electrophysiological evidence exists, is also involved in the control of the extinction of cardiovascular responses elicited by conditioned fear. Hypofunction of the PFC, particularly the infralimbic area, appears to be associated with increased susceptibility for affective disorders such as posttraumatic stress disorder. Changes in neural excitability and epileptic-like hyperactivity of the SNS or dysinhibition of the PNS in the CAN underlie the generation of fatal tachyarrhythmias. Transient inactivation of the amygdala prevents tachyarrhythmias elicited by psychological stress in pigs with compromised heart function. Tachyarrhythmias are implicated in sudden cardiac death based on episodic central autonomic dysregulation, and the risk for sudden cardiac death is increased during emotional challenge as documented in epidemiological studies.

The limited understanding of the exact role of specific brain areas stems from the complexity of the cardiovascular system and its function under physiological condition. We attempt to isolate contributions of individual brain areas to specific responses. Thereby, we often ignore feedback mechanisms that continuously contribute to dynamic heart rate and blood pressure regulation that play important roles in the maintenance of basal cardiovascular function and adjustments by emotional challenges.

Conclusions

Cardiovascular parameters are extremely useful to investigate especially emotional learning-induced adjustments that can complement and extend behavioral studies and improve our understanding of the neural substrates that partly differentially contribute to cardiovascular versus behavioral responses. Yet, there is a need for a more detailed understanding of the brain–heart interaction for the expression of learned and innate cardiovascular responses. To better understand the role of individual brain areas of the CAN for cardiovascular baseline regulation and stimulus-induced adjustments (partly through cognitive processes), a shift is required to physiologically relevant experimental conditions and analyses beyond the linear domain. This is necessary since cardiovascular regulation involves complex feedback mechanisms acting on multiple timescales from fast baroreflex feedback to slow circadian hormone fluctuation-related effects. The progress in the analysis of dynamic properties of cardiovascular measures by nonlinear measures will provide for invaluable new insights in neurocardiology, the translation of results across species, and an increased sensitivity of diagnostic power to identify pathological states of autonomic dysregulation in affective disorders. Taken together, this will add to an increasingly complex picture of the tight coupling of brain and heart function.

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See also: Animal Tests for Anxiety; Cerebellum: Associative Learning; Fear Conditioning.

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Cerebellum: Associative Learning

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Glossary

- Conditioned response (CR)** – A learned behavioral response to a conditioned stimulus resulting from an acquired association with an unconditioned stimulus.
- Conditioned stimulus (CS)** – A stimulus that does not elicit any overt behavioral response prior to associative learning.
- Expansion recoding** – The means of transforming, or expanding, a given input into a sparse or distributed representation to make the problem of pattern separation and recognition easier to solve.
- Somatotopy** – The correspondence between areas of the body and neural regions that exert control over or are responsive to stimulation of those areas.
- Unconditioned response (UR)** – The reflexive behavioral response evoked by certain stimuli.
- Unconditioned stimulus (US)** – A stimulus that elicits an innate or reflexive response.

the nature of the information acquired and the response itself. Certainly, sensorimotor systems are required, first, to transduce the information related to the critical stimuli and then, subsequently, to perform the sequence of actions reflective of the memory, but it is the integrative neural trace of the association itself that is the focus of learning-related research. Several structures have emerged as being the critical loci of plasticity underlying specific types of associative memory. For example, emotion-based memory often involves the amygdala and the amygdalar nuclei are critical for the acquisition of conditioned fear – a process wherein neutral stimuli become associated with a fearful event. Similarly, the medial temporal lobe is involved in some forms of associative learning as well as long-term storage of associative information. Structures within this region show changes in neural activity at time points that precede and/or coincide with the behavioral expression of newly acquired associations between visual stimuli, as well as novel spatial and temporal relationships between task-relevant environmental features. But, perhaps, the most exhaustive and complete description of the neural instantiation of memory in the mammalian brain is that involving a simple form of associative memory localized to the cerebellum – namely, classical conditioning of discrete reflexes to behaviorally neutral stimuli. That the cerebellum is involved in cognitive processes at all was met with some resistance originally, although it is now widely accepted that it is the essential neural substrate for the acquisition and expression of this associative memory.

Introduction

Associative learning is the process through which organisms acquire information about relationships between events or entities in their environment. It is expressed as the modification of existing behaviors, or the development of novel behaviors, that reflects the conscious or unconscious recognition of a contingency. It is the contingent, and contiguous, relationship among stimuli that is a hallmark of associative learning – a meaningful temporal or spatial proximity of A and B and the perceived consequent occurrence of B if A. As such, it is fundamental to our sense of causality and is the basis of much of our understanding of the external world. Associative learning also underlies the majority of our adaptive behavior when the association is recognized to have either positive or negative consequences. Adaptive changes in behavior can be triggered by both aversive and appetitive stimuli and can thus enable the organism to avoid negative outcomes or to increase the probability of obtaining a reward. This type of associative learning depends on the presence of signaled reinforcement.

However, there are many forms of associative learning, and the brain regions that support the acquisition and expression of these learned behaviors are determined by

Cerebellum in Motor Control

Historically, the cerebellum was viewed primarily as a structure that simply modulated or refined the execution of extant motor programs. Evidence for the involvement of the cerebellum in motor control came initially from studies of patients in the nineteenth and early twentieth century in whom cerebellar damage correlated with a loss of coordinated movement. Generally, it was observed that lesions to the cerebellum resulted in deficits in the movement of limbs ipsilateral to the site of damage. However, a myriad of movement-related abnormalities were reported, including extensor rigidity, dysmetria, and dysfunctions in motor-sequence execution, equilibrium, and gait control. Additional evidence for the role of the cerebellum in motor function came from studies in which

stimulation of cerebellar regions elicited movements. Although there was not a single, incontrovertible interpretation of how the cerebellum contributed to motor control, it was clear that this structure played a significant role in the coordination, timing, and execution of movements. This accurate, but somewhat limited, view of cerebellar function dominated and circumscribed research until the middle of the twentieth century. In addition, this view still holds considerable influence as evidenced by the many current descriptions of the cerebellum primarily as a facilitator of motor coordination. However, we now know that the cerebellum is capable of encoding new associations and plays a significant role in motor and nonmotor learning, as well as related processes including timing and attention. Parallel lines of evidence from anatomical, theoretical, and behavioral studies in support of this more expansive view of cerebellar function are detailed below.

Cerebellar Anatomy

Cerebellar Cortex

Since the development of an influential theory of cerebellar was based on a close analysis of the relevant circuitry, a brief overview of the anatomy is necessary to

ground any discussion of cerebellar function. The cerebellum is a deeply foliated structure in which a structurally uniform and functionally heterogeneous cortical layer overlies extensive white matter and deep nuclei. Sensory information is relayed to the cerebellum via the pontine nuclei and inferior olive. Axons from principal excitatory neurons in each of these structures terminate on neurons in both – the cortex and the deep nuclei (**Figure 1**). Pontine nuclei receive visual, auditory, and somatosensory information from cortical and subcortical regions which is transmitted via mossy fiber axons to cortical granule cells, the most numerous cells in the cerebellum. Axons of granule cells bifurcate in the upper layers of the cerebellar cortex and extend transversely through the dendritic arbors of the Purkinje cells to make synaptic contacts on the spiny dendrites. Climbing fibers carry somatosensory information from the inferior olive to deep nuclear cells and Purkinje neurons in the cerebellar cortex. Purkinje neurons have a distinctive morphology due to an extensive and planar dendritic arbor positioned orthogonal to the longitudinal axis of the folia. Purkinje cells receive up to 150 000 excitatory synaptic contacts from nearly the same number of parallel fibers. These cells also receive hundreds of excitatory synaptic connections from a single climbing fiber – the

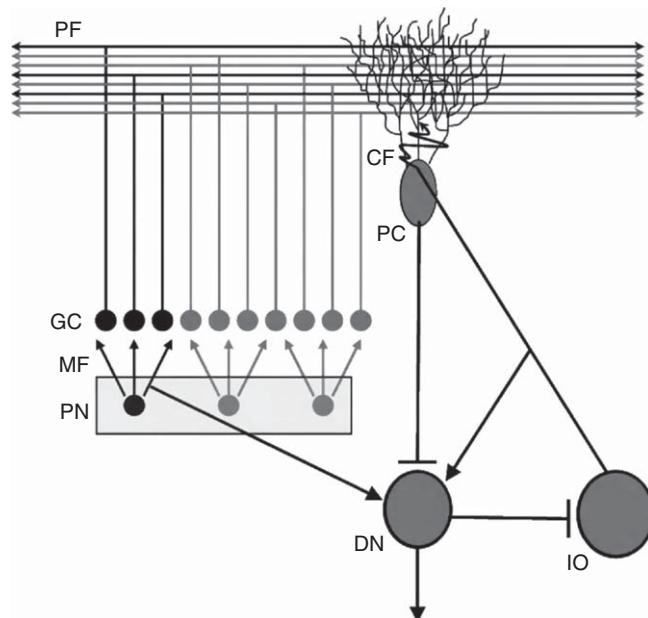


Figure 1 Simplified diagram of cerebellar circuitry (cortical interneurons have been omitted) to illustrate critical paths for integration of information required in associative learning. Two key sites of putative integration are the Purkinje cell (PC) and the deep nuclei (DN). Both receive inputs from the pontine nuclei (PN) and inferior olive (IO) which carry information related to the CS and US respectively. Mossy fibers (MF) and climbing fibers (CF) send collaterals to the deep nuclei, as well as primary projections to the Purkinje cell layer. CS information is believed to undergo expansion recoding via the granule cells (GC) within the cerebellar cortex. A simplistic illustration of this process is shown by the black-filled granule cells that send bifurcating axons, parallel fibers (PF), to synaptic targets on the extensive dendritic arbor of the Purkinje cell resulting in a sparse representation of contextual information. Climbing fibers contact a single Purkinje neuron, densely innervating the base of the dendritic tree. Arrowheads indicate excitatory connections and terminal bars indicate inhibitory projections.

ascending axon of a cell in the inferior olive complex. Climbing fibers wrap around the smooth segments of the Purkinje cell's dendritic arbor and a single action potential in an inferior olivary cell will elicit a massive postsynaptic depolarization resulting in a multiphasic action potential known as a complex spike. In addition to these excitatory connections, there are several types of inhibitory interneurons that form feedforward and feedback loops within the cortex. Purkinje cells are thus positioned to receive and process a wealth of sensory information in distinct synaptic patterns. The output of the cerebellar cortex is mediated exclusively through the Purkinje cells, which are γ -aminobutyric acid (GABA)-ergic neurons and form inhibitory synapses on neurons in the deep cerebellar and vestibular nuclei.

Deep Nuclei

There are three pairs of deep nuclei in the cerebellum: the fastigial, the interpositus, and the dentate. Functionally, the vestibular nuclei in the brainstem are analogous to the cerebellar deep nuclei and are generally included in a description of cerebellar circuitry. Roughly, these nuclei are organized from medial to lateral in correspondence with the innervating region of the cortex, the efferent targets, and somatic region of control – that is, the fastigial nuclei are the most medial and control the trunk of the body; the interposed nuclei (which exist as the globose and emboliform nuclei in some species) control proximal limbs; and the dentate nuclei, which are located most lateral, control distal limbs. The vestibular nuclei receive direct projections from the flocculonodular lobe of the cortex and are involved in eye movements, balance, and gait control. The deep and vestibular nuclei receive excitatory connections from the mossy fibers and inhibitory input from the Purkinje cells. Primary excitatory efferents from the deep nuclei innervate many cortical and subcortical regions including the spinal cord, reticular formation, vestibular nuclei, the red nucleus, primary and premotor cortex, and thalamic nuclei. Deep nuclei also send inhibitory projections to the inferior olive, creating a topographic feedback loop with the cerebellar cortex. In addition, the deep nuclei have a strict somatotopy and microstimulation of regions within the nuclei can elicit discrete movements.

Cerebellar Learning Theory

In contrast to many neural systems for which theories of function are developed incrementally to explain experimental results, a theory of cerebellar learning largely preceded any direct empirical evidence. In 1969, David Marr published a seminal paper proposing that one of the central functions of the cerebellum was to learn to

perform motor skills. Marr based his theory on the unique architecture of the cerebellar cortex – remarkable for its singular geometric regularity and simplicity. John Eccles had recently published a detailed account of cerebellar anatomy and this work – in conjunction with an earlier suggestion by Giles Brindley that the cerebellum functioned to encode new motor skills – inspired Marr to develop a computational theory of cerebellar learning. Importantly, Marr predicted that synapses within the cerebellar cortex were capable of plasticity through conjoint activation of parallel fibers and climbing fibers, and could thus store context-specific information. Although Marr predicted that this plasticity would take the form of a strengthened synapse, his theory was later extended by James Albus who posited, correctly, that the direction of plasticity would take the form of a learning-related depression in synaptic efficacy. Because Purkinje neurons are inhibitory and fire at a high rate spontaneously, a well-timed decrease in the synaptic drive could result in an adaptively timed disinhibition of the deep nuclei.

The cellular arrangement of the cerebellar cortex appeared to map perfectly onto the critical features of neurally inspired computational models of pattern recognition. In brief, expansion recoding mediated by the granule cells could transform information about the environment relayed from the pons into sparse patterns of activation in the parallel fiber–Purkinje cell synapses. In other words, the relatively vast numbers of granule cells, and thus parallel fibers, in the cortex could serve to create more unique, and therefore more easily separable, activity patterns by distributing information over a larger network of axons. Climbing fibers were then poised to act as a teaching signal to reinforce the encoding of these synapse-specific activation patterns. Massive depolarization induced by climbing fiber activity results in a complex spike and, subsequently, a brief cessation in Purkinje cell firing. Essentially, Albus proposed that the coincident activation of parallel and climbing fibers entrained the Purkinje cells to respond to specific patterns of activation with a transient decrease in activity so that, once learned, the activity patterns from the parallel fibers alone would be sufficient to suppress firing. This transient suppression could release the deep nuclei from tonic inhibition, resulting in the well-timed execution of a discrete movement. By viewing the cerebellum from a computational perspective as an information-processing machine, Marr and Albus developed a cohesive account of putative input–output functions that could support the encoding of novel associations at the neural level.

Critically, the associative nature of cerebellar-mediated learning was deeply embedded in the tenets of the computational theory. Because the theory required temporal proximity of parallel fiber and climbing fiber activation, it was now possible to interpret the Purkinje neuron as a cellular agent of coincidence detection. It was

this belief that the cerebellar cortex was a putative site of integration that was instrumental in transforming the view of the cerebellum from a simple motor-program facilitator to a site of intrinsic plasticity and that of the origin of directed behavior.

Over a decade later, the Marr-Albus theory of cerebellar learning gained significant empirical support with the discovery of long-term depression (LTD) at the parallel fiber–Purkinje cell synapse. Furthermore, it was shown that this plasticity could be evoked through the conjunctive activation of parallel and climbing fibers. Remarkably, the theoretical prediction based on the perceived similarity between abstract computational models and cerebellar anatomy was confirmed experimentally through electrophysiological recordings. This was a powerful convergence that elevated the status of the theory and contributed to its deep and sustained influence in the field of cerebellar research.

Cerebellar-Mediated Behavior

Aversive Conditioning

Around the same time as the discovery of LTD at cortical synapses, the initial reports of cerebellar involvement in the classical conditioning of a discrete motor reflex – the eyeblink response – appeared. Eyeblink conditioning is the paradigmatic example of associative learning in the cerebellum. As in all forms of classical or Pavlovian conditioning, eyeblink conditioning develops through an association of two previously unrelated stimuli – the unconditioned stimulus (US), which elicits a reflexive or unconditioned response (UR), and the conditioned stimulus (CS), which does not elicit an overt behavioral response. If the CS and US are repeatedly paired in close temporal proximity so that the CS precedes and predicts the occurrence of the US, the CS acquires properties sufficient to evoke a behavioral response. This acquired response – the CR – is anticipatory, reflects the organism's recognition that the US is imminent, and is triggered by the presence of the CS alone. In the most basic and commonly employed model of eyeblink conditioning, rabbits are trained with a tone or light CS which is paired with a periorbital shock or puff of air to the cornea as the US. In the standard delay protocol, the CS precedes the onset of the US and the two stimuli co-terminate. As learning occurs, CRs begin to appear during the interstimulus interval, eventually developing the appropriate timing to peak at the onset of the US. In a variant of this task – trace eyeblink conditioning – there is a stimulus-free interval between the offset of the CS and the onset of the US. Although trace eyeblink conditioning also critically involves the cerebellum, the introduction of a trace-interval requiring the animal to associate temporally distinct stimuli also requires the involvement of the

hippocampus. Because of its reliance on extracerebellar structures, trace conditioning is mechanistically distinct from delay conditioning. The discussion below is focused on the latter as it illustrates a form of learning in which the critical association depends solely on the cerebellum.

In 1981, Richard F. Thompson and colleagues published the first report that the cerebellum was involved in the acquisition of the novel association introduced during delay eyeblink conditioning. Lesions of the cortex and deep nuclei ipsilateral to the trained eye resulted in the abolition of learned behavior and prevented the acquisition of the conditioned eyeblink. Further refinement of cerebellar lesions and reversible inactivation revealed a critical and highly circumscribed region of the interpositus nucleus that was essential to the acquisition and expression of the CR. In addition, electrophysiological recordings from the interpositus nucleus revealed learning-related increases in neuronal activity that preceded the onset of conditioned behavior within a single trial and modeled the magnitude and temporal form of the behavior itself. Extensive research has now culminated in overwhelming evidence that the cerebellum – and in particular, the anterior lateral region of the interpositus – is the essential neural substrate for the memory trace in this form of associative learning.

Evidence from studies of human subjects and patients is in agreement with the findings from the nonhuman animal literature. Imaging studies have consistently shown cerebellar activation during acquisition of delay eyeblink conditioning. Lesions of the appropriate regions of the cerebellum – critically, the deep nuclei – prevents the acquisition of this associative memory trace. The role of the cerebellum in this form of associative learning appears to be evolutionarily conserved across species and supports the investigation of cerebellar function in this animal model. It is also important to note that even though eyeblink conditioning has been the most widely studied model, the results from these studies apply to the classical conditioning of any discrete behavior response with an appropriate US.

These empirical data from behavioral studies could be viewed as further confirmation of the predictions derived from the cerebellar learning theory. Specifically, the apparent integrative role of the cerebellum in associating distinct and previously unrelated stimuli was in accord with the observation of intrinsic plasticity and the requisite anatomical circuitry to deliver information related to the CS and US. Indeed, as was suggested in the formulation of the Marr–Albus theory, the sensory information related to the CS is relayed to the cerebellum via the pons, culminating in a pattern of activation in the parallel fibers reflective of the context. The pontine nuclei receive projections from auditory, visual, somatosensory, and association systems – both cortical and subcortical – and could thus support conditioning to a wide range of

stimuli. Empirically, it has been shown that appropriate lesions of the pontine nuclei can prevent expression of the CR in a modality-specific manner. Stimulation of the pons can serve as an effective CS and the results of many studies identify the pontine nuclei-mossy fibers as the essential CS pathway.

Similarly, evidence indicates that the climbing fiber system originating in the inferior olive supports the delivery of information concerning the US to the cerebellum. For eyeblink conditioning, the critical region of the inferior olive is the dorsal accessory olive – which relays somatosensory and nociceptive input from the spinal cord and cranial nuclei. One of the key predictions in the Marr-Albus theory was that climbing fiber activation served as an error signal to guide the physical imprinting of contextual information through the induction of plasticity in critical neurons. In this sense, the climbing fibers were thought to manifest the reinforcement that is integral to this type of associative learning. Consistent with this idea, lesions of the critical region of the inferior olive completely prevent learning if made before training and result in the extinction of the CR if made after training. Importantly, the US evokes activity in the inferior olive that decreases as the animal learns – that is, when the errors have decreased and the need for a teaching signal is lessened. As with the pontine-mediated CS circuitry, electrical microstimulation of this region serves as a very effective US. These results argue that the inferior olive-climbing fibers system is the essential US reinforcing pathway for the learning of discrete responses.

Although the anatomical mapping of CS and US signals to the cerebellum and its associated circuitry appears congruent to the suppositions made in the theoretical model, an important distinction remained – namely, that the Marr-Albus theory explicitly described the associative mechanism as existing exclusively in the cerebellar cortex. On the one hand, the data from eyeblink conditioning confirmed that the cerebellum was exemplary of a neural circuit primed to learn associations, while on the other, the emphasis on the cortex as the primary site of integration did not account for the critical role of the deep nuclei. As the research progressed in identifying the relevant circuitry for the learned association in the classical conditioning of the eyeblink response, the critical role of the interpositus was clear and unequivocal, while that of the cerebellar cortex is much less certain. Although the nature of cortical involvement in classical conditioning is not yet understood fully, data from studies using lesions, inactivations, and genetic manipulations suggest that the cerebellar cortex does contribute substantially to the development of the CR under normal conditions. Effects on the timing, amplitude, and frequency of the CR – as well as the rate at which the CR is acquired – have all been reported following cortical manipulations. However, one problem in identifying the role of the cortex in this

form of associative learning is that the organization of cortical regions does not share the well-delineated somatotopy of the deep nuclei. While stimulation and lesion studies have resulted in the identification of distinct regions of the deep nuclei that correspond to sites of peripheral stimulation, the cortex appears to have a redundant and fractured representation of somatic regions. This anatomical complexity hinders the straightforward investigation of cortical modulation of the classical conditioning of discrete reflexes.

Given the evidence suggesting the primacy of the interpositus in classical eyeblink conditioning, it is somewhat paradoxical that the most intense investigation has focused on the mechanisms through which the cortex may facilitate learning, while there remains a relative paucity of data describing synaptic changes in the interpositus. However, if understood in a historical context, this is not so surprising. With the theoretical and early empirical data in close agreement, it became generally accepted that cortical plasticity in the form of LTD could provide a plausible and likely mechanism for the emergence of the CR. In addition, it is true that since then a steadily increasing body of data has shown a correlation between learning deficits and compromised LTD in the cerebellar cortex. Genetic manipulations affecting cortical LTD can impair learning. However, mice in which Purkinje cells are selectively eliminated still exhibit significant, although dramatically impaired, learning in the absence of any cortical output to the deep nuclei. Interestingly, the associative memory derived from trace eyeblink conditioning depends on the cerebellar deep nuclei and extracerebellar structures but may not require the involvement of cerebellar cortex at all. In sum, the cerebellar circuitry can support the learning of new associations in this basic task but the site of integration is not restricted to the cerebellar cortex.

Predictions from the Marr-Albus theory were strikingly accurate in many ways but may have overstated the role of the cortex in cerebellar-mediated learning. Recently, however, there has been an increased focus on additional sites of plasticity in the cerebellum and changes in efficacy have been reported at mossy fiber-granule cell synapses, GABA interneuron-Purkinje neuron synapses, Purkinje neuron-nuclear cell synapses, parallel fiber-Purkinje dendrite synapses, climbing fiber-Purkinje dendrite synapses, and Purkinje neuron-nuclear cell synapses. Intrinsic changes in excitability have been identified in granule cells and nuclear cells. Due to the behavioral results implicating the deep nuclei in the formation of the associative memory trace, and the anatomical inputs that would support integration of the conditioning stimuli, one would expect plasticity to occur at synapses in this region. Evidence does suggest that eyeblink conditioning can increase the number of excitatory synapses between mossy fibers and the neurons in the

interpositus and there was an early report of LTP at the mossy fiber–nuclear synapses. Although the mechanisms through which the deep nuclei may encode the integrative memory trace have yet to be described in full, it is clear that the cerebellum and its related circuitry are both necessary and sufficient to support this form of associative learning.

Appetitive Conditioning

Building upon the research that demonstrated a role for the cerebellum in aversive classical conditioning, cerebellar contributions to other forms of associative learning have also been tested, with varying results. Appetitive conditioning employs reward as the reinforcing, motivational element in the learning task. To date, results from investigations of cerebellar involvement in appetitive conditioning have been mixed or negative. Pavlovian appetitive conditioning appears not to require the cerebellum. Even if procedural elements are controlled such that the performance requirements are the same for avoidance- and appetitive-operant tasks, it appears that the cerebellum is preferentially involved in acquiring the association in the aversive condition. Lesions to the cerebellum and deep nuclei can thus block avoidance learning in a bar-pressing task while having no effect on appetitive learning. In these instances, it would seem that the value, and perhaps the valence, of the reinforcer determine the degree of cerebellar involvement in learning the association. However, in an appetitive visuolocomotor task, rats with hemicerebellar lesions were delayed in the acquisition of a novel object–reward association. Nevertheless, lesioned animals were capable of generalizing feature discrimination to new object pairs, thereby demonstrating intact performance once the procedural strategy had been acquired. Similarly, results from studies of nonhuman primates have suggested that the cerebellum is minimally involved in the expression or retention of visuomotor associations.

That the cerebellum may not be required for maintaining or expressing some reward-based associations could be related to a transient need for the error signal mediated by climbing fiber activation. In other words, when animals are performing at near asymptotic levels, the utility and frequency of the error signal decreases to reflect a successful encoding of an appetitively based association, the expression of which may rely on extracerebellar structures. Performance degradation in an intact system would presumably reinstate the need for error-based teaching signals but long-term studies of cerebellar involvement in appetitive conditioning have not been done. It is therefore premature to speculate about the possibility of a time-limited role of the cerebellum in such tasks. And, as discussed above, the memory trace for aversive classical conditioning of motor reflexes requires

the ongoing involvement of the cerebellum – that is, insofar as it has been tested, there is no temporal window that constrains the essential role of the cerebellum. Furthermore, recent studies of human patients with focal cerebellar lesions have shown nearly the opposite effect. While patients were able to acquire information about reward contingencies in a probabilistic associative learning task, they were impaired in reward-based reversal learning and learning of a second reward-based task. While this is a promising area of future research, much work needs to be done to identify the nature and degree of the cerebellar contribution to associative learning with appetitive stimuli.

Conclusion

Although it is clear that the cerebellum can support associative learning, it is not yet clear whether the cerebellum is involved only in specific forms of associative learning and whether there is some fundamental property of the learning task that would signal cerebellar involvement. It does seem to be the case that the cerebellum is more significantly involved in aversive, as opposed to appetitive, conditioning. Strikingly, empirical results have largely confirmed the early predictions that the cerebellum is a neural machine primed to learn, and more specifically, to associate information of relevance to an organism in learning to negotiate its environment. However, it is not a generic learning machine capable of supporting any new associative memory trace. The mechanics of the intracerebellar circuitry that so inspired early theorists may be free of content in design but are not in practice. Information processing is constrained by the input to the system. The nature of the information received, in conjunction with the unique processing capabilities of the machinery, will largely determine the extent to which a neural system contributes to the encoding of memory in nonpathological conditions. Because the cerebellum receives afferent signals from widespread cortical and subcortical regions, further research is necessary to identify the extent of cerebellar-based learning. In addition, cerebellar modulation of nonmotor learning is also a topic of increasing relevance. Converging evidence indicates that the cerebellum is involved in many aspects of cognition such as linguistic processing, mental imaging, timing, verbal memory, working memory, and attention. It remains to be seen how well the traditional computational description of the cerebellum and its intrinsic circuitry will be able to account for this expanded view of cerebellar function.

This article has focused on classical delay eyeblink conditioning as it is the most extensively studied form of associative learning mediated by the cerebellum. It is important to remember, though, that eyeblink

conditioning utilizes only one cerebellar-based circuit of potential behavioral control. Nevertheless, the detailed overview of this form of learning is important to illustrate that the cerebellum is, in fact, a structure that can support the encoding of novel associations. Far from being a system employed only to refine the execution of coordinated movement, it has the circuitry necessary to integrate stimulus-specific information at the cellular level, as well as a capacity for modifiable synaptic connections – two features suggested to be constitutive of neural structures involved in associative learning.

See also: Animal Models of Learning and Memory; Cognition: Learning and Memory: Pavlovian; Eyelid Classical Conditioning; Implicit Learning and Memory: Psychological and Neural Aspects; Learning and Memory: Computational Models; Motor Learning in the Vestibulo-Ocular Reflex; Neural Basis of Classical Conditioning.

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<http://www.neuroanatomy.wisc.edu> – Neuroscience resource page.
<http://capsule.brain.riken.jp> – Online simulator of computational models of the cerebellum.

Contribution of Split-Brain Studies to the Evolution of the Concept of Hemispheric Specialization

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Glossary

Bilateral advantage – Improved performance when the two hemispheres are simultaneously stimulated compared with monohemispheric stimulation. The distribution of processing across both hemispheres is thought to be advantageous, since it allows for the combination of all available resources. In this case, bilateral stimulation is advantageous, because the stimulus input is initially distributed over both hemispheres, which reduces the amount of callosal transfer.

Corpus callosum – It corresponds to the important white matter tract connecting the two halves of the brain. It represents the main interhemispheric commissure. The corpus callosum mediates interhemispheric communication mainly between homotopic cortical areas of the right and left cerebral hemispheres.

Hemispheric specialization – Mainly based on the observation of distinctive impacts of lateralized brain lesions on behavior and cognition, the concept of hemispheric specialization expresses the fact that each cerebral hemisphere has developed distinct specialized abilities for different aspects of behavior, perception, and cognition. Initially thought to occur at a functional level (music, language, etc.), hemispheric specialization is now conceived at the level of some specific subcognitive processing.

Interhemispherical dynamic – A complex exchange of information between the two hemispheres thought to allow for the full deployment of computational and cognitive processing. Interhemispherical dynamic is somewhat viewed as opposed to a static conception of hemispheric specialization. It stresses a dynamic, complementary sharing of labor, and a cooperative functioning relying on neural networks spread over the two hemispheres.

Lateralized stimulation – It has been used as a means of inducing the initial stimulation to one hemisphere only. It has been used in split-brain individuals as well as normal individuals (along with tachistoscopic presentations; see below) to study and compare the ability of each hemisphere individually. This method relies on the characteristics of most sensory and sensorial systems that project the stimulation to the contralateral hemisphere. It is the case for the visual

system (divided visual field presentations) as well as for the tactile system; given the presence of a large bilateral component, the auditory stimulation has a tendency to be more represented to the contralateral hemisphere only in the presence of a bilateral competition (dichotic listening). Finally, the olfactory central projection is uncrossed.

Split-brain individuals – Those who have suffered a surgical section of a part or all of the corpus callosum (callosotomy) dictated by therapeutic reasons. Indeed, in some rare cases of intractable and pharmacoresistant epilepsy, the section of the corpus callosum diminishes the frequency and intensity of epileptic seizures, probably because it interferes with the epileptic activity of malfunctioning bilaterally distributed neural networks. Initially, total callosotomy has become more and more partial. Individuals with callosotomy have provided a unique opportunity to study the functions of the two hemispheres in isolation.

Tachistoscopic presentation – Projection of a visual stimulus for a short time. This type of presentation is used to prevent the subject from using an eye saccade in order to centrally capture a lateralized visual stimulation. Once done using a mechanical shutter, tachistoscopic presentations are now done using computer screens having a few limitations due to the frequency of screen refreshment and the possible occurrence of remanence.

Historical Context

Although the cerebral hemispheres are relatively comparable in terms of size, weight, and surface area, centuries of studies on the effects of unilateral brain damage have shown major functional differences between them. In this regard, it was the study of language impairments following a lateralized lesion that provided the classic evidence that the left hemisphere is the primary locus of language. As Hughlings Jackson put it as early as 1864, the left and right hemispheres are to be conceived of as functionally complementary since the human brain cannot be duplicating functions in both hemispheres. In this regard, Hughlings Jackson later proposed that the right

hemisphere specialized in other functions such as the perception of visual objects. This conception of the two complementary hemispheres was more or less ignored until the 1930s, when the use of psychological tests in large patient groups revealed the specialized roles of the two hemispheres; more recently, the use of functional neuroimaging, such as functional magnetic resonance imaging (fMRI), has produced further data.

However, one of the most significant contributions to our understanding of the complementary functional contributions of each cerebral hemisphere came from the observation of individuals whose corpus callosum had been rendered nonfunctional. The two hemispheres are connected through the brain's largest bundle of fibers, the corpus callosum (CC). Containing more than 300 million axons, the CC forms a massive neural pathway between the two cerebral hemispheres, allowing excitatory or inhibitory transfers to occur between them.

Quite early in the history of the study of hemispheric specialization, the systematic analysis of the impact of a disruption in the flow of information between the two hemispheres allowed unique insights into the functional characteristics of each hemisphere. Over the years, three sources have provided such unique evidence: (1) individuals with agenesis of the CC, (2) individuals with a more or less complete lesion of the CC, and (3) individuals who must undergo a surgical separation of the two hemispheres, known as callosotomy.

Callosal agenesis. Agenesis of the CC stems from an *in utero* dysfunction in the growth of the central nervous system. The agenesis can be partial or complete depending on the stage at which the defect occurs. The first case of agenesis of the CC was described following an autopsy by Reil in 1812. The condition has been described as "a natural model of the split brain." There are definite signs of callosal disconnection in callosal agenesis, such as a lack of interhemispheric transfer of motor skills or sensory impairments when midline functions are involved. However, at the same time, individuals with agenesis of the CC frequently show other signs of brain insult or dysfunction. Moreover, since it is present at birth, individuals with callosal agenesis appear to develop a number of compensatory pathways or mechanisms.

Lesion of the corpus callosum. Dejerine was probably the first researcher to report the specific impact of a lesion to the splenium of the CC, combined with a left occipital lesion, on written language abilities (pure alexia without agraphia). In 1907, Liepmann and Maas reported a case of left apraxia accompanied by left-hand agraphia following a callosal lesion. Cases of callosal lesion are still reported today, and now neuroimaging can verify the lesion. For example, Habib, Ceccaldi, and Poncet reported the case of a young woman with a nearly complete callosal lesion. Apart from the usual aphasic and apraxic signs, the authors reported the presence of some strange inner

conflicts that raise questions about the integrity and uniqueness of the self in those patients.

Callosotomy. The surgical separation of the two hemispheres was introduced in 1939 by Van Wagenen and Herren as a drastic solution to help individuals with otherwise untreatable epileptic seizures. The first behavioral analyses of these patients by Akelaitis did not take advantage of the surgical separation of the two hemispheres to study their specialization. Two contemporary neurosurgeons, Phillip Vogel and Joseph Boghen, invited Roger Sperry and Michael Gazzaniga to examine some of their callosotomized young patients. The explosion of knowledge that resulted from this interdisciplinary approach was massive, as innovative methods of stimulus presentation (e.g., lateralized visual field presentation) made it possible to test each hemisphere separately. Roger Sperry received a Nobel prize in 1981 for his unique series of neuropsychological investigations of callosotomized patients that opened up a new era in the study of mental unity and the lateralization of cognitive functions. Rarely has a single paradigm in neuropsychology and the cognitive neurosciences represented such a leap forward in the evolution of the concept of hemispheric specialization and cooperation. After a brief presentation of the different behavioral approaches used to selectively examine each hemisphere, this article summarizes the most important discoveries about hemispheric specialization and cooperation in key cognitive domains.

Methodology

Studies that describe the respective cognitive potential of each hemisphere make full use of the fact that nearly all sensory and motor pathways are crossed. Thus, each cerebral hemisphere receives information primarily from the opposite half of the body and the corresponding sensory hemifield. Special stimulus presentation techniques have been introduced to confine sensory inputs and motor responses to one hemisphere in split-brain participants. The resulting restriction of stimuli to one hemisphere has allowed researchers to describe some of the specialized functions of the two hemispheres.

Lateralized Visual Field Presentation

The study of hemispheric specialization based on visually presented stimuli is based on the anatomical properties of the visual system, which is divided into left and right hemifields on either side of a small binocular zone. Thus, due to the partial chiasmatic reorganization of the nasal hemiretinas, the central visual pathways convey a complete visual hemifield to the lateral geniculate nucleus, which finally reaches the primary visual cortex

through the optic radiation. The restriction of visual presentation to one hemisphere is thus possible if one respects two conditions: the visual stimulus must be at a visual angle of at least 2.5° outside the fovea area (which projects to both the hemispheres), and the time of presentation must be less than the time necessary (estimated at between 80 and 220 ms) for an eye saccade that would allow the stimulus to be positioned in the central visual field and achieve bilateral visual representation. In early studies, this was managed using a mechanically driven tachistoscope. The participant would sit in front of a screen with a small black dot in the center and the tachistoscope (rapid shutter presentation) allowed the experimenter to precisely control the duration of presentation. In split-brain participants, this procedure was often used to compare the ability of each isolated hemisphere to process verbal and nonverbal stimuli. More recently, lateralized computer presentations have replaced the tachistoscope. Divided visual fields are also frequently used to test normal participants, although the fact that the two hemispheres are still connected imposes a fine-grained analysis of the respective combinations of visual field and response time for each hand in order to infer which hemisphere is best suited to process a given visual stimulus.

Auditory Dichotic Presentation

Unlike the visual pathways, the central auditory pathways are not anatomically lateralized. Indeed, each ear projects information to both the hemispheres following a complex multirelay and multicrossing brainstem pathway. However, probably because the contralateral projections are somewhat greater, there is evidence of a functional lateralization of the central auditory pathways when two different stimuli are presented simultaneously to both ears. This technique referred to as dichotic listening makes it possible to present auditory stimuli primarily to the contralateral hemisphere. As Kimura suggested, under dichotic presentation conditions, a modest group-based right-ear advantage emerges for verbal stimulation. Thus, in dichotic listening condition, a right-handed participant would report the syllables presented to the right ear first and more accurately because of its privileged functional connection with the left hemisphere. In this condition, dichotic listening for verbal stimuli results in a right-ear advantage, suggesting that the left hemisphere is superior at processing such stimuli. In split-brain participants, because the verbal response emanates from the left hemisphere, only the right-ear sound is reported.

Lateralized Tactile Stimulation

Tactile, or haptic, stimulation of one hand projects primarily to the contralateral cerebral hemisphere. The

usual testing arrangement combines lateralized visual or tactile stimuli and tactile responses. Such a set-up was used to investigate the contribution of each cerebral hemisphere in the categorization of tactile information, for example. Tactile information is kept within one hemisphere by presenting tactile stimuli out of sight. Under such conditions, for example, split-brain participants are incapable of comparing an object held by the left hand (right hemisphere) with a visual stimulus flashed in the right visual field (left hemisphere). Moreover, the participant is only able to name the object processed by the left hemisphere.

Lateralized Olfactory Stimulation

The central olfactory tracts first divide into medial and lateral stria, the latter of which end up in the prepyriform cortex and parts of the amygdala of the temporal lobe corresponding to the primary olfactory cortex. Unlike vision, hearing, and somesthesia, the olfactory system is essentially uncrossed: the left nostril projects to the left hemisphere, and the right nostril to the right hemisphere. Studies of normal subjects suggest that odor discrimination and intensity judgments show an advantage in the right nostril (right hemisphere), but odor naming shows a left nostril superiority. However, for split-brain subjects, odors identified through one nostril are not recognized through the other. These subjects fail to identify by verbal report the objects smelled through the right nostril, which are experienced within the right hemisphere.

The Contribution of Split-Brain Studies to the Comprehension of Functional Specialization

Complete severing of the CC drastically blocks the interhemispheric transmission of sensory, perceptual, motor, and other types of information. Yet, cursory observation of split-brain patients usually fails to demonstrate that surgical disconnection of the hemispheres has a significant impact on their behavior, cognition, affect, or sense of self. Nevertheless, under specific testing conditions, they can exhibit dramatic hemispheric asymmetries.

Motor Control

According to the split-brain literature, each hemisphere can control bilateral proximal muscles (muscles closest to the center of the body), but only exerts control over contralateral distal muscles (muscles farthest away from the center of the body). For instance, a disconnected hemisphere can control both arms (proximal muscles), but only the opposite hand (distal muscles). Nonetheless, the disconnected left hemisphere often shows an advantage

for motor planning of proximal movements (e.g., appropriately reaching for an object). While split-brain patients usually experience difficulty with bimanual asynchronous, simultaneous movements, they can often use both hands in a seemingly coordinated fashion when performing a task that requires the integration of both. They can also use both hands to execute simultaneous conflicting spatial programs, like drawing two different stimuli concurrently with both hands, whereas normal individuals are impaired in such tasks. However, in rare instances of diagnostic apraxia (alien-hand syndrome), the patient's hands may perform conflicting actions following callosotomy, such as opening a door with one hand while the other hand tries to close it.

Perception, Attention, and Visuospatial Processes

Somatosensory processes are largely lateralized. Thus, in split-brain patients, tactile information processed by one hand is not available to the ipsilateral hemisphere. As a result, split-brain individuals are unable to compare items held in each hand, even though they are aware that both hands are holding something. Likewise, visual information presented to one hemisphere is not available to the other disconnected hemisphere for perceptual analysis. Moreover, tactile information presented to one hemisphere cannot be cross-integrated with visual information presented to the opposite hemisphere. On the other hand, noxious somatosensory information (e.g., pain) is projected to both hemispheres, thus allowing bilateral perception of unilateral adverse stimulation. With regard to basic perceptual processes, the functions of both hemispheres appear to be duplicated in split-brain patients and may proceed independently. Some attentional processes also seem to function independently. For instance, split-brain studies have shown that each hemisphere is able to maintain an independent focus of attention in a visual search task. Accordingly, each hemisphere is able to concurrently and independently direct its attention to its own visual field. Therefore, when scanning a bilateral stimulus array, split-brain patients frequently outperform neurologically normal individuals. Likewise, they usually perform better in a test of visual retention when the information is distributed across both visual half-fields.

Split-brain studies often evince a right-hemisphere advantage for many aspects of visuospatial processing (even in right-handed individuals). For instance, drawings produced by the right hand of split-brain patients (left hemisphere) often lack spatial coherence, whereas those produced by the left hand (right hemisphere) show appropriate spatial organization. At the perceptual level, facial recognition of upright faces is more efficient when processed by the right hemisphere. The right hemisphere

also demonstrates an advantage for perceptual grouping processes, which involves the ability to assemble complex visual features into a combined representation of objects and surfaces. Although both hemispheres are able to perceive illusory contours (perception of a visual contour in the absence of lightness or color in a stimulus), only the right hemisphere performs well at amodal completion (perceiving a partly occluded stimulus as a complete whole). Sophisticated visual matching abilities, such as deciding whether two visual images are identical or mirror reverse, are also more efficient when executed by the right hemisphere. Moreover, the right hemisphere of split-brain patients is typically better than the left at performing higher-order cognitive tasks, such as the block design subtest of the Wechsler intelligence scale.

Language

The assessment of behavioral asymmetries for linguistic or verbal processing in split-brain patients has greatly contributed to our understanding of the hemispheric specialization of language. A common observation from such studies is that the left hemisphere plays a dominant role in linguistic or verbal processing for the majority of right- and left-handers. For instance, split-brain patients can easily tell the name of an object presented in the right hand or the right visual field (left hemisphere). However, they are typically unable name an object when it is presented in the left hand or the left visual field (right hemisphere), even though they seem to have full knowledge of what it is. Such results indicate that the left hemisphere is specialized for speech production. Moreover, the left hemisphere possesses a lexicon (meaning associated with words) and a complete grammar (rule-based system) and is fully capable of comprehending all aspects of language. Thus, in split-brain patients, only the left hemisphere is typically able to make judgments requiring phonological decoding (e.g., indicating whether two differently spelled words rhyme with each other) or converting graphemes (fundamental unit of written language) to phonemes (fundamental unit in the sound system of language). Nevertheless, split-brain studies have shown that the right hemisphere does have some linguistic abilities. For instance, it has the capacity to read whole words, primarily if they are concrete, picturable, and frequent, and may even have limited access to grammar.

Learning and Memory

It is not uncommon for split-brain patients to present memory problems. For example, there have been reports of short-term memory deficits following surgical sectioning of the CC, which seem to be related to the joint

suppression of the anterior commissure. Some studies also suggest that commissurotomy has detrimental consequences on free recall abilities (remembering without any clues to help retrieval), although recognition processes for previously learned material remain unaffected. Split-brain research also suggests that each hemisphere tends to demonstrate some material-specific abilities. For instance, the left hemisphere is better at encoding verbal material (e.g., words), while the right hemisphere is more proficient at remembering nonverbal information (e.g., faces). Hemispheric processing differences have been noted as well; the isolated right hemisphere performs better than the left at discriminating previously learned items from new items, such as words, faces, and abstract visual forms. Even so, both hemispheres are able to use procedural memory (implicit long-term memory of motor, perceptual, and cognitive skills), although the ensuing learning is limited to each isolated hemisphere.

Emotion and Sense of Self

When confronted with negative events, split-brain patients do not show the typical reactions associated with negative emotions (e.g., sadness). Even though they express a definite dissatisfaction, their reactions appear somehow diminished or distant. Yet, their personality characteristics have been described as relatively more positive than negative and they remain verbally expressive. Likewise, both isolated hemispheres are able to generate spontaneous facial expressions, although the left is dominant for voluntary facial expressions.

Following callosotomy, each hemisphere is able to operate independently without a contribution from or awareness of the other. Given that this surgical procedure apparently produces a division of conscious experience, it has raised questions about the possibility that each hemisphere may possess its own mind and sense of self. There is no clear evidence supporting such duality. Split-brain patients usually appear to have an unaffected sense of self. It has been suggested that this apparent unity may be attributed to the left hemisphere's ability to formulate hypotheses and generate explanations about the environment and itself, which has led Gazzaniga and colleagues to label the left hemisphere as the 'interpreter.' Since the right hemisphere is unable to do so, at least using articulated language, the interpreter (left hemisphere) would provide a sense of unified self. On the other hand, some split-brain data suggest that both hemispheres possess the ability to self-recognize, although only the right can recognize familiar others. Other evidence suggests that the left hemisphere requires less information than the right for self-recognition.

Other Aspects of Cognition

Split-brain studies have also contributed to the exploration of hemispheric specialization for other aspects of cognition. For instance, a few studies have shown that the left hemisphere is usually more efficient at performing mathematical operations (e.g., calculations) than the right hemisphere, even though both are able to compare numerical representations. There are also evidences that the isolated right hemisphere is superior at making temporal judgments, such as making fine temporal discrimination, which contrast with divided visual field studies of neurologically normal adults suggesting a left-hemisphere advantage. On the other hand, split-brain studies have also helped us understand the contribution of subcortical structures and different portions of the CC. For instance, subcortical structures are able to transfer some information to both isolated hemispheres, such as contextual information and a few aspects of spatial localization.

Theories of Callosal Dynamics

Three main classes of models are widely considered to explain why lateralized presentation results in hemispheric performance. The first two, the direct-access model and the callosal-relay model, are anatomically motivated and assume that the information transfer through the corpus callosum results in measurable losses of time and stimulus quality. A third class of models of laterality effects, first proposed by Kinsbourne in 1975, involves a dynamic shift of hemispheric control.

Direct-Access and Callosal-Relay Models

The direct-access model assumes that information will be processed by the hemisphere that first receives it, regardless of the difference in ability that may exist between the hemispheres. The hemisphere that receives the sensory information first will process it, for better or for worse. Laterality differences here are due to relative differences in the perceptual–cognitive competencies of the two hemispheres. The direct-access model predicts an advantage in performance for information that reaches the appropriately specialized hemisphere first, since processing by that hemisphere presumably would be better than processing by the other hemisphere. This model is often suggested by split-brain and lesion studies showing different performance styles in the two hemispheres for certain cognitive tasks.

The callosal-relay model, on the other hand, assumes that information is always processed by the more specialized hemisphere. If sensory information reaches the other hemisphere first, it will have to

shuttle across the CC to the specialized hemisphere prior to processing. In this case, the transfer results in some loss of clarity of information and an advantage would be found for stimuli that reach the specialized hemisphere directly.

A direct-access task usually shows small and comparable behavioral laterality effects in split and normal brains since no callosal transfer is involved. A callosal-relay task, on the contrary, will show a massive laterality effect in the split brain but a relatively small, even if significant, laterality effect in the normal brain.

The Interhemispheric Dynamic: A Modularity View

Kinsbourne suggests that hemispheric activation depends on psychological set effects and thus on a host of task parameters. In this view, dual-task priming can increase hemispheric activation, but overloading can eventually shift control to the other hemisphere. Dynamic-shift models utilize both the direct-access assumption of bilateral competence and the callosal-relay emphasis on cross-callosal transfer.

Banich and colleagues proposed the most elaborate account of interhemispheric interaction in decision making. They suggested that the processing required for decision making may be divided between the two cerebral hemispheres. If one postulates that each hemisphere is an independent processor, dividing and coordinating processing across the hemispheres may be advantageous in certain situations. These theoretical considerations were influenced in large part by experiments using a letter-matching paradigm, which allows one to compare performance when a match decision initially involves one hemisphere and when a match decision necessitates the division of processing across the two hemispheres, because of the level of complexity (simple vs. difficult). The results highlight a shift in efficiency from within- to across-hemisphere processing. For less complex tasks, processing is more efficient when information is directed to a single hemisphere (i.e., a within-hemisphere advantage). By contrast, when computational complexity is relatively high, the within-hemisphere advantage is diminished, and across-hemisphere processing is more beneficial. Interhemispheric processing was found to aid performance for the more complex task but not for the less complex one. These studies suggest that interhemispheric interaction (IHI) is a flexible mechanism, whose role in the performance of a task changes dynamically depending on what the task demands. Whether and how much IHI aids task performance depends on the relative balance between two factors, the division of processing between the cerebral hemispheres and coordination of processing, which is carried out by the CC. When the

computational complexity is relatively low, the benefits associated with the greater computational power afforded by dividing processing between the hemispheres are not large enough to outweigh the costs associated with integrating information across the hemispheres. Thus, processing is more efficient when all the information is directed to a single hemisphere, and IHI may be deleterious to task performance. By contrast, when the computational complexity is relatively high, the benefits associated with greater computational power begin to outweigh the costs of integrating processing between the hemispheres. Studies like those by Banich and her colleagues demonstrated that IHI increases the brain's information-processing capability during complex tasks by allowing the distribution of computations between the two hemispheres.

See also: Agnosia; Animal Models of Learning and Memory; Attention and Speed of Information Processing; Behavior Adaptation and Selection; Behavioral Planning: Neurophysiological Approach of the Frontal Lobe Function in Primates; Brain Imaging; Cognition: Learning and Memory: Spatial; Declarative Memory; Development and Language; Disorders of Face Processing; Emotions; Emotion–Cognition Interactions; Episodic and Autobiographical Memory: Psychological and Neural Aspects; Evolution of Emotions; From Sensation to Perception; Hemispheric Specialization: Language, Space, and Sexual Differentiation; Implicit Learning and Memory: Psychological and Neural Aspects; Language and Communication – Brain Substrate; Neural Basis of Working Memory; Orientation and Navigation; Plasticity in the Primary Auditory Cortex: Substrate of Specific Long-Term Memory Traces; Physical Cognition and Reasoning; Role of Neuronal Synchrony in Normal and Pathological Brain Functions; Short-Term Memory: Psychological and Neural Aspects; Vision; Voluntary Movement: Control, Learning and Memory.

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Control of Food Intake

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Glossary

Hormone (from Greek ὄρμη, meaning impetus) – A chemical released by cells that affects cells in other parts of the body. Endocrine hormones are released into the bloodstream. They typically bind to receptor proteins, resulting in the activation of a signal transduction mechanism and cell-type-specific responses.

Hypothalamus (from Greek ὑποθαλαμος, meaning under the thalamus) – A portion of the brain situated beneath the thalamus in the ventral diencephalon, which coordinates numerous endocrine processes, autonomic nervous system activities, and behavioral functions. The hypothalamus, sensitive to numerous forms of information, subserves many homeostatic processes, in part by synthesizing and secreting neuropeptides, including hypothalamic-releasing hormones that stimulate or inhibit the secretion of pituitary hormones.

Nucleus accumbens – Also known as the nucleus accumbens septi (from Latin, ‘nucleus leaning against the septum’), it is a collection of predominantly gamma-aminobutyric acid (GABA)ergic spiny neurons within the forebrain thought to play important roles in reward and motivation processes. The nucleus accumbens is composed of at least two structures – the nucleus accumbens core and the nucleus accumbens shell – with different morphology and function. The nucleus accumbens and the olfactory tubercle collectively form the ventral striatum, which is a part of the basal ganglia.

Peptide (from Greek πεπτίδια, meaning small digestibles) – An amino-acid-sequence polymer of ~50 residue length or shorter, in which the link between one amino acid residue and the next is an amide bond.

Vagus nerve – Also called the pneumogastric nerve or cranial nerve X, the vagus nerve is the 10th of the 12 paired cranial nerves and is the only nerve that originates in the medulla oblongata and projects, through the jugular foramen, to the neck, chest, and abdomen, where it contributes to the innervation of the viscera. Besides output to the various organs, the vagus nerve conveys sensory information about the state of the body’s organs to the central nervous system.

Indeed, 80–90% of the nerve fibers in the vagus nerve are afferent (sensory) nerves communicating the state of the viscera to the brain.

Peripheral Signals

Gut hormones play a major role in the regulation of food intake by signaling short- and long-term energy needs to the brainstem and mediobasal hypothalamus. Appetite-regulating neuropeptide systems in the brainstem and hypothalamus are activated or inhibited according to peripheral signals (Table 1), several of which are discussed below. Peripheral signals from the gastrointestinal tract, pancreas, liver, and adipose tissue allow for homeostatic control of appetite and energy expenditure.

Gastrointestinal Tract

Ghrelin

Ghrelin is a 28-amino-acid peptide hormone released from programmed death receptor 1 (P/D1) cells of the stomach fundus and, to a lesser degree, epsilon cells of the pancreas. Ghrelin is posttranslationally acylated at serine residue three by ghrelin O-acetyltransferase, a modification needed for its potent agonist activity at the G-protein-coupled growth-hormone-secretagog receptor (GHS-R). Central or peripheral injection of acylated, but not *des*-acyl, ghrelin rapidly initiates food intake and growth hormone release in animals, and chronic administration leads to weight gain and fat accumulation. Ghrelin administration in humans increases feelings of hunger. Ghrelin levels rise with fasting or weight loss, and fall following refeeding, leading to its proposed role as a meal initiator.

The orexigenic effects of ghrelin are mediated in part by activation of neuropeptide Y (NPY)- and agouti-related protein (AgRP)-expressing neurons in the arcuate nucleus (Arc) of the hypothalamus. GHS-R mRNA is expressed in many arcuate NPY/AgRP neurons, and ghrelin injection increases Fos expression in NPY/AgRP neurons. Intercerebroventricular injection of ghrelin increases NPY

Table 1 Major sites of synthesis and actions of selected appetite-regulating energy homeostatic molecules

<i>Site of Synthesis</i>	<i>Effect on Feeding</i>
<i>Gastrointestinal tract</i>	
Ghrelin	↑
Peptide YY	↓
Cholecystokinin	↓
Glucagon-like peptide-1	↓
Oxyntomodulin	↓
Enterostatin	↓
Bombesin-related peptides (gastrin-releasing peptide, neuromedins B and C)	↓
<i>Pancreas</i>	
Insulin	↓
Pancreatic polypeptide	↓
Amylin	↓
Glucagon	↓
<i>Adipose Tissue</i>	
Leptin	↓
<i>Hypothalamus</i>	
Neuropeptide Y	↑
Orexins	↑
Melanin concentrating hormone	↑
Agouti-related protein	↑
Galanin	↑
α-Melanocyte-stimulating hormone (from POMC)	↓
Corticotropin-releasing factor	↓
Urocortins 1, 2, and 3	↓
Cocaine and amphetamine-related transcript	↓
Thyrotrophin-releasing hormone	↓

expression in the hypothalamus and the orexigenic effects of ghrelin are absent in NPY/AgRP double null mutant mice and blocked by administration of a Y1 receptor antagonist. Ghrelin acts as an opponent to the anorectic adipocyte hormone leptin, disinhibiting NPY neurons and inhibiting pro-opiomelanocortin (POMC) neurons. The orexigenic response to ghrelin also involves specific inhibition of fatty acid biosynthesis resulting from AMP-activated protein kinase (AMPK) activation, which leads to decreased hypothalamic levels of malonyl-CoA and increased carnitine palmitoyltransferase 1 (CPT1) activity. An intact cannabinoid type 1 receptor-signaling pathway also is necessary for the stimulatory effects of ghrelin on AMPK activity and food intake.

In addition to activating neurons in the Arc of the hypothalamus, ghrelin has been shown to act on GHS-Rs in brainstem vagal afferent fibers. It was previously thought that ghrelin stimulated feeding by entering the brain through the blood-brain barrier, but recent studies emphasize that the orexigenic effects of ghrelin require the vagus nerve. Vagus nerve deafferentation abolishes the feeding effects of ghrelin. Binding, immunohistochemistry, and Fos expression studies also implicate vagal afferent fibers in the ability of ghrelin to induce growth hormone secretion and activate hypothalamic NPY neurons.

The hindbrain has also been shown to be involved in the effects of ghrelin. GHS-Rs are expressed in neurons in the

nucleus of the solitary tract (NTS) of the caudal brainstem. Correspondingly, local administration of ghrelin into the fourth ventricle or directly into the dorsal vagal complex increases food intake. Reciprocal connections are present between the NTS and hypothalamus, including an ascending noradrenergic pathway from the hindbrain to the hypothalamus. Thus, a component of ghrelin's orexigenic action following hindbrain administration may be mediated indirectly through activation of forebrain hypothalamic neurons. On the other hand, fourth ventricle injection of ghrelin increases Fos expression in the NTS, but not arcuate or paraventricular nucleus (PVN), suggesting that orexigenic effects of hindbrain ghrelin administration do not require hypothalamic activation.

In addition to increasing appetite, ghrelin also decreases fat expenditure and promotes fat storage. Conversely, ghrelin/GHS-R double null mutant mice exhibit decreased body weight, increased energy expenditure, and increased motor activity. Similarly, active immunization against the conserved N-terminal ghrelin residues slowed fat accrual in rats through a metabolic mechanism, with similar retardation of body weight gain reported in pigs.

Peptide YY

Peptide YY (PYY) is a 36-amino-acid gut hormone released by L cells of the ileum/colon in proportion to

ingested calories. PYY is functionally related to NPY and binds to the Y family of receptors, Y1R–Y6R. Most circulating PYY is the truncated form, PYY_{3–36}, a cleavage product yielded by dipeptidyl peptidase IV. PYY is a putative satiety signal released following a meal, decreasing food intake. Levels of PYY are reduced following a fast. Peripheral injection of PYY_{3–36} reduces food intake in rodents and humans, but anorectic effects of PYY are highly sensitive to mild stressors such as a new environment. PYY is hypothesized to enter the brain through the area postrema and NTS. Peripheral administration of PYY increases Fos immunoreactivity in the NTS and area postrema, and anorectic effects of PYY are diminished following transection of brainstem–hypothalamus connections. Anorectic effects are suppressed by a Y2R antagonist, suggesting that PYY_{3–36} acts through the Y2R receptor.

Cholecystokinin

Cholecystokinin (CCK) and related bioactive fragments such as the C-terminal sulfated octapeptide CCK-8 are released from I cells in the mucosal epithelium of the small intestine following a meal. CCK peptides can bind two G-protein-coupled receptors with nanomolar affinity (CCK1 and CCK2). CCK1 is abundant in peripheral organs, including the pancreas, gall bladder, pyloris, intestine, and vagus nerve, as well as in a few discrete brain regions, whereas CCK2 is the predominant CNS subtype, with limited distribution in the stomach and vagus nerve in some species. CCK secretion causes the release of digestive enzymes from the pancreas and bile from the gall bladder and inhibits gastric emptying. CCK is released postprandially, suppressing hunger and reducing food intake in part through CCK1 receptors on the vagus nerve. Accordingly, mice that lack CCK1 receptors are insensitive to the anorectic actions of CCK8. CCK1 antagonists increase food intake, and Otsuka Long-Evans Tokushima Fatty rats, which lack functional CCK1 receptors, are obese secondary to hyperphagia. CCK2 receptors do not appear to be essential for maintenance of normal body weight, though they help stimulate gastric acid secretion.

CCK1 systems synergistically and reciprocally interact with the adipocyte leptin hormone to curtail food intake. For example, CCK1 receptor agonists increase permeability of the blood–brain barrier to leptin, and leptin administration enhances the sensitivity of gastric and duodenal vagal afferents to CCK, thereby facilitating meal termination.

Glucagon-like peptide-1

Glucagon-like peptide-1 (GLP-1) is derived from the precursor protein preproglucagon and is secreted by L cells in the small intestine following food intake. The most common form of GLP-1 *in vivo* is GLP-1_{7–36}amide,

which can act both peripherally and centrally. Peripheral GLP-1 is an incretin, acting on GLP-1 receptors (Glp1r) in the beta cells of the pancreas to stimulate insulin secretion and decrease glucagon secretion. Central GLP-1 is localized in the NTS and area postrema and acts as a neuropeptide to control food intake and whole body glucose in hyperglycemic conditions. It decreases food intake and feelings of hunger, and also has effects on autonomic function. The GLP-1 competitive antagonist exendin-4_{9–39}, a truncated form of the GLP-1 agonist exendin-4, increases food intake when administered acutely and increases body weight when administered chronically.

Oxyntomodulin

Oxyntomodulin is a 37-amino-acid peptide product of a preproglucagon precursor molecule that is released after a meal by oxyntic cells in the oxyntic mucosa of the colon. It appears to act through GLP-1 receptors and, like GLP-1, reduces food intake. The anorectic effects of oxyntomodulin are abolished in GLP-1 receptor knockout mice and are blocked following administration of exendin-4_{9–39} administration to the Arc. Chronic administration of oxyntomodulin leads to weight loss and may increase energy expenditure.

Enterostatin

Enterostatin (the amino-acid sequence APGPR) is a pentapeptide derived from the precursor protein procalipase, produced by the exocrine pancreas, and expressed in the gastric mucosa of the gastrointestinal tract. Procalipase is also expressed centrally, in the PVN, amygdala, and cortex, and APGPR mRNA is expressed in the Arc. APGPR reduces insulin secretion and stimulates adrenal corticosteroid secretion. It leads to a feeling of fullness and reduces consumption of fat, specifically. Central injection of APGPR potently decreases intake of a high-fat diet, but has no effect on intake of a high-carbohydrate, low-fat diet. The effects of APGPR on fat intake are mediated by the melanocortin system, with studies showing the involvement of melanocortin receptor 4 (MC4R). The serotonin system is also involved. Injection of APGPR to the amygdala activates neurons in serotonergic brain regions that innervate the PVN. Administration of a serotonin 5HT1B receptor antagonist blocks the anorectic effects of injection of APGPR to the amygdala.

Enterostatin also reduces serum cholesterol levels, an effect that is blocked by administration of a CCK1R antagonist, suggesting involvement of the CCK system.

Gastrin-releasing peptide/neuromedin/bombesin family

Bombesin (BN) is an amphibian tetradecapeptide that shares a common C-terminal heptapeptide sequence with the mammalian peptides, gastrin-releasing peptide

(GRP_{1–27}) and neuromedin C (GRP_{18–27}). The C-terminal heptapeptide sequence of neuromedin B (NMB), which occurs in 30- and 32-residue precursors and in a 10-amino-acid residue mature form, differs by two amino acids from BN (i.e., Thr¹⁰ and Phe¹³ vs. Val¹⁰ and Leu¹³, respectively).

Central or systemic administration of BN, GRP, and NMB all decrease feeding in rodents, with the BN family peptides showing greater anorectic potency when administered into the cerebral ventricles than peripherally. The biological actions of BN are mediated through two pharmacologically distinct, cloned G-protein-coupled receptors, BB₁, also known as the NMB-preferring receptor, and BB₂, also known as the GRP-preferring receptor. During feeding, GRP immunoreactivity increases in the hypothalamus and central amygdala as well as in the antrum of the stomach. GRP has therefore been hypothesized to act as a negative feedback satiety signal in mammals through its neuronal and humoral effects. Consistent with this possibility, mice that lack BB₂ receptors (GRP-preferring receptors) eat more food than wild-type mice and show impaired glucose tolerance, with impaired early insulin and GLP-1 responses. These functions correspond to the high expression of BB₂ receptors in human pancreas, stomach, adrenal cortex, and brain. Altered function of GRP and/or BB₂ receptors also has been implicated in anorexia nervosa, bulimia, and mood disorders.

Consistent with the observed pharmacology, NMB anorexia, in contrast to GRP anorexia, occurs independent of BB₂ stimulation and is thought to be BB₁ mediated. Two association studies suggest a role of NMB genes in human eating disorders and obesity.

In addition to the BB₁ and BB₂ receptor subtypes, an orphan BN receptor subtype 3 (BB₃), identified in guinea pig uterus, is widely distributed in feeding-regulatory centers, including hypothalamic nuclei. The endogenous natural ligand for BB₃ remains unclear, because the receptor shows little affinity for BN, GRP, or NMB. Animal models indicate that the BB₃ subtype may have roles in energy balance and glucose homeostasis, but a Japanese association study suggests that BB₃ receptor gene mutations are unlikely to be a major cause of obesity in humans.

Pancreas

Insulin

Insulin is a 51-amino-acid peptide hormone synthesized in the pancreas and released from pancreatic β -cells following a meal. It signals cells to take up glucose from the blood, storing it as glycogen. Insulin reduces food intake and is an adiposity signal, increasing with increases in body fat stores. Insulin resistance can occur with excessive weight gain. If the pancreas fails to compensate for insulin

resistance by raising basal insulin levels as well as raising insulin levels postprandially, then hyperglycemia can occur. This relationship contributes to the association of type 2 diabetes with obesity. Insulin receptors in the hypothalamus inhibit neuropeptide systems that increase food intake, leading to a decrease in food intake when insulin is detected.

Pancreatic polypeptide

Pancreatic polypeptide is synthesized in and released from the endocrine pancreas after a meal and reduces appetite. Like PYY and NPY, pancreatic polypeptide binds to the Y family of receptors, preferentially to Y4 and Y5. Peripheral injection of pancreatic polypeptide reduces food intake, and chronic administration decreases body weight in obese mice. Mice that overexpress pancreatic polypeptide show reduced food intake and body weight, as well as reduced gastric emptying.

Amylin

Amylin, also known as islet amyloid polypeptide, is a 37-amino-acid peptide that is released from pancreatic β -cells along with insulin following a meal. It is thought to be involved in glucose homeostasis and also reduces food intake when administered at high levels peripherally.

Glucagon

Glucagon is a hormone released by alpha cells in the endocrine portion of the pancreas when blood glucose levels are low. It binds to G-protein-coupled glucagon receptors on hepatocytes, causing the liver to convert glycogen to glucose and release glucose into the bloodstream. Glucagon acts as an opponent to insulin, which signals cells to take up glucose in times of satiation, to maintain blood glucose homeostasis.

Adipose Tissue

Leptin

Leptin is an important protein hormone produced by adipose tissue. It binds to six receptor types, LepRa–LepRf, but LepRb is the only receptor that contains active intracellular signaling domains. Leptin levels in the blood are proportional to body fat content. An increase in body fat leads to an increase in leptin, which leads to a decrease in food intake and increase in energy expenditure. As with insulin receptors, LepRb receptors are abundant in the hypothalamus and inhibit neuropeptide systems that induce food intake, leading to decreased food intake when leptin is detected. Inadequate leptin signaling, whether due to leptin deficiency (e.g., *ob/ob* mice) or mutated leptin receptors (e.g., *db/db* mice, obese [*fa/fa*] Zucker rats), causes obesity and hyperphagia in both rodents and humans. Leptin resistance develops with obesity and is common in obese humans. Leptin levels

decrease during weight loss, leading to increased food intake.

Brainstem

The brainstem receives peripheral information about the current state of energy balance from reviewed peripheral endocrine feedback signals as well as direct neural innervations. Brainstem circuitry integrates these neural and hormonal indicators from the gut and elsewhere, communicates with other brain regions such as the hypothalamus, and regulates food intake according to energy needs. Brainstem motor systems involving the nucleus tractus solitarius (NTS) are involved in the control of feeding and respond to gut hormones through cranial nerves. The dorsal vagal complex is also of particular importance in the integration of neural signals.

Brainstem Motor Systems and Peripheral Signaling

Brainstem motor systems control eating behavior and food digestion. The trigeminal, facial, and hypoglossal cranial nuclei in the brainstem are responsible for the chewing and swallowing motor behaviors involved in eating. The dorsal motor nucleus of the vagus (DMX) contains motoneurons that project to the gut, liver, and pancreas to control gastric motility and secretion. Neurons in the DMX are organized topographically, with descending fiber projections organized in distinct columns that control different parts of the gut. Positive feed-forward information signaling the presence of food in the mouth or stomach is relayed from the gut to cortex, amygdala, and hypothalamus, and then transmitted to the brainstem. The brainstem integrates information and sends efferent signals to initiate chewing and gastric motility when appropriate.

Negative feedback from the gut is processed by intermediate, subpostrema, and caudal NTS in the brainstem. Tracing studies have shown topographically organized projections from the gut to NTS. The NTS receives mechanical and chemical satiety signals through the mechanosensitive and chemosensitive vagal fibers in the stomach. Mechanosensitive fibers respond to stomach distension and changes in gastric load following a meal, but do not detect nutrient content. Distension of the duodenum can also reduce feeding through satiety signals, but depends upon nutrient content rather than only mechanical stretch. Ingestion of food rich in amino acids, glucose, or lipids induces Fos activity in the NTS. Nutrient ingestion leads to the release of CCK from the duodenum, which signals to the brainstem through vagal afferent fibers. Vagal afferents terminate in the medial nucleus of the NTS, which is part of the dorsal vagal

complex in the medulla oblongata. The dorsal vagal complex is bidirectionally connected to the hypothalamus, making the brainstem an important link between peripheral hormones and hypothalamic neuropeptides. In addition to vagal input to the NTS, cervical ganglion and cervical spinal afferents play an important role in transducing peripheral feedback through direct neural mechanisms.

Chronically maintained decerebrate rats, whose caudal brainstem is dissociated from the forebrain, are able to regulate meal size and show similar energetic response to food deprivation as control rats, with both groups showing reduced energy expenditure and respiratory quotient. Decerebrate and control rats also respond to insulin administration with increased intraoral intake, and both groups respond to administration of the competitive glucose analog 2-deoxy-D-glucose (2DG) with sympathoadrenal hyperglycemia. Thus, the caudal brainstem is sufficient to maintain some aspects of the control of food intake and energy expenditure.

Hypothalamic Control of Food Intake

Along with the brainstem, the hypothalamus is a crucial brain region for the regulation of food intake and energy homeostasis, integrating signals from the hindbrain, periphery, and limbic regions. In the dual-center model of appetite control, the ventromedial hypothalamic nucleus (VMH) was hypothesized to be the satiety center of the brain, whereas the lateral hypothalamic nucleus (LH) was represented as the hunger center. While these regions are still recognized to have important roles in energy homeostasis, a more complex appetite-regulating network has been identified in the hypothalamus that involves not only the VMH and LH, but also the major roles of the Arc and the PVN. The hypothalamic control of food intake is achieved through the transmission of orexigenic or anorexigenic neuropeptides from neurons localized in these and other hypothalamic nuclei, as reviewed in this section.

Orexigenic Neuropeptides

Neuropeptide Y

Neuropeptide Y (NPY) is found in high concentrations in the hypothalamus and is the major orexigenic peptide in the brain. It is a 36-amino-acid peptide with six receptor types, Y1–Y6. Only the Y1 and Y5 subtypes have been shown to be involved in the orexigenic effects of NPY. Hypothalamic NPY is synthesized in large part by neurons in the Arc, which project to PVN, dorsomedial nucleus (DMN), and the median preoptic area. Neurons expressing NPY also express AgRP, another potent orexigenic neuropeptide.

Injection of NPY into the ventricles or hypothalamus increases food intake, increases meal duration, reduces latency to eat, increases the motivation to obtain food, and delays satiety. Neurons in the Arc, PVN, and hindbrain detect available energy through interactions with peripheral hormones and central peptides, and activate the NPY system if not enough energy is available to fulfill the body's metabolic needs. Thus, overnight food deprivation increases NPY expression in the Arc and PVN, and NPY immunoreactivity in hindbrain and hypothalamus increases with chronic food deprivation. NPY levels quickly return to normal following refeeding. The NPY system is activated by ghrelin, which is released in association with feelings of hunger and increases appetite. Orexigenic effects of ghrelin are suppressed when NPY or AgRP is blocked.

NPY neurons in the Arc contain leptin, insulin, and glucocorticoid receptors, allowing them to detect changes in hormone levels that occur with food deprivation, energy repletion, and changes in adiposity. Leptin and insulin form a negative feedback loop with NPY to regulate energy homeostasis. In times of negative energy balance (food deprivation and active depletion of body fat), leptin levels decrease, thus leading to increased NPY gene expression and increased food intake. In times of positive energy balance (excess energy intake and gains in adiposity), leptin levels increase, thus leading to reduced NPY levels and decreased food intake. Hyperphagia is often seen in diabetic states because the low levels of insulin (or insulin action) are unable to inhibit NPY neurons. Accordingly, treatment of diabetes with insulin can normalize NPY activity in the hypothalamus and decrease food intake.

Orexins

Orexins, also known as hypocretins, are orexigenic neuropeptides involved in the regulation of the sleep-wake cycle and feeding. Two hypocretins, orexin A, a 28-amino-acid peptide, and orexin B, a 33-amino-acid peptide, are synthesized in LH. The orexins bind to two orexin receptor subtypes, OX1-R in VMH and Arc and OX2-R in the PVN and hindbrain. Injection of orexin A and B into the ventricles or hypothalamus can increase food intake, but less potently than NPY. Orexins also increase drinking, food seeking, and spontaneous activity.

The orexin system is bidirectionally connected to the NPY system. NPY expression is increased with intraventricular injection of orexins, and NPY Y1 and Y5 receptor antagonists reduce the orexigenic effects of orexin injection. GABA is also involved in the modulation of orexin activation. GABA neurons co-express orexin, and orexin neurons are activated by a GABA agonist.

Orexin neurons are glucosensitive and respond to changes in blood glucose levels rapidly, making them an early hypothalamic factor for triggering food ingestion.

Glucosensitivity makes orexins highly sensitive to changes in food intake. A reduction in food intake leads to increased orexin concentrations in the LH, increased orexin gene expression, and increased expression of orexin receptors. As with NPY, orexins are also sensitive to changes in leptin levels. Leptin inhibits orexin gene expression, so an increase in leptin due to satiety or increased adiposity suppresses orexin activity in the hypothalamus, leading to decreased food intake.

Melanin-concentrating hormone

Melanin-concentrating hormone (MCH) is a 19-amino-acid peptide that is synthesized in the LH and binds to two receptor types, MCH-R1 and MCH-R2. MCH neurons are directly connected to orexin neurons and contain OX1-R receptors, as well as cannabinoid CB1 receptors.

Central injection of MCH increases food intake, and overexpression of MCH leads to obesity and insulin resistance. MCH is less potent than NPY, but as potent as orexins. Chronic MCH administration increases food intake and leads to weight gain when animals are fed a high-fat diet. Increased food intake is caused by action at the MCH-R1 receptor, which is widely distributed in the brain.

MCH neurons are not glucosensitive, but they are affected by food restriction. Fasting for 24 h leads to increased expression of MCH. As with NPY and orexins, MCH is inhibited by leptin. Central injection of leptin leads to decreased MCH expression, decreasing orexigenic signaling. MCH also interacts with other hypothalamic neuropeptides. For example, injection of MCH increases NPY expression and stimulates the release of NPY.

Agouti-related protein

Agouti-related protein (AgRP) is a neuropeptide released by NPY/AgRP neurons in the Arc of the hypothalamus. It plays a significant role in energy balance, in part through its inverse agonist action at anorectic melanocortin receptors 3 and 4. AgRP binds to MC3-R and MC4-R, antagonizing anorectic melanocortins such as α -melanocyte-stimulating hormone (α -MSH) that bind to MC3-R and MC4-R. Central administration of AgRP increases food intake potently and with long-lasting effects. Obese mice have increased levels of hypothalamic AgRP, and mice overexpressing AgRP are obese and hyperphagic. Like NPY, AgRP is downregulated by leptin and insulin, with increased leptin and insulin levels leading to decreased AgRP levels. Conversely, administration of the appetite-stimulating hormone ghrelin upregulates AgRP expression and induces Fos expression in NPY/AgRP neurons.

Galanin

Galanin is 29-amino-acid peptide formed by the cleavage of a prepropeptide encoded by the GAL gene. It binds to three receptor types, GALR1-3. Galanin plays a role in the regulation of several neuroendocrine functions in the hypothalamus, anterior pituitary, and peripheral organs. It acts as an orexigenic neuropeptide in the regulation of food intake. Neurons expressing galanin are found throughout the hypothalamus, including the paraventricular nucleus and Arc. Administration of galanin to the PVN increases food intake without increasing feeding-related behaviors such as drinking and grooming. Orexigenic effects of galanin are not as strong or long lasting as the effects of NPY however. The effects of chronic administration of galanin depend on the conditions of the administration. An early study showed that chronic administration did not lead to obesity, but chronic administration to mice lacking endogenous galanin did lead to increased food intake and obesity. Galanin appears to be related to fatty acid metabolism, as female rats fed a high-fat, but not high-carbohydrate or high-protein, diet have highly elevated levels of galanin in the PVN. In addition, injection of galanin to the PVN increases body fat accrual in rats on a high-fat diet, suggesting that galanin may directly influence fat oxidation or fat lipogenesis.

Anorectic Neuropeptides

Anorectic neuropeptides found in the hypothalamus include the melanocortin α -melanocyte-stimulating hormone (α -MSH), corticotropin-releasing factor (CRF), urocortins 1, 2, and 3, thyrotropin-releasing hormone (TRH) and cocaine- and amphetamine-regulated transcript (CART). Below we summarize findings concerning the important melanocortin control of food intake.

Melanocortins

Melanocortins are anorexigenic peptides cleaved from the POMC precursor polypeptide that can bind to MC1-MC4 receptors. The most abundant melanocortin found in the hypothalamus is α -MSH. POMC-expressing neurons in the Arc comprise a more distinct population of neurons than those that express NPY/AgRP and also co-express CART. Similar to NPY/AgRP neurons, POMC neurons appear to be nodal points of peripheral feedback to the CNS, but in this case subserving an anorectic role, and show major ascending projections to the PVN and LH.

Activation of the POMC system leads to anorexia. Synthetic MC3 and MC4 receptor agonists suppress food intake, whereas antagonists for those receptors increase intake. AgRP is a natural antagonist for melanocortin receptors and thereby increases food intake. POMC/CART neurons contain leptin and insulin receptors and are thus sensitive to changes in leptin and insulin

levels. Unlike NPY, POMC expression is increased with increases in leptin levels that come as a result of overfeeding and weight gain. Injection of leptin into the Arc also increases POMC expression. Increased POMC expression results in reduced food intake. Leptin or insulin deficiency decreases POMC expression, leading to promotion of food intake. Anorexia induced by increased leptin levels can be reversed by administration of a melanocortin receptor antagonist.

Other appetite regulatory peptide systems interact with melanocortin systems. For example, MCH inhibits the release of α -MSH. Some evidence suggests that PYY₃₋₃₆ reduces food intake by inhibiting melanocortin neurons, but PYY₃₋₃₆ reduces intake in the absence of melanocortin signaling, suggesting that while melanocortins may play a role in PYY action, they are not necessary for it.

As shown in **Figure 1**, POMC neurons receive inhibitory GABAergic collateral input from appetite-stimulatory NPY/AgRP neurons such that stimulation of NPY/AgRP neurons facilitates food intake both through direct effects of the orexigenic pathway and by inhibiting the anorexigenic pathway. A reciprocal inhibition of NPY/AgRP neurons by POMC neurons has not been identified, an absent regulatory mechanism that some have speculated contributes to the less strict control over positive energy balance.

Role of Reward Systems in Control of Food Intake

While food intake is regulated homeostatically by the interaction of peripheral hormones with hypothalamic neuropeptides, it is also modulated in partly nonhomeostatic fashion by corticolimbic reward pathways. Hunger and satiety signals from the periphery and hypothalamus are integrated with motivationally relevant reward and pleasure signals from the ventral tegmental area (VTA), nucleus accumbens (NAcc), and ventral pallidum (VP), among other brain regions, as briefly summarized below.

Dopamine

It is widely accepted that dopamine plays a significant role in reward and motivation. Dopamine neurons in the VTA project to the NAcc and cortex, creating a mesocorticolimbic dopamine circuit. Dopamine is released with the consumption of pleasurable food and other rewards. When animals seek and receive food rewards, dopamine neurons are activated and fire more in relation to preferred food than nonpreferred food. Food reward is enhanced with the activation of dopamine neurons.

However, the exact nature of dopamine's role in reward is controversial. It has been hypothesized that dopamine may mediate the pleasure in a reward, or liking;

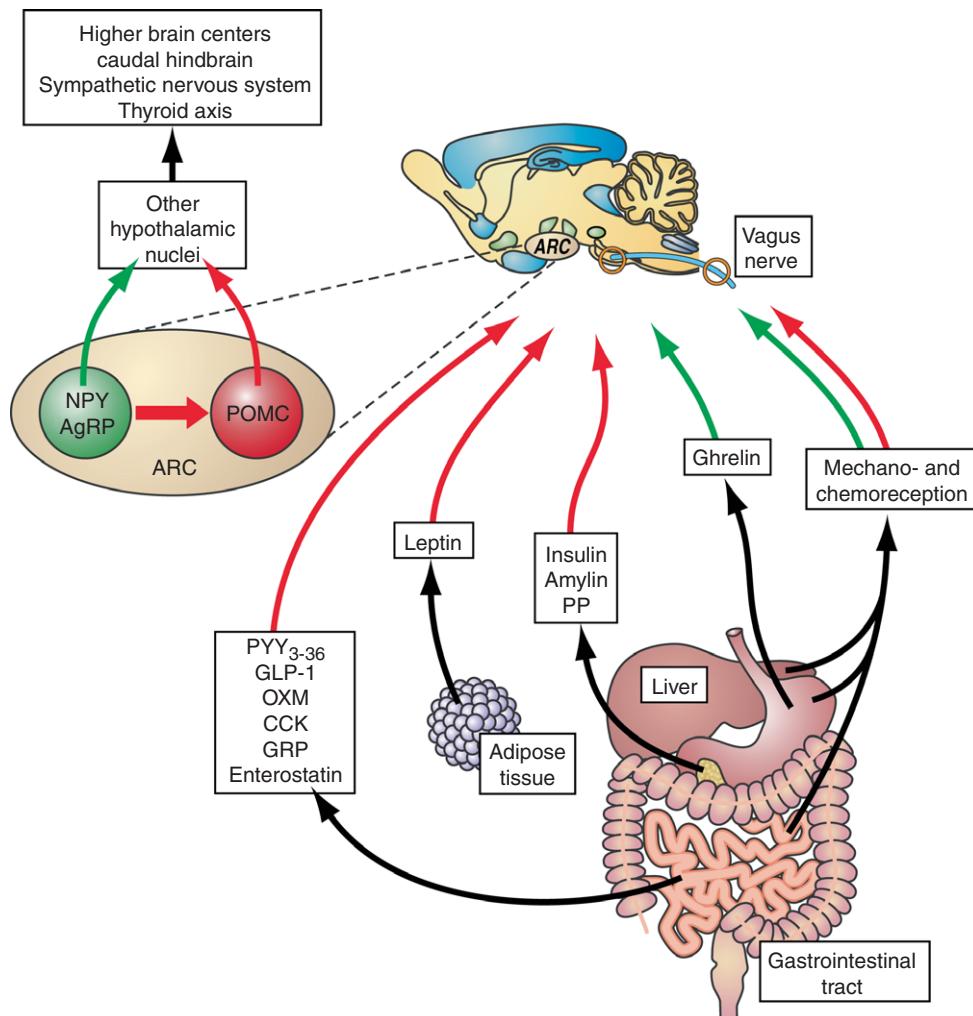


Figure 1 Role of peripheral signals in the regulation of food intake. Following a meal, PYY₃₋₃₆, glucagon-like peptide-1 (GLP-1), oxyntomodulin (OXM), cholecystokinin (CCK), gastrin-releasing peptide (GRP), and enterostatin are putatively released from the gastrointestinal tract, while insulin, amylin, and pancreatic polypeptide (PP) are released from the pancreas, signaling to the brain to inhibit food intake. The anorectic signal leptin is released from adipose tissue in proportion to body fat. During a fast, ghrelin is released from the stomach to stimulate food intake. Feeding-relevant signals encoded by mechanoreceptors (e.g., stretch/pressure) and chemoreceptors (e.g., pH and osmolarity) in the stomach and liver are relayed by the vagus nerve and splanchnic nerve fibers that project to the caudal hindbrain. The arcuate nucleus of the hypothalamus (Arc) helps integrate blood-borne peripheral signals, and NPY/AgRP and POMC neurons in the Arc signal to other hypothalamic nuclei and brain regions. Green arrows indicate stimulatory effects and red arrows indicate inhibitory effects on appetite. Adapted with permission from Murphy KG and Bloom SR (2006) Gut hormones and the regulation of energy homeostasis. *Nature* 444: 854–859.

it may regulate learning of predictions of future reward; or it may mediate the drive or desire to obtain a reward, or wanting. Taste reactivity studies monitoring the reaction of rats to different foods are able to identify when a rat likes or dislikes a food. Such studies have shown that the animal's liking of a food is not affected by the loss of striatal dopamine neurons or by the blockade of dopamine transmission. Electrophysiology studies in monkeys have shown that dopamine neurons do not fire in response to juice reward after the reward prediction is learned. Thus, dopamine is not a pleasure neurotransmitter and does not mediate the hedonic value of foods.

Instead, recent evidence has implicated dopamine as a motivational neurotransmitter, mediating the wanting of a reward. It is thought that activation of dopamine neurons enhances the desire for a reward through incentive salience, a process by which salience is attributed to a stimulus, making it wanted. Motivational value is assigned to the stimulus, causing the animal to seek it. Incentive salience is related to the sensory representation of the stimulus (i.e., how the food tastes, smells, and looks), what has been learned about the stimulus, and the current energy needs. Dopaminergic incentive salience interacts with peripheral and hypothalamic energy regulatory

signals. For example, after food deprivation, the incentive salience of food increases, making the subject want it more. The concept of incentive salience can explain how dopaminergic mesolimbic systems participate in motivation without encoding the hedonic value of a stimulus.

Opioids

Brain opioid systems also regulate food intake by mediating the rewarding properties of food. Opioids have been shown to be involved in the pleasure or liking of a food, as well as wanting of a food. Opioid transmission in a highly interconnected network of forebrain regions increases the hedonic value of foods, enhancing food reward. In particular, hedonic hot spots of μ -opioid receptors in the NAcc shell and VP have been identified as critical for liking reactions. Taste reactivity studies have shown that μ -opioid stimulation of a cubic millimeter in rostromedial NAcc shell and in posterior VP increase liking facial reactions to foods. In addition, injections of opioids into the NAcc and VP hot spots, as well as the other areas in NAcc, increase food intake, potentially consistent with an increase in wanting. In this case, there was no wanting hot spot in the NAcc, but instead a diffuse opioid network throughout NAcc that increased food intake. Thus, NAcc and VP are involved in both hedonic and motivational aspects of food reward. The NAcc and VP are highly interconnected and work together to enhance the hedonic value of food. The two regions modulate Fos expression in each other and μ -opioid stimulation in both regions simultaneously is necessary for enhancement of liking reactions to food. However, the NAcc appears to dominate the actual consumption of food, with opioid

injection into the NAcc alone increasing food intake. Thus, the opioid system in the ventral striatum is a key component of reward-related control of food intake.

Endocannabinoids

Endogenous cannabinoids (ECs) are thought to interact with energy regulatory systems in the periphery and hypothalamus, as well as in reward systems in limbic regions. The endocannabinoids anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) have been shown to interact with leptin, the peripheral adiposity signal that decreases food intake. Leptin deficient mice (*ob/ob*) and leptin receptor signaling-deficient rats (*Zucker fa/fa*) have increased levels of 2-AG in the hypothalamus, while intraperitoneal administration of leptin decreases EC levels in the hypothalamus. ECs have been also shown to affect regulation of food intake. CB1 receptor antagonists decrease food intake as well as abdominal adiposity. LH-stimulated food intake is enhanced by the CB1 agonist tetrahydrocannabinol (THC), and blocked by CB1 antagonists.

EC transmission also appears to mediate the hedonic value of food. Microinjection of AEA into the medial shell of the NAcc increases liking reactions to sucrose taste. CB1 receptor density is decreased in rats fed a palatable diet, which may indicate increased EC activity with intake of pleasurable food. Further interactions with limbic reward systems were shown in studies that demonstrated increases in AEA and 2-AG levels in limbic regions after food deprivation and an increase in food intake after injection of 2-AG into the NAcc shell. Because of their role in both hypothalamic and limbic

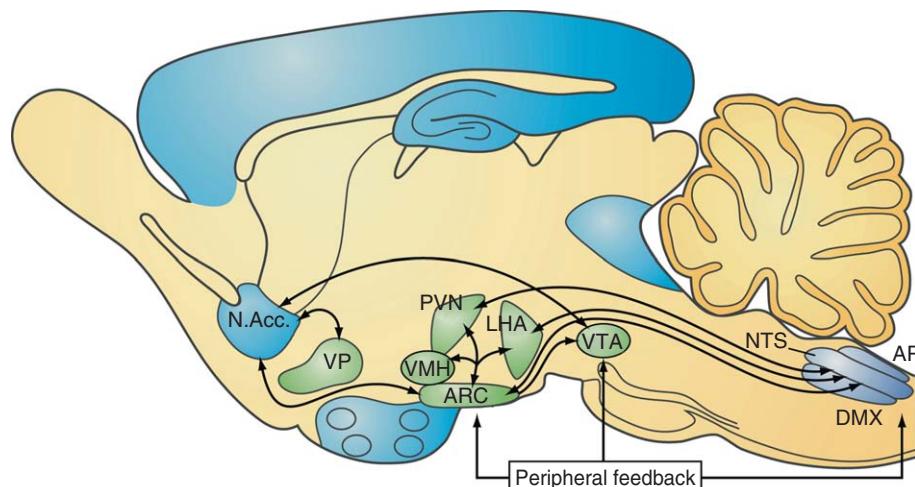


Figure 2 Neural circuitry that regulates food intake and energy homeostasis. Peripheral feedback signals converge on the arcuate nucleus of the hypothalamus (ARC), the ventral tegmental area (VTA), and the nucleus of the solitary tract (NTS). Reciprocal connections among hypothalamic nuclei, reward pathways, and the caudal brainstem allow for coordinated control of food intake and energy homeostasis. AP, area postrema; DMX, dorsal motor nucleus of the vagus nerve; LHA, lateral hypothalamus; VMH, ventromedial hypothalamus; PVN, paraventricular nucleus; VP, ventral pallidum; N. Acc, Nucleus accumbens. Adapted with permission from Morton GJ, Cummings DE, Baskin DG, Barsh GS, and Schwartz MW (2006) Central nervous system control of food intake and body weight. *Nature* 443: 289–295.

feeding-regulatory systems, ECs have been proposed to integrate energy homeostatic- and reward-based aspects of food intake control.

Summary

Figure 1 summarizes the manner in which peripheral gut-, pancreas-, and adipose tissue-derived endocrine hormones and visceral neural feedback signal the brain to stimulate (green arrows) or inhibit (red arrows) appetite in the service of energy homeostasis. **Figure 2** emphasizes that much of this peripheral feedback directly converges on the medio-basal hypothalamus, including the Arc of the hypothalamus and its ascending projections to other hypothalamic nuclei; the caudal brainstem, through vagal and splanchnic afferents and endocrine transmission; and brain reward pathways, such as the mesolimbic dopamine system. These feedback integration sites in the CNS intercommunicate to achieve a coordinated control of food intake with both energy and nonenergy homeostatic components.

Acknowledgments

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See also: From Sensation to Perception.

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Relevant Websites

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- <http://web.sfn.org> – Society for Neuroscience – Appetite and Food Intake.
- <http://www.obesity.org> – The Obesity Society – Links
- <http://www.ssib.org> – The Society for the Study of Ingestive Behavior.
- <http://www.yaleruddcenter.org> – Yale Rudd Center for Food Policy and Obesity.

Cytokine Effects on Neuronal Processes and on Behavior

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Glossary

Acute-phase proteins – The serum proteins (e.g., haptoglobin, α 1-antitrypsin, α 1 and α 2 macroglobulin, and C-reactive protein) released from the liver that increase during acute inflammation. Negative acute-phase proteins (e.g., albumin), in contrast, decrease during inflammation.

Adrenocorticotrophic hormone (ACTH) – The polypeptide hormone secreted by the anterior pituitary; stimulates cells of the adrenal cortex to secrete cortisol.

Anhedonia – The loss of interest or pleasure in activities that would ordinarily be perceived as rewarding.

Antigen – Any foreign material that is specifically bound by a specific antibody or specific lymphocytes.

Apoptosis – Programmed cell death in which the chromatin becomes condensed and the DNA is degraded. No inflammation is typically observed, unlike passive death processes where leakage of organelles and intracellular proteases induce inflammatory reactions.

Autocrine – The action of a hormone on the same cell that synthesized it.

Chemokines – A family of 8- to 10-kDa cytokines that stimulate leukocyte movement and directed movement.

Corticotropin-releasing hormone (CRH) – Hypothalamic hormone that stimulates ACTH secretion by the anterior pituitary and acts as a neurotransmitter in the brain.

Cytokines – Immune system proteins that are biological response modifiers. They coordinate antibody and T-cell immune system interactions and amplify immune reactivity. Proinflammatory cytokines trigger inflammation. Cytokines include monokines (e.g., interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF α)) synthesized by macrophages, and lymphokines (e.g., interleukins, gamma interferon, and lymphotoxin) produced by activated T-lymphocytes and natural killer (NK) cells.

Glucocorticoid – One of a group of hormones (e.g., cortisol) of the adrenal cortex that are important in protein and carbohydrate metabolism, secreted especially in times of stress.

IL-6 – A cytokine, formerly known as B-cell differentiation factor, produced by vascular endothelial cells, phagocytes, fibroblasts, activated T-lymphocytes, and neoplasms. IL-6 is secreted in response to IL-1 or

TNF. It mainly acts on B cells and hepatocytes. Its main function is to cause B lymphocytes to differentiate into cells that synthesize antibodies. IL-6 also induces hepatocytes to form acute-phase proteins including fibrinogen.

Interferon-gamma (IFN- γ) – A cytokine synthesized mainly by activated T-helper cells; it activates killer T-cells, NK cells, and macrophages.

Interleukin (IL)-1 – Produced by monocytes and macrophages, it stimulates acute-phase protein synthesis and promotes T-cell proliferation; it is also an endogenous fever-producing substance. IL-1 is comprised of two principal polypeptides: IL-1 α and IL-1 β .

Interleukins – A group of cytokines synthesized by lymphocytes, monocytes, and other cells, which promote growth of T- and B-cells and other hematopoietic stem cells, and have a variety of other biological functions.

Leukocyte – White blood cell. The principal types include the granulocytes (neutrophils, eosinophils, and basophils), lymphocytes (T- and B-cells), and monocytes (macrophages).

Lymphocytes – Small cells with virtually no cytoplasm, and bearing antigen-specific receptors, which are found in blood, in all tissue, and in lymphoid organs, such as lymph nodes, spleen, and Peyer's patches. Lymphocytes are divided into two principal groups: T-and B-lymphocytes. In addition, NK cells, which are large granular lymphocytes, comprise a small percentage of the lymphocyte population. Lymphocytes are distinguished by the expression of distinctive surface molecules that have precise roles in immune reaction.

Microglia – A type of glial cell present within the central nervous system that serves in a defensive capacity, constantly seeking damaged neurons, plaques, and infectious agents.

Motivation – A central state that organizes perception and action.

Myeloid cells – An immature bone marrow cell that is a precursor of the polymorphonuclear (multi-nucleated) leukocytes. Typical polymorphonuclear cells include neutrophils, eosinophils, and basophils, which are all present in the peripheral blood.

Natural killer (NK) cells – The cells that attack and destroy tumor cells and certain virus-infected cells. These cells induce lysis through the action of antibody.

Immunologic memory is not involved, in that previous contact with the antigen is not necessary.

Paracrine – The local effects of a hormone acting on cells in its immediate vicinity.

Prostaglandins – Biologically active lipids, with hormonal actions, derived from arachidonic acid by actions of the enzyme, cyclooxygenase. These are found in virtually all Mammalian tissues. Prostaglandins have numerous actions including decreasing blood pressure, stimulating muscle contraction, regulating inflammation and blood clotting, and modulating immune responses.

Pyrogen – A substance that induces fever. It may be endogenously produced, such as by IL-1, or may result from exogenous infection (e.g., bacterial or viral insults).

Transforming growth factor-beta (TGF- β) – A cytokine that induces the proliferation of many cell types, including epidermal and epithelial cells. It also facilitates the growth of selected transformed cells.

Tumor necrosis factor- α (TNF- α) – A monokine produced by macrophages. It participates in inflammation, wound healing, and tissue remodeling, and has potent antitumor properties and is associated with cachexia. More recently, this cytokine has been shown to be active in the central nervous system.

instance, recently identified interleukins, including IL-18 and IL-23, may contribute to neurodegenerative process that occur in multiple sclerosis, Alzheimer's disease (AD), and stroke. Conversely, other trophic cytokines, such as granulocyte colony-stimulating factor (G-CSF) and TGF- β , impart neuroprotective effects in models of stroke, traumatic head injury, and Parkinson's disease (PD).

Interleukin-1 β

The actions of cytokines, such as IL-1 β , involve an interaction between its type I receptor and the IL-1 β receptor accessory protein, which are not only found on immune cells, but also on neurons and glial cells within the brain. The actions of IL-1 β are regulated by activation of anti-inflammatory cytokines. In addition, an endogenous antagonistic cytokine, namely the IL-1 β receptor antagonist (IL-1ra), competes with IL-1 for binding sites, essentially terminating the effects of IL-1 β . In addition, there exists a second IL-1 receptor (type II) that acts as a decoy receptor, as it does not transduce a signal following binding with the cytokine. In effect, there are multiple checks and balances that determine whether and to what extent IL-1 receptor activation will occur. Once the type I IL-1 receptor is stimulated, a cascade of events ensues culminating in the phosphorylation and degradation of the endogenous inhibitory factor kappa B (I κ B). This results in nuclear factor kappa B (NF κ B) being translocated to the nucleus, where it promotes the expression of genes important in neuronal homeostasis and inflammatory processes. Similarly, IL-18 is a member of the IL-1 β cytokine superfamily and like IL-1 β , signals mainly through NF κ B-dependent processes. Specifically, IL-18 triggers intracellular cascades that ultimately induce the production of IFN- γ and other inflammatory factors through promotion of NF κ B-dependent gene transcription, often coupled with mitogen-activated protein kinase (MAPK) signaling.

Interleukin-6

A second essential cytokine, IL-6, is also secreted from macrophages and from T-lymphocytes following antigenic challenge. The receptors for this cytokine are present on numerous peripheral nonimmune cells (e.g., fibroblasts, adipocytes, hepatocytes, and endothelial cells) as well as on brain microglia, astrocytes, and neurons. Binding of IL-6 to its receptor results in a relatively low-affinity structure that requires recruitment of glycoprotein-130 (gp130) to create a functional receptor complex, thereby promoting a signaling cascade involving the stimulation of various protein kinases. Signaling through the IL-6 receptor-gp 130 complex primarily involves activation of the Janus kinase (JAK) signal and signal transducer and activators of transcription (STAT)

Cytokines as Signaling Molecules

In response to foreign particles (antigens), different immune cells (T- and B-lymphocytes, macrophages, as well as endothelial cells) involved in defense of the organism are activated. These cells communicate with one another and mobilize innate and adaptive immunological responses through signaling molecules that are referred to as cytokines. Several cytokines have been identified that include the interleukins (ILs), tumor necrosis factors (TNFs), interferons (IFNs), chemokines, and growth- and cell-stimulating factors. These cytokines have largely been classified on the basis of their molecular structure, and their physiological actions, including the production of fever (pyrogenicity) or inflammation (i.e., swelling resulting from leukocyte infiltration, redness due to local vasodilatation, heat, and pain). Of the various cytokines whose behavioral actions have been assessed, greatest attention has been devoted to the pro-inflammatory cytokines (IL-1 β , TNF- α , and IL-6) that are released from monocytes and macrophages, and the anti-inflammatory cytokines, IL-4 and IL-10, released from T-cells. Of course, numerous other cytokines have been found to influence brain pathology and have been implicated in acute and chronic neurodegeneration. For

pathway. Essentially, activation of the IL-6 receptor complex triggers conformational changes in the cytoplasmic domain of the receptor, resulting in JAK aggregation and subsequent phosphorylation of various STATs. Thereafter, nuclear translocation of STAT homo- or heterodimers provokes the transcription of a number of immune and central nervous system (CNS) regulatory proteins.

Other cytokines, including those with anti-inflammatory properties, particularly IL-4 and IL-10, also influence CNS processes by stimulating the JAK–STAT pathway. Although IL-6 can have proinflammatory actions, such as the provocation of acute phase proteins, this cytokine also shares anti-inflammatory functions with IL-10, including the inhibition of macrophages and TNF- α . In fact, IL-6 and IL-10 share many common effects that likely stem from similar signaling mechanisms that primarily involve activation of elements of the JAK–STAT pathway, particularly STAT3. Unlike STAT1 (induced by IFN- γ), which promotes inflammatory and proapoptotic responses, STAT3 counteracts inflammation and promotes cell survival. STAT3 phosphorylation may result in neuroprotective effects, either through the production of trophic or anti-apoptotic factors or by buffering the impact of extracellular excitotoxic/oxidative species.

Tumor Necrosis Factor- α

Like IL-1 β and IL-6, TNF- α is produced by macrophages, and excites receptors present peripherally (e.g., blood cells, fibroblasts, and insulin-sensitive adipocytes) and on brain neurons and glial cells. The actions of TNF- α are mediated by two receptor subtypes, termed p55 and p75. It seems that p55 contributes to apoptosis and cellular necrosis, and p75 is involved in cellular nutritive functions. TNF- α possesses a unique apoptotic signaling mechanism involving a death domain region intracellularly attached to its p55 receptor. Upon activation, this death domain triggers the recruitment of caspases 1 and 8, which engender cytotoxic consequences. The intracellular actions of p55 are, to some degree, counterbalanced by p75 receptor activation that promotes stimulation of the transcription factor, NF κ B, which may have inhibitory effects on apoptosis. In addition, there are endogenous antagonists for TNF- α , including α -melanocyte stimulating hormone (α -MSH), which contribute to the regulation of this cytokine.

Interferon

In addition to the aforementioned cytokines, IFNs are also considered within the proinflammatory category. In general, IFNs are divided into type I IFNs (including the IFN- α and IFN- β isoforms) and the structurally

unrelated type II form, IFN- γ , which possesses several functional actions similar to those of the type I IFNs. All IFNs act as signaling molecules of the immune system and control infection by blocking viral replication and promoting immune responses against infected cells. Importantly, through stimulation of STAT1 signaling, IFN- γ primes the antigen-presenting cells (e.g., macrophages and microglia) and renders them receptive to later antigenic challenges. Once bound to their respective receptor chains, the presence of an accessory protein is necessary for functional outcomes. The primary signaling pathway for the IFNs is the JAK–STAT pathway, resulting in phosphorylation by intracellular protein kinases. Moreover, the type I IFNs stimulate phosphatidylinositol 3-kinase (PI3K) and MAPK pathways, which might contribute to the ability of these IFNs to interfere with viral replication and promote the apoptosis of tumor and other cells.

Cytokines Influence CNS Processes

Cytokines may influence a variety of cell types, and affect physiological processes, such as cellular proliferation, immune and inflammatory responses, and behavioral states. Ordinarily, low levels of cytokines are present, primarily acting locally (i.e., in an autocrine fashion) or upon cells in close proximity to where they are produced (i.e., functioning in a paracrine manner), and rarely act on cells located some distance away, particularly as spillover into the circulation is rapidly diluted. Under certain conditions, such as severe immunological challenge (e.g., leading to septic shock), marked increases of circulating proinflammatory cytokines levels are provoked, causing the liberation of acute-phase proteins and, as will be seen shortly, promoting utilization of steroid hormones and increased central neurotransmitter activity.

Cytokines comprise relatively large molecules that ordinarily do not readily gain access to the brain. Nevertheless, there are several ways by which peripheral cytokines can influence brain functioning. For instance, cytokines may stimulate fibers projecting from the periphery to the CNS. For instance, IL-1 β and TNF- α stimulate visceral branches of the vagus nerve, thereby provoking altered neuroendocrine and neurochemical functioning and promoting behavioral changes. In addition, despite their large size, cytokines may gain entry to the brain at sites where the blood–brain barrier (BBB) is less effective, specifically at circumventricular organs (median eminence, subfornical organ, area postrema, and organum vasculosum of the lamina terminalis). Saturable transport mechanisms exist that can move IL-1 β and TNF- α into the brain, albeit in low concentrations. Interestingly, certain immunologic challenges or stressful events may undermine the BBB, thus permitting

greater cytokine entry to the CNS. In this regard, it was proposed that the release of corticotropin-releasing hormone (CRH) promotes brain mast cell activation, which in turn increases the release of IL-6, IL-8, and vascular endothelial growth factor. Likewise, proinflammatory cytokines themselves have such an action, as do chemokines (chemo-attractant cytokines). These chemokine actions may promote the formation of vasogenic brain edema that may be evident in neuropathological conditions, such as brain trauma, ischemia, CNS infection, presence of brain tumors, and recurrent flares in multiple sclerosis patients.

It is particularly significant that cytokines and their receptors are endogenously expressed in the brain, being synthesized by microglia, astrocytes, and perhaps by neurons as well. Thus, having gained access to the brain, cytokines may stimulate receptors on cells lining the BBB, particularly around the meninges as well as the vascular areas of the brain, and ultimately, through volume diffusion, receptors may be stimulated at hypothalamic, amygdaloid, and brainstem sites. Besides, cytokines may promote activation of secondary mediators (e.g., prostaglandins) that contribute to fever and possibly other actions elicited by inflammatory factors. In addition to their peripheral activation following infectious challenges, cytokine-producing leukocytes are also mobilized and may enter the brain as a result of such events. Under these conditions, peripheral immune cells can interact with local glia (e.g., through the release of cytokines), thereby affecting neuronal functioning and possibly survival. In addition, direct cell-to-cell interactions between leukocytes and microglia can influence the production of oxidative species important for neuropathology.

Production of both pro- and anti-inflammatory cytokine levels is provoked by traumatic insults and by psychological and physical stressors. It seems that microglia, which serve as specialized immune cells within the brain, are responsible for the synthesis of the majority of these cytokines following head injury, stroke, and neurotoxin treatments. It is less certain what function these cytokines play within the brain (e.g., are they neuroprotective, neurodestructive, or merely bystanders?). It is likely, however, that the actions of the cytokines may be determined by their concentrations (possibly being protective at relatively low concentrations, and destructive at high concentrations). Moreover, as indicated earlier, there is ample reason to believe that they may be involved in the provocation of a variety of pathological states of a psychological nature (e.g., depression) as well as those that involve degenerative processes (PD, AD, multiple sclerosis, as well as cardiovascular disease such as atherosclerosis). In addition, they may contribute to the comorbidities that have been observed between psychological and neurodegenerative disorders and heart disease.

Neurochemical Effects of Cytokines and the Implications for Depressive Illness

Activation of the immune system, as alluded to earlier, may affect multiple hormonal and neurotransmitter systems, and it has been suggested that the immune system acts like a sensory organ to inform the brain about the presence of antigenic challenges. Moreover, cytokines play a particularly essential role in this regard, acting as a messenger between the immune system and the brain (i.e., serving as an immunotransmitter), although this does not preclude involvement of other factors (e.g., cyclooxygenase-2 (COX-2)-derived products or even classical biogenic amines).

Neuroendocrine and Brain Neurotransmitter Changes Elicited by IL-1 β , IL-6, and TNF- α

Studies in rodents have shown that proinflammatory cytokines increase hypothalamic–pituitary–adrenal (HPA) functioning, and influence a range of monoaminergic and peptidergic neurotransmitter changes at hypothalamic and extrahypothalamic sites. Specifically, proinflammatory cytokines increase the release of CRH from paraventricular nucleus (PVN) terminals within the hypothalamus, leading to increased release of pituitary adrenocorticotrophic hormone (ACTH), thereby promoting elevated circulating levels of corticosterone in laboratory rodents and cortisol in human beings. Studies conducted in postmortem tissue as well as *in vivo* have shown that these cytokines increase the utilization of 5-hydroxytryptamine (5-HT) and norepinephrine (NE) within the hypothalamus, and increase NE and/or 5-HT utilization in the medial prefrontal cortex (PFC), hippocampus and the central amygdala, regions that are implicated in cognitive, affective and emotional (anxiety) processes.

These effects of cytokines are not unlike those provoked by physical and psychological stressors, and cytokine manipulations can moderate the impact of traditional stressors. For instance, inhibition of endogenously produced IL-1 β action by an exogenously administered IL-1 receptor antagonist (IL-1ra) attenuated the monoamine changes otherwise elicited by an immobilization stressor in rats. Likewise, several of the behavioral and neuroendocrine effects of a chronic mild stressor were precluded among IL-1 β type I receptor knock-out mice. Interestingly, mice lacking the IL-1 β receptor did not exhibit the reduction of hippocampal neurogenesis that was evident in wild-type mice that had been exposed to a stressor.

Inasmuch as these effects, as well as several behavioral actions of cytokines, are similar to those provoked by psychological and physical stressors, it was suggested

that activation of the inflammatory immune system might be translated much the same as other stressors. This should not be misinterpreted as suggesting that cytokine and stressor actions on biological systems are identical. Aside from the fact that diverse stressors engage different neuronal pathways, different appraisal and coping processes might be engaged to deal with cytokines versus psychosocial stressors. Most certainly, the presence of bacterial or viral infections does not promote cognitive information processing in the same way as psychosocial stressors, although it is possible that cytokine activation may affect appraisal of concurrent psychological stressors.

Neuroendocrine and Brain Neurotransmitter Changes Elicited by IFN- α

Although the extensive use of IFN- α in clinical practice for the treatment of viral diseases (e.g., hepatitis C) and cancer (e.g., metastatic melanoma) is known to be associated with a number of behavioral and psychiatric side effects, the central neurochemical and behavioral actions of this cytokine have not been assessed as extensively as those of other proinflammatory cytokines. It was reported that in rodents, *in vitro*, IFN- α stimulated CRH release from amygdala and hypothalamic neurons, and elicited several *in vivo* brain neurochemical and hormonal changes, although these effects were modest relative to those provoked by IL-1 β . Nevertheless, IFN- α treatment increased hypothalamic and hippocampal NE utilization, reduced DA concentrations within the amygdala, and stimulated activity of the amino acid transmitters, γ -aminobutyric acid (GABA) and glutamate, within limbic and hypothalamic regions.

Many of the effects of IFN- α stem from its central action; IFN- α administered directly into the brain of rodents provoked fever, anorexia, and analgesia, as well as changes of hypothalamic neuronal firing. This treatment also reduced 5-HT concentrations in the PFC, increased hippocampal dopaminergic activity, and increased expression of the serotonin transporter (5-HTT) at the messenger RNA (mRNA) level, which ought to promote reduced serotonin (5-hydroxytryptamine, 5-HT) availability. Moreover, this treatment provoked increased 5-HT_{2C} receptor mRNA editing, resulting in receptor downregulation. It is of particular relevance that 5-HT_{2C} receptor functioning has been associated with anxiety and depressive symptoms, and it was reported that depression/suicide was accompanied by altered 5-HT_{2C} editing.

The effects of chronic IFN- α treatments have not been assessed extensively, despite the fact that this cytokine is administered chronically in the treatment of several human conditions. Nevertheless, the available data using rodents indicate that when IFN- α was administered repeatedly, several 5-HT changes were induced

(e.g., diminished 5-HT levels and turnover in PFC, increased 5-HT turnover within the amygdala, and increased low-affinity 5-HT_{1A} receptor sites). In addition, the effects of acute IFN- α on plasma corticosterone levels diminished with repeated treatment, although cytokine mRNA changes that occurred in brain persisted. Not unlike some of the effects seen in rodents, acute IFN- α administration to healthy volunteers and to patients being treated for hepatitis C increased plasma levels of ACTH, cortisol, and IL-6, but as treatment continued, the increased cortisol and ACTH response was attenuated.

The impact of cytokines on brain monoamine functioning may come about in different ways. For instance, CRH activation evoked by cytokine administration may come to affect forebrain 5-HT functioning, by stimulating receptors present on the dorsal raphe. Alternatively, IFN- α stimulates indoleamine-2,3-dioxygenase (IDO), which is responsible for the catabolism of tryptophan to kynurene. Thus, the IFN- α -provoked increase of IDO will have the effect of reducing tryptophan availability, and hence diminish 5-HT availability. Indeed, it has been shown that depression provoked by IFN- α was accompanied by elevated levels of kynurene and reduced tryptophan. Beyond these actions of IDO, the elevated levels of kynurene are formed into the oxidative metabolites, 3-hydroxy-kynurene, and quinolinic acid (which are elevated in depression) that may have neurotoxic actions. The resulting diminution of neurons might thus favor the emergence of major depressive disorders. In this regard, parenthetically, it seems that these kynurene metabolites synergistically induce free-radical generation, and might also contribute to neurodegenerative diseases such as Huntington's, Parkinson's, and the acquired immune deficiency syndrome (AIDS) dementia.

Sensitization of Processes Related to Cytokine and Stressor-Provoked Neurochemical Changes

In most studies concerning the impact of cytokines (as well as stressors) on neurochemical and behavioral changes, these analyses were conducted at relatively brief intervals following the initial treatment. Yet, there is compelling evidence indicating that stressful experiences may result in the sensitization of neuronal processes, thus augmented behavioral and neurochemical changes (monoamine functioning and corticoid variations) are elicited upon later challenges with either similar or dissimilar stressors (including cytokines). It has likewise been shown that cytokine challenges (particularly IL-1 and TNF- α) result in the sensitization of neurochemical processes, and that some of these effects (e.g., the corticosterone variations) become progressively greater with the passage of time, at least over the 28 days following the initial treatment.

There are several nonmutually exclusive ways by which such a sensitization may come about, including altered receptor sensitivity, metabolic changes, or the induction of neurotrophic factors such as basic fibroblast growth factor (bFGF). Likewise, it is possible that the initial challenge may set in motion a cascade of phenotypic changes that culminate in altered circulating hormone levels. For example, arginine vasopressin (AVP) is ordinarily co-expressed in a modest number of CRH neurons of the PVN of the hypothalamus that terminate within the external zone of the median eminence. With the passage of time following a stressor or cytokine challenge, the co-expression of AVP and CRH increases. Upon later stressor or cytokine treatments, AVP and CRH are co-released and synergistically stimulate ACTH release from the anterior pituitary, leading to adrenal cortisol release. As alluded to above, several processes may be at work in promoting sensitization effects, and the processes governing hormonal changes may be distinct from those associated with neurochemical changes. Irrespective of the process by which a sensitization occurs, the fact is that cytokines may have proactive effects long after the initial effects of the treatment have subsided. Thus, it is possible that stressors and inflammatory immune challenges may increase vulnerability to pathology at lengthy intervals following the initial insult.

Of course, there are other related processes that exist that could influence the way in which a challenge comes to affect disease states. For example, inflammatory factors may become sensitized with their repeated activation, and during the course of a neurodegenerative disease activation of inflammatory processes may exacerbate the pathology. In effect, a disease itself may prime the brain for the damaging effects of inflammatory factors.

Cytokine-Induced Behavioral Changes

Sickness Behaviors in Animal Studies

Activation of the innate immune system, either through the administration of a bacterial endotoxin such as lipopolysaccharide (LPS), or by IL-1 β or TNF- α , provokes behavioral symptoms in rats and mice (e.g., soporific effects, ptosis, anorexia, fever, fatigue, reduced motor activity, and curled body posture) that have been referred to as ‘sickness behaviors.’ In addition, these treatments disrupt relatively complex behaviors, such as operant responding for food reward and disturb exploration and social interaction. Many of these symptoms can be elicited by administering cytokines directly into the brain and hence it is likely that the sickness is centrally mediated. It has been suggested that sickness, following immune challenge, represents an adaptive response to a pathogen, in the sense that it serves to diminish energy expenditure. Yet, the view has also been expressed that

the sickness associated with immune activation may reflect depressive-like behavior given that some of these symptoms are attenuated by antidepressant treatments.

Motivational Changes and Anhedonia

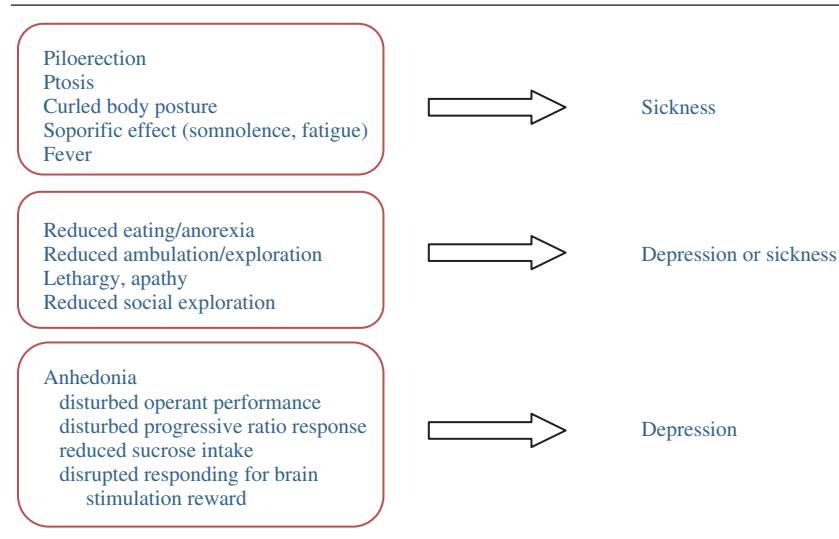
The findings using animal models of depression, coupled with the effects of antidepressants on sickness behaviors and other behavioral disturbances associated with cytokine challenges, are consistent with a role for inflammatory factors in mediating a depressive-like state. Indeed, besides sickness behaviors, cytokines and bacterial endotoxins may elicit anhedonia in mice and rats, a key symptom of depression. For example, LPS disrupted responding for rewarding brain stimulation, and diminished the motivation to work for a food reward. Specifically, using a progressive ratio (PR) schedule (i.e., progressively increasing the number of operant responses that are required for a fixed amount of reward) for a highly preferred treat, namely sucrose reinforcement, it was found that injection of IL-1 β to rats reduced both PR performance, just as free-chow consumption was diminished. Significantly, chronic antidepressant (fluoxetine) treatment attenuated the performance deficit elicited by the cytokine without altering the diminished free chow consumption provoked by the cytokine. Evidently, the cytokine promotes anhedonia independent of the anorexic actions that are induced.

As shown in **Table 1**, of the behavioral effects elicited by cytokines, on the surface, some of these reflect malaise or sickness elicited by the treatments, others reflect depressive symptoms, and still others might reflect sickness and depression. It should be underscored that even some of the symptoms that might be taken to reflect sickness, can be attenuated by repeated treatment with an antidepressant; thus, even these sickness symptoms could involve processes associated with depression.

Behavioral Effects and Correlates of Inflammatory Factors in Humans

Inflammatory Correlates of Depression

Studies in humans have been consistent with the view that inflammatory factors are involved in major depressive disorders. Specifically, it has frequently been reported that depressive symptoms were accompanied by indices of an acute-phase response, such as elevated complement protein levels (C3 and C4) and positive acute-phase proteins, haptoglobin, α 1-antitrypsin, α 1 and α 2 macroglobulin, and C-reactive protein (CRP), whereas negative acute-phase proteins were reduced. It has also been noted that, among depressed patients, activated T-cells (CD25 $^{+}$ and HLA-DR $^{+}$), secretion of neopterin, prostaglandin E2, and thromboxane were elevated. These markers were also

Table 1 Sickness and/or depressive symptoms elicited by cytokines

accompanied by several changes related to circulating or mitogen-stimulated cytokines, particularly among patients presenting with melancholic depression. These included elevations of IL-2 and its soluble receptors (sIL-2R), IL-1 β , IL-1ra, IL-6, sIL-6R, and IFN- γ , and the circadian variations of IL-6 were disturbed. Consistent with the view that cytokines and depression were related, it was reported that the elevated levels of IL-1 β , IL-6, TNF- α , and IFN- γ normalized with antidepressant; however, it was also reported that many of these effects were not apparent with successful pharmacotherapy. Still, the possibility cannot be dismissed that cytokine normalization might occur with more protracted treatment, or alternatively, that cytokine normalization might occur in a subset of individuals, and that the absence of such a normalization might be a marker for illness recurrence. It is also possible that cytokine variations and the normalization of cytokine levels might vary with particular symptoms of depression (or particular subtypes of the illness, e.g., typical vs. atypical depression). For example, it is possible that proinflammatory cytokines, such as IL-1 contribute to the fatigue and increased sleep ordinarily associated with atypical depression.

Reports that cytokine levels are associated with depression do not, of course, indicate that cytokines provoked the depressive state. To be sure, it is equally possible that depressive mood (or the stress associated with depression) altered circulating cytokine levels. It is likewise possible that any number of factors secondary to (or related to) depression provoked the cytokine variations. For instance, factors that promoted depression (e.g., antecedent conditions, such as recent stressful experiences, stressors encountered early in life, and altered

coping with stressors) could have influenced cytokine levels. In addition, institutionalization (and the social and environmental change related to this) of depressed patients may itself have contributed to cytokine variations, as would an increase of drug use (including nicotine, alcohol, as well as illicit drugs). Indeed, it was reported that when age and body mass index were statistically controlled, the differences in circulating cytokine levels between controls and depressed patients were absent. Furthermore, it is possible that certain neurovegetative features of typical major depression (reduced eating and weight loss, reduced sleep) promote altered cytokine levels, as might gender, smoking habits, recent infection or prior medication, as well as altered circadian cycles. Finally, the cytokine variations might stem from any of the many pathologies that have been shown to be comorbid with depression.

Cytokines Promote Depressive States

There is ample reason to believe that the depressive state brought on by immunotherapy with IFN- α in hepatitis-C and cancer patients reflects a genuine major depressive episode rather than being secondary to malaise associated with the treatment. In this regard, the development of depression following IFN- α therapy was aligned with many of the features associated with depression that occur in the absence of cytokine immunotherapy (e.g., stress-related depression). For example, major depressive symptoms in response to IFN- α were most prominent among patients who presented with sub-syndromal levels of depression prior to immunotherapy, those who showed symptoms such as sadness, pessimistic thoughts, and sleep disturbances, as well as individuals with poor social

support. Furthermore, paralleling the mood changes brought on following depletion of tryptophan, depressive symptoms elicited by IFN- α were most prominent among women and those with a history of depression, among individuals with low tryptophan levels prior to treatment, or those with relatively high baseline levels of sIL-2r, IL-6, and IL-10.

Finally, it is significant that selective serotonin reuptake inhibitors or serotonin-specific reuptake inhibitors (SSRIs) influenced the expression of several cytokines and generated marked anti-inflammatory effects. Moreover, it was shown that the depression brought about by IFN- α therapy could be attenuated by SSRIs (e.g., sertraline, paroxetine, and citalopram). In this regard, paroxetine primarily affected the mood-related symptoms, with only minor effects on fatigue and anorexia. Furthermore, it was shown that SSRIs influenced the expression of several cytokines and generated marked anti-inflammatory effects. Together, these findings indicate that the relationship between cytokines and depression is not simply one of a correlational nature, but possibly through effects on 5-HT functioning, cytokines may provoke the emergence of depressive symptoms.

Although 5-HT activity possibly contributes to the effects of inflammatory factors on depressive symptoms, other neurochemical factors may also play a prominent role in this regard. Specifically, like the SSRIs, the phosphodiesterase IV inhibitor (e.g., rolipram), which acts as an antidepressant, likewise diminished cytokine production in response to LPS in rodents. Further, administration of 5-HT itself reduced the expression of TNF- α and IFN- γ , and this outcome could be attenuated by CRH treatment. Thus, it is likely that 5-HT and CRH-related neuronal activity might reciprocally affect one another, thereby influencing depressive mood.

It would be expected that if inflammatory factors contribute to depressive symptoms, then anti-inflammatory compounds might themselves possess antidepressant properties, or enhance the effects of conventional antidepressants. In fact, COX-2 antagonists, normally used as nonsteroidal anti-inflammatory drugs (NSAIDs), attenuated the depressive-like behavioral and neurochemical alterations associated with both LPS and IFN- α treatment in animal models of depression, and also diminished glucocorticoid responses to social stressors. Moreover, it has been shown that a COX-2 inhibitor enhanced the effectiveness of antidepressant medication in treating depression in humans; however, the usefulness of combining NSAIDs with antidepressant medication is limited by the increased risk of gastrointestinal disturbances that may ensue.

Effects beyond Major Depressive Disorder

In addition to mood changes, IFN- α may engender disturbed vigilance, alertness, and may promote some

memory problems. In addition, IFN- α immunotherapy was accompanied by disrupted neurocognitive functioning (retarded cognitive processing and impaired executive functioning, concentration and memory, as well as irritability and anxiety), and at high doses, a confusional state characterized by disorientation as well as psychotic-like features was apparent. These features (e.g., disturbances of immediate recall) may become more pronounced with treatment continuation. The nonspecificity of symptoms begs the question as to whether or not the effects observed are a genuine manifestation of neurochemical changes that underlie depression, or whether the constellation of symptoms is more a reflection of general malaise or toxicity provoked by the treatment.

Comorbidity with Other Illnesses

Comorbidity between depression and neurological conditions is common, with two or more pathological states often influencing one another's course. Indeed, the presence of depression may augment the neurological decline in PD patients and hampered functional recovery in stroke patients. In some instances, the development of comorbid depression might result from the psychological distress or major life changes and psychosocial factors associated with the neurological condition. However, in other instances, the depressive symptoms might emerge as part of underlying processes that are in common with the neurological disturbances. For instance, stressor-provoked elevations of corticosterone may instigate hippocampal neuronal damage, and may contribute to both depression and chronic neurodegenerative diseases. Likewise, cytokines and associated inflammatory factors (e.g., COX-2 and inducible nitric oxide synthase (iNOS)) have been implicated in both depression and PD and might thus contribute to the comorbidity.

Interestingly, besides affecting mood, antidepressants such as rolipram and bupropion have anti-inflammatory properties, largely by increasing the release of the anti-inflammatory cytokine, IL-10, while reducing levels of the proinflammatory cytokines, TNF- α and IFN- γ . In addition, antidepressants, such as imipramine, were reported to have neuroprotective consequences against the impact of high doses of inflammatory agents. Similarly, anti-inflammatory drugs that inhibit COX-2 production have also been shown to be protective in animal models of neurodegeneration relevant to PD and AD, just as they enhanced the effects of SSRIs in reducing depressive symptoms. Evidently, multiple links may exist between activation of the inflammatory system and the development of depressive and neurodegenerative pathology.

In the case of acute neurological conditions, such as traumatic head injury and stroke, depression is often present, and the depressive symptoms are more resistant to treatment relative to that evident in the absence of comorbidity. It seems that stressor and stroke-induced elevations of IL-1 β , TNF- α , and IL-18 influence some of the same enzymatic pathways, including IDO, resulting in 5-HT depletion. Conversely, stressors that increase IL-1 β or TNF- α have been reported to increase infarct size caused by later middle cerebral artery occlusion. Finally, in addition to CNS pathologies, it was observed that depression was associated with increased likelihood of cardiovascular disease, and conversely myocardial events were predictive of subsequent depression.

See also: Brain Aging: Structural Imaging Biomarkers of Risk of Alzheimer's Disease; Comorbidity – Depression; Depression; Drug Sensitization and Drug Abuse; Environmental Influences on Adult Neurogenesis; Fear, Anxiety, and Defensive Behaviors in Animals; Feeding; Genes and Behavior: Animal Models; Hormonal Contributions to Arousal and Motivation; Incentive Motivation and Incentive Salience; Motivation; Neural Substrates of Unconditioned Fear, Defense, and Anxiety; Neural Systems of Motivation; Parkinson's Disease; Psychiatric and Substance Use Disorder Comorbidity; Psychoneuroendocrinology of Stress; Psychosocial Influences on Immunity; Rewarding Brain Stimulation; Stress and Emotionality; Stress and Energy Homeostasis; Stress and Reward.

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Episodic and Autobiographical Memory: Psychological and Neural Aspects

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Glossary

Phenomenology – It is the subjective mental experiences of observers during cognition or perception. These experiences are assumed reportable, although the nature of the experiences may be influenced by the requirement to report them, and the veracity and reliability of reporting is often of concern.

Semantic knowledge – It is the long-term knowledge about the properties of objects and canonical features of events. This form of memory is typically spared by damage to medial temporal lobe and midline diencephalic brain regions that support the memory for specific events and experiences.

for episodic memory designs, which often test for the remembrance of arbitrary associations or contextual associations, for example, the ability to remember that the word ‘cabbage’ was previously part of an animacy rating task as opposed to a subjective pleasantness rating task (**Figure 1**). This type of mini-event is not associated with a routine experience and therefore observers are limited in their ability to reconstruct the event from semantic knowledge stores. Regardless of these differences, episodic and autobiographical memory are generally considered highly overlapping memory capacities, and the term episodic memory will be taken here to refer collectively to both.

Conceptualizations of Episodic and Autobiographical Memory

The ability to recover information about unique prior experiences in order to guide current choices is a cornerstone of human adaptive behavior. When memories are temporally remote, personally significant, and encompass extended events (e.g., one’s high school graduation), this skill is referred to as autobiographical memory. In contrast, when the remembered events are highly controlled laboratory experiences with verifiable characteristics (e.g., retrieval of newly learned word associations), the skill is typically referred to as episodic memory. The distinction between autobiographical and episodic memory is largely quantitative and not qualitative. In general, autobiographical memories contain considerably more emotional content and visual imagery than episodic remembrances, although it is sometimes unclear the degree to which autobiographical reports rest, not on specific remembrances, but on the observer’s knowledge of the typical characteristics of the targeted experiences. For example, when asked to describe one’s high school graduation, one can rely on the features common to all high school graduations when attempting to report the event. To control for this, autobiographical designs typically score reports for their specificity and degree of idiosyncratic content, in hopes of attributing the report to a genuine remembrance. This reconstruction problem is typically less of a concern

Contrasts with Other Forms of Memory

The distinction between episodic memory and other kinds of memory has been conceptualized along many dimensions, such as phenomenology, content, persistence, behavioral control, and, most recently, neural substrate. Phenomenology was perhaps the earliest characteristic used to parse putative types of memory, dating back to the Ancient Greeks, and it contributes to modern conceptualizations as well. For example, one fundamental modern distinction is between declarative memory (making declarations about the past, or ‘knowing that’) and procedural memory (carrying out actions based on past learning, or ‘knowing how’). The latter encompasses a broad range of skills and abilities that rely upon processes observers are often unable to describe or characterize, such as motor skills like riding a bike and tying one’s shoes. In contrast, knowledge that is accessible to conscious awareness falls under the category of declarative memory. Within declarative memory, an important distinction arises between episodic and semantic memory. One’s general knowledge of the world, such as knowing that lions have manes or that breakfast occurs in the morning, constitutes one’s semantic memory. In contrast, it is also possible to report the details of a particular episode in which say a lion was viewed, or a specific breakfast was consumed. Critically, accessing semantic memory does not require retrieval of the original time or place of learning, as opposed to episodic retrieval. As a particular type of episode recurs across the lifespan, it is

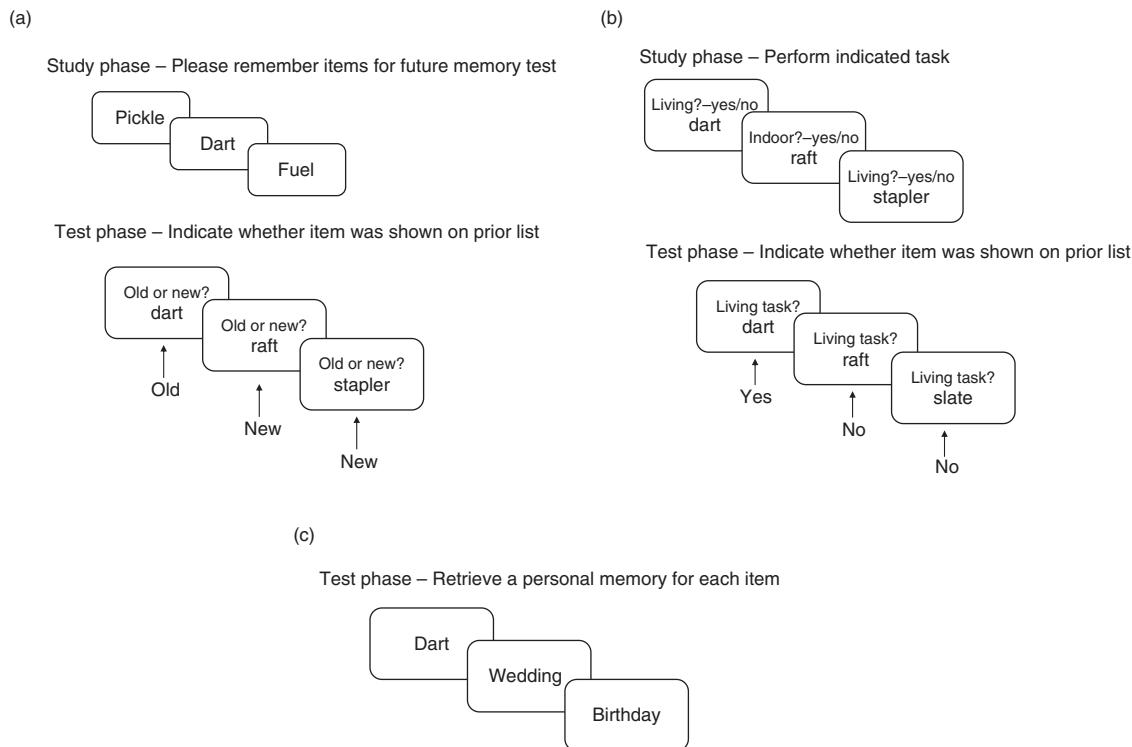


Figure 1 Examples of experimental episodic- and autobiographical-memory tasks. (a) Item recognition. Participants are asked to remember presented words for an unspecified number of memory tasks. During testing they are prompted with studied or novel items and asked to indicate whether items originated from the study list. (b) Context/source task. Participants encounter words during two different encoding tasks. During testing they are required to identify items from the study phase and further to distinguish between items originating from each context task. (c) Autobiographical Crovitz-Galton word-cueing task. Participants are asked to generate memories from their own life in response to word cues.

generally assumed that its canonical features are incorporated into the semantic knowledge store.

Episodic and semantic memory reflect examples of long-term memory, since retention lasts more than several seconds and survives intervening experiences. In contrast, the concept of working memory describes a collection of temporary memory processes in which information is maintained and manipulated in an active or labile state for a short period of time. It is an essential part of current conscious awareness, with important connections to attention and mental ability. Working-memory tasks usually require one to maintain and transform information, keep track of changes, divide attention, and make comparative judgments. Originally thought to function independently of episodic representations, recent conceptualizations of working memory have introduced an episodic buffer which enables the integration of information from perceptual and linguistic operations and long-term memory. The component is episodic because it is assumed to bind different features of an event into a unitary episodic representation; however, it differs from episodic memory in that the episodic buffer is a temporary store.

As is clear from the above, the clarity of the theoretical boundaries between episodic memory and other kinds of memory vary. In most instances, experimental tests of memory cannot be assumed to be ‘process pure’ in that they likely recruit several partially or fully independent memory processes. Nonetheless, through behavioral experimental dissociations, animal lesion studies, the examination of special populations, and, most recently, functional imaging of the brain, psychologists and neuroscientists have amassed evidence for an episodic memory system that is at least partially dissociable from other forms of memory.

Neuroanatomical Frameworks of Episodic Memory

The Amnesic Syndrome

The strongest evidence for a unique episodic memory capacity arose from neuropsychological research examining the patterns of spared versus disrupted memory capacities following neurological insult. In the late 1950s, seminal case-study research examining

a 27-year-old patient undergoing experimental surgical treatment for intractable epilepsy demonstrated that bilateral damage to the hippocampus and surrounding cortical structures resulted in a global amnesia syndrome characterized by severely impaired memory for events occurring up to 3 years before the surgery (retrograde amnesia) and a virtually complete inability to remember new experiences following the surgery (anterograde amnesia) (Figure 2). The severity of the syndrome is easy to underestimate and such patients often demonstrate complete forgetting of to-be-remembered material when distracted for mere seconds with a secondary task. Despite this profound memory impairment for episodes, other intellectual domains appear spared and this has been used as a defining characteristic of the amnesia syndrome, namely, an abnormally low standardized memory score in relation to a preserved estimate of general intelligence. Subsequent animal research and lesions of opportunity in human subjects have also implicated other medial brain structures outside of the medial temporal lobes (MTLs) as critical, including the thalamus, mamillary bodies, fornix, basal forebrain, and retrosplenial areas. Damage to any of these regions can result in a profound global amnesia with preserved intellectual functioning in other domains, such as reasoning, the expression of general semantic knowledge about the world, and the learning of new motor skills.

Perception and Short-Term Visual Memory

Although global amnesia is defined as gross episodic memory impairment in concert with preserved intellectual functioning in other domains, closer examination of patients with damage to MTL regions suggests that not all nonepisodic functions may be fully preserved. Both animal and human neuropsychological studies indicate that the ability to discriminate perceptually similar visual exemplars over short periods may be compromised by damage to MTL regions. During these paradigms, subjects are typically shown a visual sample, required to wait for a brief unfilled delay (e.g., 4–8 s), and then asked to identify the target probe among visually similar lures. Such tasks were thought to fall fully within the realm of working-memory maintenance, that is, the form of memory supporting the maintenance of items in the mind during brief unfilled delays, such as when one holds a phone number in mind before dialing. Previous extensive work with amnesic patients suggested that visual and verbal short-term working memory was spared; however, these designs typically did not use highly visually confusable stimuli. Nonetheless, damage to the MTLs does yield impairment on such tasks, although the cause of the impairment remains controversial. From one viewpoint the compromised short-term working memory suggests a link between working memory and the MTL regions. In contrast, the deficit may reflect the fact that control subjects may occasionally rely upon episodic memory to overcome occasional lapses or disruptions of working-

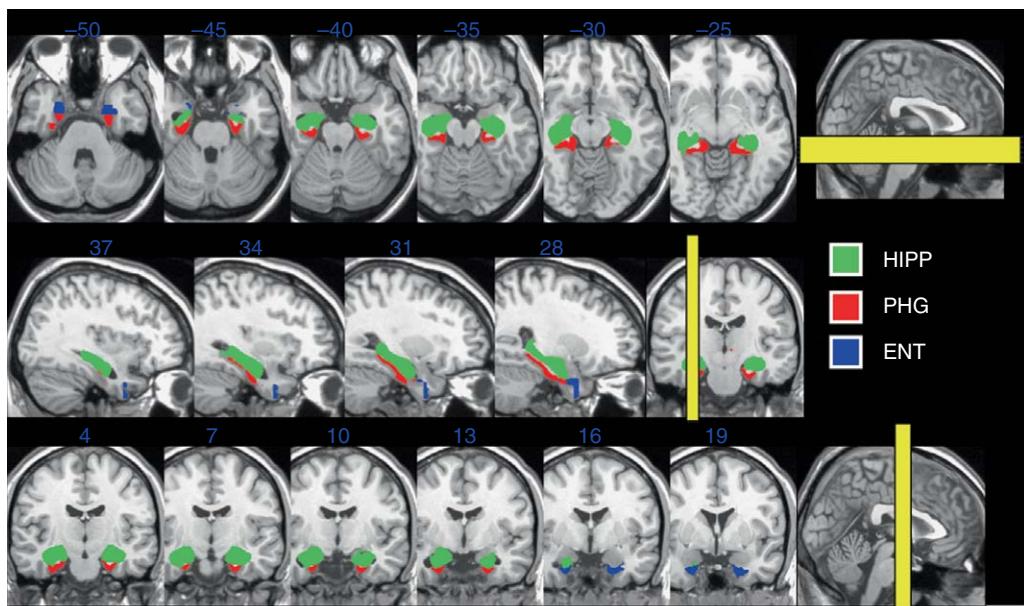


Figure 2 Schematic rendering of regions of the medial temporal lobes (MTLs) critical for episodic memory. Green regions approximate the hippocampus. Red regions encompass the parahippocampal gyrus, the anterior extent of which is often referred to as perirhinal cortex. Blue regions illustrate the entorhinal area. The filled yellow area on the three cardinal views indicates the range from which the partially overlaid slices originated.

memory maintenance. This latter account fits with the observation that even brief attentional distraction in global amnesic subjects is sufficient to cause complete forgetting, which in turn suggests that hippocampally supported retrieval processes are important for bridging even brief delays when attention has been captured or otherwise diverted.

Transitive Inference and Flexible Reasoning

Episodic memory is a vital contributor to flexible, adaptive behavior. For example, determining the correct course of action can be facilitated by remembering that alternative courses of action previously led to undesirable outcomes in similar situations. Here episodic memory does not directly inform the most adaptive behavioral choice, but it can be used to inform reasoning about the relative merit of options. The simplest tests capturing this type of flexibility are known as transitive inference problems. During such problems, observers are selectively reinforced for responses to pairs of stimuli. For example, the bolded items in the following set would lead to reinforcement when selected (**A–B; B–C; C–D; D–E**). Critically, the redundancy of the pairs means that the reinforcement status of B, C, and D items is entirely contextually dependent; thus, the acquisition of the appropriate response requires configural-level knowledge. Furthermore, these designs allow testing with critical probes (e.g., B–D) that have never been reinforced, but which should lead to a preference (B) if a hierarchical episodic representation has been encoded ($A > B > C > D$) or if observers can inductively construct such a representation given the individual learning of the pairs. Animal, neuropsychological, and functional imaging research implicates the hippocampus as important during both the acquisition and critical probe periods of transitive inference problems. However, despite this convergence of evidence, the level of conscious awareness required to solve such problems remains in dispute. Although episodic memory is often considered to pertain to conscious recollection of prior events (or reasoning based on conscious recollection), and is known to require the hippocampus, this does not logically require that all hippocampal-dependent tasks be accompanied by conscious recollection. This argument is bolstered by findings in repeated visual search and visual-inspection paradigms, where hippocampally dependent improvements in detecting target stimuli enmeshed in visual arrays may occur outside of observer awareness that the arrays have been repeated.

Developmental Trends and Dependencies

At both ends of the developmental spectrum, episodic memory is less efficient when compared with that of young adults. In the case of early development, the

study of episodic memory has been challenging since instruction and verbal report measures are inappropriate. However, mimicry paradigms that can be accomplished by pre-verbal children suggest that episodic remembering occurs in children as early as 2 years of age. Although such research has demonstrated that episodic-memory functioning occurs earlier than once thought, it nonetheless remains the case that a major portion of early childhood experience, up until approximately age 3 or 4 years is later either wholly inaccessible to most adults, or when available is quite fragmentary and impoverished. This phenomenon, termed infantile or childhood amnesia, suggests that the co-development of semantic knowledge and other representational systems may be critical for adult levels of episodic memory capabilities. Although widely debated, one current hypothesis is that prior to age 3 or 4 years, children often do not actively construct larger more distinctive representations of ongoing experiences and for the same reason also do not adopt reconstructive strategies during attempted retrieval. The ability to precisely encode the relationships between event elements and to situate events into larger experiences likely relies upon the pre-frontal cortex (PFC) function and is conceptually linked to the idea of an episodic buffer within working memory. This hypothesis receives further support from functional brain imaging research demonstrating PFC recruitment during both initial encoding and later retrieval in the kinds of memory tasks with which young children experience the most difficulty. For example, although children below the age of four are rapidly acquiring large amounts of semantic information, behavioral experiments demonstrate they are impaired in the ability to later remember where and under what circumstances this information was acquired, a phenomenon known as source amnesia. The demonstration, using functional imaging, that PFC regions are heavily involved in source memory tasks (Figure 3), and the known late developmental maturation of PFC cortex in humans, jointly suggest that in this region may underlie the emerging ability to distinctively encode and selectively recover extended events and experiences in childhood.

At the other end of the developmental continuum, impairments in episodic remembering are also prominent. Indeed, even during healthy aging, lapses in episodic memory are one of the most prominent complaints of older adults. As with early childhood, a portion of the impairment likely reflects impairments in organization or processing of information during the encoding of experiences. For example, although older adults experience relative decline in memory performance for even basic item-recognition tasks compared to young adult controls, a large portion of this decline is eliminated when participants are provided explicit strategies that encourage reflecting upon the semantic and unique characteristics of each stimulus during initial study. This has led to one

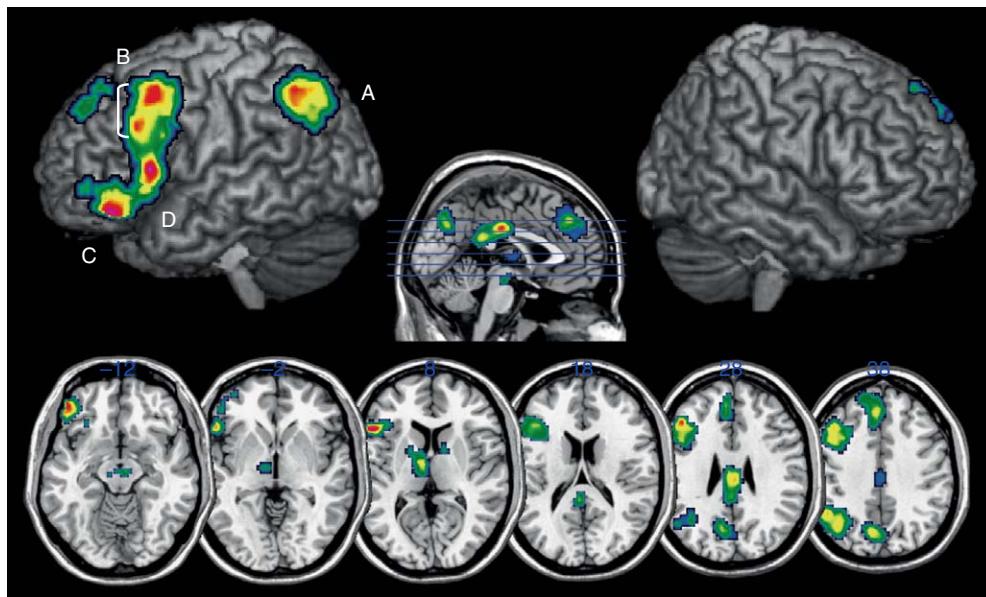


Figure 3 An example of the network implicated in context/source–memory retrieval relative to simple item recognition. Source–memory retrieval increasingly activates left lateral parietal (A), dorsolateral PFC (B), and ventrolateral PFC (C and D) regions along with midline areas.

major theoretical account of age-related memory decline that stresses the role of self-initiated distinctive processing in creating durable episodic memories. Although the root cause for the failure of older adults to spontaneously process materials and events in a distinctive manner remains debated, as with the relative episodic impairment in children, it has been linked to PFC. Consistent with a common PFC locus, older adults, as with children, demonstrate considerable difficulty in source–memory paradigms that require recovery of the contextual specifics of prior encounters and experiences. Despite the evidence for a gross role of PFC in the memory impairments observed at both ends of the developmental spectrum, the precise neurological causes for the failure to recruit PFC, while unknown, presumably arise from different root causes.

Functional Magnetic Resonance Imaging Data

Subsequent Memory and Episodic Encoding

Forming a new memory depends upon the neural mechanisms set in motion at the moment the event is experienced. An influential approach to the study of memory formation or encoding is the subsequent memory paradigm. Neural activity is recorded while participants study a sequence of items, after which memory for the items is tested. The neural responses elicited during prior study are retrospectively coded according to whether the items were later remembered or forgotten, with

differences between cortical responses for later remembered versus forgotten items taken as correlates of successful memory encoding. Data from electrophysiological event-related potentials (ERPs) and functional magnetic resonance imaging (fMRI) suggest that the neural mechanisms that mediate encoding depend upon several factors including the nature of the stimulus, the goals or tasks during encoding, and individual differences in the tendency to adopt particular processing strategies. For example, successful word encoding is associated with activation in the left ventrolateral PFC, MTL, and fusiform gyrus. In contrast, successful encoding of complex visual scenes has been associated with right inferior PFC and bilateral MTL. Aside from these simple stimulus differences, the specific PFC–MTL circuits that covary with successful encoding depend on the particular task and the subject's goals during learning. Subsequent memory designs in which the semantic features of study materials are processed have implicated bilateral PFC and left MTL regions, but when the same items are studied in a perceptual manner, only a subset of these regions is activated and predictive of later memory. Differences in the degree to which these regions are spontaneously activated also vary considerably across subjects and populations. For example, when the encoding tasks are largely unconstrained and participants are simply instructed to memorize meaningful materials, there are considerable individual differences later reported in terms of adopted strategies, with some participants reporting perceptual or imagery strategies, and others reporting adopting complex semantic judgments

and construction of sentences or stories, for example. Critically, these differences are reflected in the pattern of activity seen during encoding and in the later level of success during memory judgments; the left ventrolateral PFC, in particular, appears associated with adopting semantic analysis strategies during encoding. The degree to which these strategies facilitate later memory is dependent upon the form of the later memory task, and tasks that require the recovery of context associations, as opposed to the simple identification of familiar stimuli, appear to benefit considerably from recruitment of PFC during encoding.

Aside from individual differences in encoding strategy, recruitment of left ventrolateral PFC during encoding also varies across age cohorts with elderly participants often demonstrating reduced recruitment compared to younger controls. Requiring all subjects to render semantic level judgments for each to-be-encoded item, in part, eliminates these age-based differences. Thus, when considering both differences across individuals and across age populations, it appears to be critical to consider the degree to which the encoding environment controls the approach to the task, since this will necessarily constrain the types of information later available for retrieval. Nonetheless, recruitment of left ventrolateral PFC and MTL regions during the initial encoding of meaningful events is associated with increased episodic memory for those experiences during later retrieval.

Retrieval Success

Along with a role in memory formation, MTL structures are associated with successful retrieval. Neuroimaging research suggests that distinct subregions of the MTL support dissociable retrieval processes with the hippocampus proper involved in the contextual recollection of past event features and extra-hippocampal regions supporting judgments based on item familiarity. Hippocampal activation is consistently reported during successful source–memory judgments, when subjects correctly retrieve the prior context of a previously encountered item, relative to correctly identifying new unstudied items or correctly identifying items as studied but failing to retrieve context information. In contrast, the surrounding perirhinal and parahippocampal cortices of the MTL region appear to show a different pattern, with the former hypothesized to support familiarity-based recognition for individual objects, independent of whether contextual retrieval is possible or required. This characterization is largely based on reports demonstrating that although the region displays increased fMRI activation for correctly identified and studied materials relative to novel materials, the level of activation for studied materials does not further differ as a function of contextual retrieval outcomes. Anatomical projection

studies in animals complement these findings by demonstrating that the perirhinal cortex receives considerable information about the properties of items to be remembered whereas the parahippocampal gyrus receives inputs about the spatial context in which the items are encountered. This coarse ‘what’ versus ‘where’ distinction is itself undergoing refinement in the literature; however, it nonetheless suggests that different regions of the extra-hippocampal portions of the MTL process different information about events. These two regions then converge in the hippocampus, potentially supporting the ability to situate objects into larger episodes at retrieval. Human neuropsychological investigations further support this two-retrieval-process interpretation with reports linking damage of the hippocampus proper to marked declines in the behavioral estimates of contextual recollection with relatively preserved scores of item-familiarity discrimination. Despite the convergent evidence suggesting separable familiarity and recollection-retrieval processes in the MTLs, the utility of this dichotomous characterization of the MTL processes has been questioned and this remains a highly active area of investigation.

Although research continues to focus on the role of MTL in successful retrieval, an emerging body of literature has also begun to focus on the putative role of left lateral parietal cortex during successful episodic retrieval (**Figures 3(A) and 4(B)**). The earliest findings suggesting a role for this region were based on ERP research, and subsequent fMRI studies have documented increased left lateral parietal responses when subjects correctly detect previously encountered items compared to the correct identification of new items. Furthermore, the inferior extent of this lateral parietal activation often extends into the region of the angular gyrus, and demonstrates activation properties consistent with a role specifically in contextual recollection. For example, the activation tracks phenomenological reports of recollection, is present for the autobiographical recall of distant prior events, distinguishes successful from unsuccessful source–memory judgments, and tracks the amount of episodic detail that can be reported about a prior event. Nonetheless, the hypothesis that left lateral parietal cortex supports a core element of recollection faces opposition. This opposition primarily arises from the fact that damage to the left lateral parietal region is not linked with a global amnesia syndrome in the neuropsychological literature. This conspicuous absence may reflect the fact that the contralateral region is capable of assuming a mnemonic role following damage to the left hemisphere, and the possibility that language and comprehension dysfunction due to damage in proximal regions may mask a more subtle memory impairment in patients. Nonetheless, the historic failure to observe striking deficits such as those that occur following insult to the MTL and midline diencephalic regions, continues to

challenge the idea that lateral parietal cortex plays a direct or critical role in recollection.

Retrieval Strategies and Decision Operations

The products of an episodic retrieval attempt are usually not immediately available. For example, in autobiographical-memory tasks, participants often take 6–8 s to recover the first piece of relevant memory content. The dependence of these types of memories on volitional and sustained retrieval attempts, and the frequent correlation between tests of episodic retrieval and general intelligence, suggest that successful episodic retrieval often depends upon effective memory search and evaluation processes. Recent functional imaging research supports this assumption, demonstrating considerable PFC activation for memory judgments that require recollection of contextual information. Critically, this activation is robust even when participants ultimately fail at the task, which further suggests that PFC supports retrieval search and evaluation processes that are recruited even when sufficient memory evidence ultimately fails to be recovered. Currently, it is assumed that distinct ventrolateral (**Figures 3(C)** and **3(D)**), dorsolateral (**Figure 3(B)**), and rostralateral (**Figure 4(A)**) regions of the PFC contribute to different search or evaluative processes, although there is considerable variety in the functional characterizations ascribed to these regions. Furthermore, because these regions are also active during a host of tasks other than episodic retrieval, any successful functional characterization must accommodate a large range of decision-making and reasoning tasks. To this end, current characterizations of the role of left ventrolateral PFC during retrieval are perhaps the most successful. Recruitment of this region during context relative to item memory judgments has been interpreted as reflecting controlled semantic operations that facilitate the recollection of contextual information by foregrounding or selecting the semantic features of retrieval probes that are potentially central to an episodic representation of the event. Consistent with

well-documented cognitive models, this is thought to improve the match between active search cues and the original event, increasing the likelihood of recollection when appropriate. Buttressing this view, activation in this region has been reported in tasks in which subjects must retrieve contextual memories about prior object encounters that are linked to the semantic properties of the memory probes (e.g., remembering having made a pleasant/unpleasant or a living/nonliving judgment at encoding for each test probe – **Figure 1(b)**). In contrast, when the to-be-remembered information is perceptual in nature (e.g., whether the object had appeared in a large or small size during prior encoding), the left ventrolateral region is not recruited, presumably reflecting the reduced relevance of the semantic or conceptual features of the memory probes during memory search. Thus activation in the left ventrolateral region potentially signifies a proactive strategy enabling the maintenance of semantic information that serves as a cue to retrieval during memory search. This characterization fits well with research directly examining semantic processing, and with research on self-initiated semantic encoding strategies, with both research domains implicating the same left ventrolateral PFC region.

In contrast to the selection or foregrounding of semantic information during memory search, dorsolateral PFC activation during retrieval instead appears to reflect the monitoring or evaluation of the products of retrieval with respect to decision standards. Thus the region is thought to underlie the ability to evaluate the products of memory retrieval with respect to their task relevance. Direct comparisons between inclusion tasks (wherein subjects must respond ‘yes’ to studied words regardless of their specific prior context) and exclusion tasks (in which subjects are asked to respond ‘yes’ only to words that are familiar and from a specific source) reveal increased dorsolateral PFC activity, during the latter. Similarly, memory tasks that require a greater number of intermediate judgments about recovered episodic information elicit greater activation than simpler tasks with matched materials requiring fewer intermediate memory judgments. Thus, activation in dorsolateral PFC appears to index either the number of judgments rendered during retrieval attempt or the complexity of the decision standard adopted by the participants when evaluating memories. From either perspective, extended retrieval tasks that require successful recovery of convergent aspects of a prior event are predicted to be the most compromised following damage to dorsolateral PFC.

The least-understood PFC region that is routinely activated during episodic retrieval tasks is rostralateral PFC. Neuropsychologically, the most prominent behavioral impairment following damage to rostralateral PFC is one of flexible planning and multitasking, not memory retrieval. Despite this, the region is frequently implicated in the functional imaging of simple item-recognition

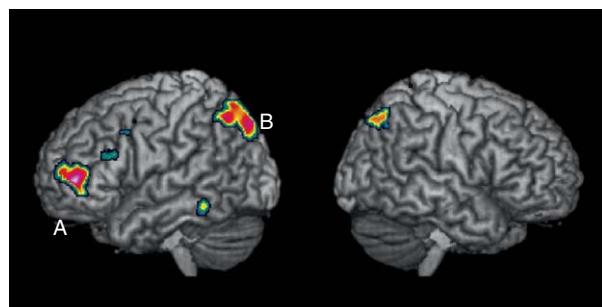


Figure 4 An example of regions implicated in simple item recognition for the contrast of correct responses to studied items (hits) vs. correct responses to new items (correct rejections). Left rostralateral PFC (A) is frequently implicated in this contrast.

memory, demonstrating greater activation for correctly recognized old items versus correctly rejected new items, typically lateralized to the left hemisphere (**Figure 4(A)**). However, this differential activation is sensitive to subjects' beliefs regarding the relative frequency of old and new items in the test list, with the differential response amplified when subjects believe old items to be relatively infrequent, even if objective frequency remains constant. Furthermore, the region has been shown to demonstrate complex temporal profiles of activation during retrieval. For example, in the right rostralateral PFC there is an increase in tonic or sustained activation across recognition trials under conditions of low discriminability during item recognition, whereas the region demonstrates an increased trial-based anticipatory response when subjects are forewarned of an upcoming context-memory demand compared to when they are forewarned of an upcoming simple recognition demand. Given the temporal complexity of the responses, combined with the dependence upon subjects' beliefs about the list structure, a consensus of the region's contribution to retrieval will likely require considerable future research. As with ventrolateral and dorsolateral PFC regions, a major challenge will be to ensure that retrieval characterizations accommodate the role of the region in nonepisodic tasks as well. For example, fMRI studies of analogical and complex reasoning also frequently implicate rostralateral PFC. At present, the only firm conclusion that can be drawn with respect to episodic memory is that activation in the region represents a complex combination of global beliefs regarding the structure or properties of the test list and the recovery of episodic information for individual items within the list.

Event Reconstruction and Forecasting

Cognitive and computational models of episodic memory have long assumed that efficient retrieval requires reconstructive processing, in which observers fill in aspects of a to-be-remembered event with semantic knowledge of its most likely components. For example, when attempting to retrieve a memory about a given breakfast, observers will often fill in details based on their semantic knowledge of the typical components of the meal, in addition to refining these components if additional constraints are also available. For example, if one is trying to remember a particular breakfast that occurred when one was in a hurry, then candidate meal items and event characteristics can be further constrained. This reconstructive process not only serves as a best guess of the likely event elements should retrieval ultimately fail, but a large body of cognitive behavioral research demonstrates that it also facilitates actual recovery of episodic information by improving the overlap between the retrieval cues actively held in working memory, and the actual event.

Based on this framework, pioneering memory researchers speculated that anticipating the future should recruit many of the same mechanisms engaged when reconstructing the past. In short, thinking about the likely constituents of a prior event should recruit much of the same processing as trying to predict the characteristics of an event that has not yet occurred.

Recent functional imaging work has supported this hypothesis by directly comparing the networks activated during autobiographical retrieval tasks to those in which subjects are instructed to imagine specific future scenarios (e.g., imagine yourself in a future beach holiday). The networks activated by these two tasks are virtually identical with both the tasks implicating left dorsal PFC, medial prefrontal and anterior cingulate areas, posterior cingulate and restrosplenial areas, and parahippocampal gyrus. Critically, this network is engaged above and beyond conditions that attempt to control for simple visual imagery. At present there are multiple ways of interpreting the high degree of similarity between the network activated during future simulation or forecasting, and that typically observed during autobiographical memory retrieval. Much of this overlap could be due to the voluntary or involuntary retrieval of episodic content when attempting to construct a future scenario. Although subjects are encouraged to create entirely new episodes this need not mean that every element is new, since the task merely requires the configuration of elements to be novel. Thus the overlap could largely result from the fact the both tasks encourage retrieval of episodic content. Somewhat similarly, both tasks have a constructive component whereupon initial descriptions are incrementally enhanced across an extended time period and this requirement would likely recruit working memory operations. Finally, both tasks may require mechanisms for overcoming episodic interference with the autobiographical task triggering multiple candidate events competing for selection and the future simulation tasks triggering episodic remembrances that potentially interfere with the goal of creating novel future scenarios. Current research is investigating these possibilities, and given the importance of accurate prediction and simulation in higher-order reasoning, this new line of research is quite important.

Acknowledgment

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See also: Cognitive Decline in Laboratory Animals: Models, Measures, and Validity.

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Gastrointestinal Peptides and the Control of Food Intake

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Glossary

Enteric neurons – Neurons that have their cell bodies and processes confined to the wall of the gastrointestinal (GI) tract. These neurons and their processes form two plexuses or nerve nets. One plexus, the submucosal plexus is immediately beneath the GI mucosa, while the other, the myenteric plexus, lies between two layers of smooth muscle that make up the wall of the GI tract.

Enteroendocrine cells – Specialized endocrine cells scattered among the absorptive cells of the gastrointestinal mucosa. These cells often are characterized as open or closed. Open enteroendocrine cells make contact with the luminal surface of the intestinal mucosa, while closed cells lie beneath the luminal mucosal surface. All enteroendocrine cells secrete one or more biologically active peptides, many of which enter the circulation and act hormonally at target sites in the GI tract and/or brain. Collectively, the enteroendocrine cells are referred to as the diffuse gut endocrine system.

Nodose ganglion – A cluster of nerve cell bodies lying next to the carotid artery at the base of the skull. This ganglion contains the cell bodies of vagal afferent neurons, many of which carry sensory signals from the gastrointestinal tract to the brain.

Paracrine – It refers to a secreted substance producing its action by acting on other cells that are accessed by diffusion of the secreted substance through the interstitial fluid. This mode of action is in contrast with an endocrine mode, in which secreted substance arrives at its target of action via the blood circulation.

Vagal afferents – Vagal fibers that carry sensory information from the internal organs, including the heart, lungs, stomach, and intestines, to the brain. The cell bodies of the vagal afferents are located in the nodose ganglion. From the nodose, each neuron cell body extends one end of an axon to terminate in the hindbrain, while the other extends to form a sensory ending in an internal organ.

History and Background of Gastrointestinal (GI) Peptides

The gastrointestinal (GI) tract accumulates food during meals and subjects it to physical and chemical processes to release absorbable products. These digestive products are transported across the intestinal mucosa and carried away by the hepatic portal circulation or intestinal lymphatics. Prior to their absorption, mechanical and chemical stimuli from ingesta trigger a variety of reflex changes in GI blood flow, motility, and secretion, as well as changes in ongoing and subsequent food intake. Thus, the GI tract is a site from which the quantity and chemical composition of foods can be pre-absorptively monitored in the interest of controlling ongoing or subsequent food intake. The manner in which the GI tract communicates intake-control signals to the central nervous system (CNS) is only partially appreciated. Nevertheless, it is apparent that peptides secreted by the GI mucosa profoundly affect food intake and may be major contributors to its control.

The epithelial lining of the GI tract, the mucosa, contains a variety of unique cell types that secrete peptide hormones. Collectively, these hormone-secreting GI mucosal cells are known as the diffuse gut-endocrine system. Indeed, the field of endocrinology itself began with the discovery that the upper small intestinal mucosa secretes a substance, secretin, that acts on the exocrine pancreas to evoke water and bicarbonate secretion. Later, the word ‘hormone’ – which literally means ‘I act’ – was coined following Gregory’s discovery of gastrin – a hormone secreted by cells in the gastric mucosa (G-cells). In 1973, Gibbs, Young, and Smith published the first evidence that a GI hormone participates in the control of food intake. They reported that injection of cholecystokinin (CCK) – a peptide secreted by the small intestinal mucosa – reduces food intake in rats and monkeys. Across more than three decades following the first reports

of CCK's effect on food intake, a number of additional GI peptides have been proposed to contribute to control of food intake. In addition, several nonpeptides, including cannabinoids, large proteins (insulin and leptin), and lipoproteins (apolipoprotein IV), are produced by or associated with the GI tract, and control food intake. These substances are not discussed in this article. Rather, coverage of nonpeptide or non-GI-related controls of food intake can be found elsewhere in this encyclopedia.

Gastrointestinal peptides appear in the systemic circulation after passage through the liver, via the hepatic portal venous drainage of the GI tract. The liver degrades a significant proportion of some of these peptides, such that plasma concentrations of many GI peptides typically are in the picomolar range. Gastrointestinal peptides in the systemic circulation act as hormones, reaching target sites in the GI tract and brain via the arterial circulation. However, one must remember that during their secretion these peptides must diffuse locally and, therefore, may exert their effects by paracrine as well as endocrine mechanisms. The GI sources of peptides discussed in this article are illustrated in **Figure 1**. An image of an immunohistochemically stained enteroendocrine cell is shown in **Figure 2**.

Cholecystokinin (CCK)

Discovery of CCK

As the first GI hormone implicated in the control of food intake, CCK's effects on feeding have been studied longer and more intensively than other GI peptides. In fact, subsequent to the first reports that exogenous CCK reduced food intake in rats and monkeys, CCK has repeatedly been found to reduce intake in rats, monkeys, the human, and other animals. The discovery of CCK activity was published, in 1928, by Ivy and Oldberg. They reported that extracts from small intestinal mucosa of the swine evoked contraction of canine gall bladder. Accordingly, they named the putative active agent CCK. In 1943, Harper and Raper reported that intestinal mucosal extracts evoked protein secretion by the exocrine pancreas, and named the active agent pancreozymin. However, by the 1960s purification and characterization of mucosal extracts in the laboratory of Viktor Mutt revealed that pancreozymin and CCK were the same substance, and cholecystokinin became its official name.

In the GI tract, CCK is synthesized and secreted by the enteroendocrine I cells located primarily in the duodenal and jejunal portions of the small intestine. Plasma CCK concentrations rise following food intake, especially when foods are high in fat or protein content. In rats plasma

Sources of Gastrointestinal Peptides controlling food Intake

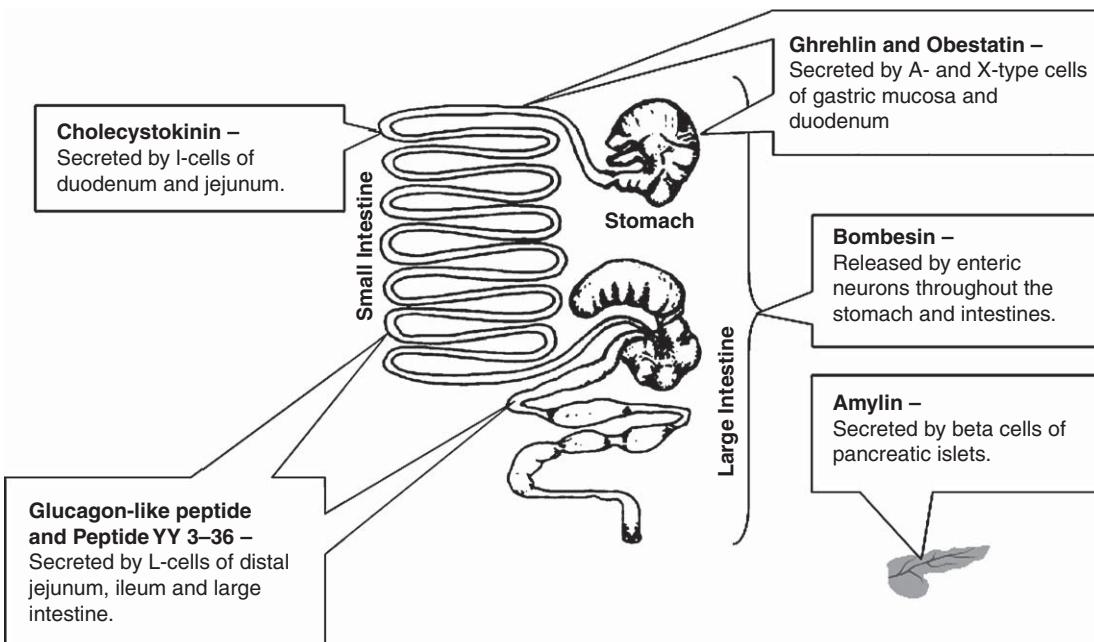


Figure 1 Sources of gastrointestinal peptides controlling food intake. Diagram of rat gastrointestinal tract is adapted with permission from Swenson MJ (1977) *Dukes' Physiology of Domestic Animals*. New York: Cornell University Press. Copyright 1933 by H. H. Dukes; Copyright © 1977 by Cornell University.

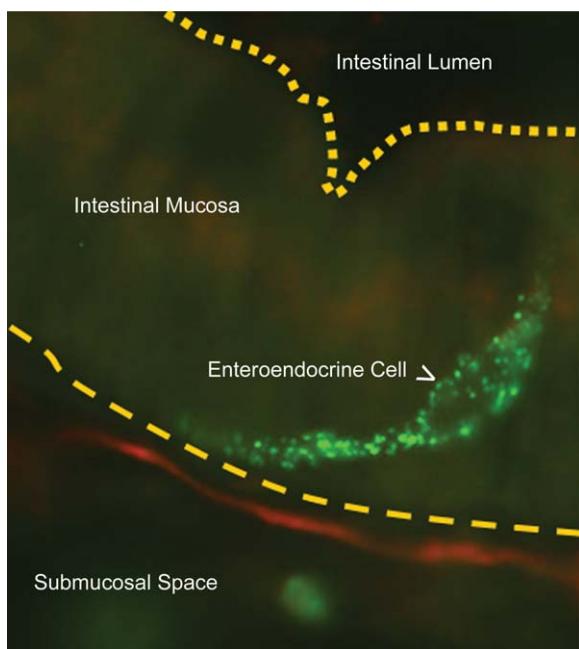


Figure 2 Fluorescence photomicrograph of rat small intestinal mucosa showing an enteroendocrine cell (L-cell) stained to reveal peptide secretory granules containing GLP-1 (green). Nutrients in the intestinal lumen stimulate these cells to secrete peptides into the submucosal space where they enter blood capillaries of the hepatic portal circulation. The secreted peptides also are likely to activate nerve fibers in the submucosa. The red linear structure in this image is a nerve fiber. Note its close proximity to the secretory process of the enteroendocrine cell. This proximity is consistent with local paracrine action of the secreted peptide to activate the fiber. Image is from C. Philes and R. Ritter, unpublished data.

CCK concentration is also elevated by direct intestinal infusion of long-chain fatty acids, protein, or peptones, but not by infusion of carbohydrate.

Forms of CCK

Cholecystokinin has been detected in the intestinal mucosa and in the circulation in a variety of molecular forms ranging from 8 to 58 amino acids, all of which are sulfated at tyrosine seven.

Because the principal identifying biological actions of endogenous CCK are mimicked by the sulfated carboxyl terminal eight-amino-acid sequence, most investigations of CCK effects on food intake have been conducted using the synthetic CCK octapeptide, sulfated CCK-8. However, Reeve and colleagues have demonstrated that the 58-amino-acid form of CCK, CCK-58, is the sole circulating form of endogenous CCK in the rat, and probably is also the principal form in other species as well. Moreover, several intriguing reports reveal that some biological actions of CCK-58 are distinct from those of shorter CCK forms, like CCK-8. Finally, because the circulating half-life of CCK-58

is significantly longer than that of CCK-8, it is likely to persist in the vicinity of its receptors for a longer period of time than do shorter CCK fragments. Whether there are quantitative or qualitative differences between CCK-58 and CCK-8 relative to control of food intake awaits further investigation.

CCK Receptors

Cholecystokinin acts via two G-protein-coupled receptors (GPCR) – the CCK-1 (formerly CCK-A) and CCK-2 (formerly CCK-B/Gastrin) receptor. Effects of CCK on food intake are mediated by CCK-1-receptor activation. Gastrin or unsulfated CCKs have activity at CCK-2 receptors, but not CCK-1 receptors. Hence, unsulfated CCK and gastrin do not significantly reduce food intake. Reductions of food intake following injection of exogenous CCK, or release of endogenous CCK after intestinal nutrient infusions, are attenuated or abolished by CCK-1 receptor antagonists in the human and the rat, while systemic administration of CCK-2-receptor antagonists are ineffectual. Therefore, it appears that reduction of food intake by both exogenous and endogenous CCK is mediated by CCK-1-receptor-dependent mechanisms.

Sites and Mechanism of CCK Action in Control of Food Intake

The vagus nerve and CCK's effects on feeding

Both *in situ* hybridization and electrophysiological studies indicate that between 30% and 40% of vagal afferents express CCK-1 receptors. Moreover, experiments using retrogradely labeled vagal afferents suggest that virtually all CCK-1-expressing vagal afferents innervate the upper GI tract. Finally, extracellular recording from vagal afferent fibers indicates that the activation of vagal afferents by intestinal stimuli is attenuated by CCK-1-receptor antagonists. These data are consistent with behavioral evidence for vagal afferent mediation of exogenous and endogenous CCK effects on food intake. Specifically, reduction of food intake by intraperitoneal injection of exogenous CCK is attenuated in rats that have undergone surgical vagal deafferentation of the GI tract or capsaicin-induced destruction of small unmyelinated vagal afferent neurons. In addition, both surgical vagal afferent denervation of the GI tract and capsaicin treatment attenuate reduction of food intake in response to intestinal nutrient infusions that stimulate CCK secretion. Thus, activation of abdominal, capsaicin-sensitive vagal afferents is necessary for the reduction of food intake by intraperitoneal CCK injections and by endogenously released CCK.

Recent reports suggest that most CCK-sensitive vagal afferents innervate the stomach and upper small intestine. In other words, it appears that the specific vagal afferent population that responds to CCK is targeted to the upper GI tract. Furthermore, intra-arterial infusion experiments

reveal that exogenous CCK infused near or directly into the celiac arterial supply to the stomach and upper small intestine reduces food intake at a dose below that required when CCK is infused into the systemic venous circulation. Finally, the infusion of a CCK-A-receptor antagonist directly into pancreaticoduodenal artery – supplying the upper small intestine – has been reported to increase meal size at doses that are ineffective when infused via the jugular vein. Taken together, these results suggest that the control of food intake by CCK is mediated specifically by the activation of peripheral vagal afferent fibers that innervate the upper GI tract.

Most of CCK's physiological actions are presumed to occur by an endocrine mechanism. That is, CCK secreted from the intestine enters the hepatic portal venous circulation and arrives at its target receptors via the arterial supply. However, some intestinal vagal afferents course through the lamina propria close to CCK-secreting I-cells, and several investigators have suggested that CCK might activate these afferents in a paracrine manner. Circumstantial evidence for a proposed paracrine mode of action comes from studies of the CCK-1 receptor state that mediates reductions of food intake. Specifically, the CCK-1 receptor exhibits both high- and low-affinity binding states. The high-affinity domain has a dissociation constant (K_d) for CCK in the picomolar range, while the low-affinity domain has a nanomolar K_d . Examinations of CCK responsiveness in cultured vagal afferents indicate that all vagal afferent responses to CCK are mediated by the low-affinity CCK-A-receptor state. Most experimental evidence indicates that plasma CCK concentrations sufficient to reduce food intake are in the range of those that would act on the low-affinity state, consistent with a paracrine mode of action. In addition, consistent with a paracrine mode of action is the fact that JMV-180 – a CCK analog that is an antagonist for the rat low-affinity CCK-1-receptor state, but an agonist for the high-affinity state – reverses reduction of food intake by exogenous CCK-8 in the rat. This result suggests that, at least in the rat, feeding effects of CCK are low-affinity-site effects. Nevertheless, if gastric as well as duodenal vagal afferents participate in CCK's effects on feeding, then endocrine CCK must play some role because, while gastric afferents are CCK sensitive, no CCK is secreted from the gastric mucosa. Hence, paracrine activation of gastric vagal afferents cannot occur.

CCK, the brain, and control of food intake

Using chronically decerebrated rats fed via cheek fistulas, Grill and colleagues demonstrated that the termination of consumption by CCK can occur in the absence of forebrain connections. While this result demonstrates dramatically that substrates for meal termination are present in the hindbrain, they do not obviate forebrain participation in affective, motivational, and metabolic

aspects of satiation. Nor do they rule out the participation of nonvagal sites of CCK action. Indeed, CCK has been shown to alter the activity of neurons in a variety of brain areas, some of which are associated with control of food intake. Likewise, CCK has been shown to modulate the activity of hindbrain neurons by acting, in part, on central terminals of vagal afferents. In addition, injection of CCK-8 into several forebrain and hindbrain sites is reported to produce small-to-moderate reductions of food intake. However, centrally applied CCK-receptor antagonists do not reverse the effects of systemically injected CCK, and do not attenuate reduction of food intake following intestinal infusions of fatty acid, which releases endogenous CCK. Therefore, it is unlikely that direct action on the brain by circulating CCK can account for CCK effects on food intake. However, CCK is synthesized by many neurons in the brain, and a role for central neuronal CCK in control of food intake should not be ruled out.

CCK and Body-Weight Control

Cholecystokinin is thought to reduce food intake by contributing to the process of satiation or meal termination. Consistent with this interpretation, rats injected with exogenous CCK reduce food intake primarily by reducing the size of individual meals (meal size), rather than decreasing the number of meals taken (meal frequency). Rats that are treated chronically with CCK exhibit reduced meal size, but tend to compensate for this reduction by increasing meal frequency. Hence, chronically infused CCK tends not to reduce long-term food intake or body weight. On the other hand, repeated administration of synthetic CCK-1-receptor agonists has been reported to reduce both food intake and body weight in rats and monkeys. Furthermore, rats that do not express CCK-1 receptors overeat and become obese. Finally, CCK-1-receptor-deficient rats do not gain excess weight if their food intake is yoked to that of normal control rats. Hence, obesity in this model appears to be entirely due to increased food intake. Unlike CCK-1-receptor-deficient rats, mice that do not express CCK-1 receptors do not overeat or become obese. However, examination of their meal patterns reveals that they exhibit increased meal size, which is compensated by decreased meal frequency. Thus, unlike rats, mice without CCK-1 receptors do have a satiation deficit, but effects of the deficit on body weight seem to be compensated by adjustments in meal frequency.

CCK and Treatment of Obesity

The prospects for CCK in the pathogenesis or treatment of obesity have risen and fallen over the decades since its effects were first recognized. The fact that several CCK-

1-receptor gene polymorphisms have been associated with excess body weight in the human suggests a link between CCK and obesity pathogenesis. In addition, plasma CCK concentrations appear to vary inversely with body mass index, suggesting that decreased CCK might play a role in sustaining increased body adiposity. On the other hand, while increased postmeal plasma CCK concentration has been reported in patients who have undergone vertical gastric banding for treatment of obesity, CCK is not generally elevated in patients who have undergone other bariatric procedures. Hence, for the most part, CCK has not been strongly implicated in loss of body weight following bariatric surgery. Trials of CCK agonists for control of food intake and body weight have, in general, been disappointing. However, several nutraceuticals that release endogenous CCK claim efficacy in the treatment of obesity, and have received limited support from controlled experiments in human volunteers.

Glucagon-Related Peptide 1

Preproglucagon Gene Products

Glucagon-related peptide 1 (GLP-1) is coded by the preproglucagon gene. The best-known product of this gene is the hormone glucagon itself, which is secreted by the pancreatic α -cells. During the 1970s, the use of antisera raised against pancreatic glucagon detected glucagon-like immunoreactivity in the GI tract. However, cloning of the preproglucagon gene in the 1980s revealed that it encoded not only glucagon, but also several other related peptides, which are generated in a tissue-specific manner by posttranslational processing. These include GLP-2 and oxyntomodulin. Of these preproglucagon-derived peptides, GLP-1 has been most extensively studied with regard to control of food intake. Systemic administration of GLP-1-receptor agonists reduces food intake in rodents, monkeys, and the human. Furthermore, in rodents, GLP-1 agonists reduce food intake when they are injected into the brain ventricles at doses that are ineffective when systemically administered. Although GLP-1-receptor knockout mice do not overeat or become obese, the fact that GLP-1 antagonist administration increases food intake supports a role for the endogenous peptide in control of food intake. Though not studied as extensively as GLP-1, oxyntomodulin is also reported to decrease food intake. At the moment, it is not entirely clear whether or not oxyntomodulin's effects are mediated by actions at the GLP-1 receptor.

Source and Disposition of Circulating GLP-1

Most GLP-1 is synthesized in the GI mucosa, where GLP-1 immunoreactivity is localized to enteroendocrine L-cells, distributed primarily in the mucosa of the distal

jejunum, ileum, and colon. In many L-cells, GLP-1 immunoreactivity is colocalized with another gut peptide, either gastric inhibitory polypeptide (GIP) or peptide YY (PYY; See below). GLP-1 secretion by L-cells is directly triggered by nutrients entering the distal small intestine and colon, but its secretion may also be triggered by endocrine/vagal reflex in response to nutrient stimulation of the proximal small intestine. GLP-1 secretion is most strongly stimulated by fat, but unlike CCK, carbohydrate also triggers GLP-1 release.

The principal biologically active forms of GLP-1 in the circulation are GLP- 1_{7-36} and GLP- 1_{7-37} amide. However, both of these GLP-1 forms are rapidly degraded by dipeptidyl peptidase IV (DPP-IV), which is present in the circulation and in the capillary endothelium adjacent to L-cells. Additional degradation of GLP-1 occurs as the peptide transits the hepatic portal circulation. Consequently, only a fraction of active GLP-1 reaches the systemic circulation, and circulating concentrations even of total GLP-1 immunoreactivity are in the low picomolar range. Because the enzyme dipeptidyl peptidase IV (DPP-IV) also rapidly inactivates exogenously administered GLP-1, many pharmacological studies of GLP-1 effects on food intake have used the GLP-1-receptor agonist, exendin 4, a DPP-IV-resistant peptide found in gila monster lizard venom.

Sites of GLP-1 Action in Controlling Food Intake

GLP-1, the brain, and control of food intake

In addition to being synthesized and secreted by intestinal L-cells, GLP-1 is synthesized and released by a small population of neurons located in the dorsal hindbrain. Interestingly, hindbrain GLP-1 neurons provide a dense projection throughout the forebrain, including areas of the hypothalamus that are involved in control of food intake and body weight. Moreover, these neurons are activated by the adipokine hormone, leptin, and by GI stimuli, such as gastric distension. Activation of hindbrain GLP-1 neurons also occurs during visceral illness, and the synaptic release of brain GLP-1 may play an important role in toxin-induced suppression of food intake and in conditioning of taste aversions. Brain GLP-1 receptors that are activated by neuronal GLP-1 might also be activated by circulating GLP-1. However, while this mode of action might account, at least in part, for actions of exogenously administered GLP-1 agonists, especially DPP-IV-resistant agonists, it seems unlikely that endogenous GLP-1 from L-cells would reach plasma concentrations sufficient to influence brain GLP-1 receptors.

The vagus nerve and GLP-1 control of feeding

The GLP-1-receptor mRNA is present in vagal afferent neurons. In addition, vagal afferent neurons in culture are

activated by GLP-1. Therefore, vagal afferent activation comprises a route by which endogenous GLP-1 could trigger reduction of food intake. Nevertheless, at this time there is only one published report suggesting that vagotomy attenuates reduction of food intake by exogenous GLP-1. This paucity of data demonstrating an exclusively peripheral site of action for GLP-1-induced reduction of food intake may be related to the short half-life of native GLP-1 in peritoneal fluid or plasma. In addition, DPP-IV-resistant GLP-1 agonists may remain in the circulation at concentrations high enough to activate central as well as vagal GLP-1 receptors, making it difficult to confirm the physiological site of GLP-1 action using pharmacological approaches.

GLP-1 Agonists and Body-Weight Control in the Human

In some, but not all, studies, chronic treatment of rodents with DDP-IV-resistant GLP-1 agonists has resulted in significant reduction of food intake and body weight. In addition, several GLP-1-receptor agonists have shown some promise for reducing food intake and body weight in the human. Exenatide, a DPP-IV-resistant GLP-1 receptor antagonist, is approved for use as an adjunct in treating diabetes mellitus. In combination with metformin or a sulfonylurea, exenatide treatment of type 2 diabetics is associated with reduced food intake and a small amount of body-weight loss. However, the drug must be injected twice a day in association with meals, and nausea is a significant side effect. Finally, exenatide is only 53% homologous with human GLP-1, and therefore its use results in the generation of antibodies against this peptide. In recent short trials, another DPP-IV-resistant GLP-1 analog, liraglutide, has been reported to produce moderate weight loss without inducing an immune response. These results and others raise the possibility that GLP-1 agonists might be useful in the treatment of nondiabetic obese individuals as well as for diabetics.

Peptide YY 3-36 (PYY 3-36)

Chemistry and Source of PYY 3-36

PYY3-36 is a member of the pancreatic polypeptide (PP)-fold family of peptides, so named because members of this family, including PYY, PP, and neuropeptide Y (NPY), have an N-terminal polyproline segment that enables it to fold back onto a C-terminal-adjacent α -helical portion of the peptide. PP-fold peptides act at a series of receptors, Y1–Y6. Notably, some members of this family, including PYY and NPY increase food intake when they bind to Y1 and Y5 receptors in the brain. On the other hand, PYY3-36 reduces food intake in rodents, the human, and nonhuman primates when it is administered systemically.

PYY3-36 is generated when an N-terminal Tyr-Pro dipeptide is cleaved from PYY1-36 by DPP-IV. This enzymatic conversion confers a relative preference for binding at Y2 receptors, and it is this preference that accounts for PYY3-36's ability to reduce food intake. As mentioned above, PYY is synthesized by enteroendocrine L-cells of the distal jejunum and large intestine. Many of these cells also produce GLP-1 and oxyntomodulin. High levels of DPP-IV are found in capillary endothelium near the base of the L-cells. Thus, it seems that the conversion of potentially orexigenic PYY to the satietogenic PYY3-36 is mediated by the same enzyme that actually destroys the satietogenic activity of GLP-1.

Sites of PYY 3-36 Action in Controlling Food Intake

Effects of intracerebral PYY 3-36 on food intake

While exogenous PYY3-36 reduces food intake following systemic administration, direct injection of the peptide into the brain has produced mixed results. Injection of the peptide into the hindbrain or the hypothalamic paraventricular nucleus (PVN) increases food intake, while injection into the arcuate nucleus (ARC) of the hypothalamus decreases food intake. This variability probably is related to the fact that, although PYY3-36 is a Y2-receptor agonist, at high concentrations it may also activate Y1 and Y5 receptors. In addition, differences in the density of Y1, Y2, and Y5 binding sites accessed by the injections may determine the sign of the behavioral response.

Vagal involvement in control of food intake by PYY 3-36

While Y2 receptors expressed in the hypothalamic ARC have been proposed as a site of PYY3-36-induced reduction of food intake, Y2 receptors are expressed by vagal afferent neurons. Furthermore, several reports indicate that vagotomy and capsaicin treatment abolish reduction of food intake by PYY3-36. Therefore, it seems that PYY3-36, like GLP-1, can reduce food intake by actions at more than a single neural substrate. Nevertheless, it is not yet clear from available data which substrates comprise physiological sites of action for circulating PYY3-36.

Possible Involvement by PYY 3-36 in Weight Gain and Weight Loss after Bariatric Surgery

As with CCK, a role for PYY3-36 in the control of body weight has not been conclusively determined. However, compared to wild-type controls, female mice that do not express the PYY gene become obese on standard rodent diet, and both males and females gain excess weight on high-fat diets. As mentioned above, the administration of

exogenous PYY3-36 reduces food intake in the human, and chronic administration reportedly results in loss of body weight. In this regard, it is intriguing that plasma concentrations of PYY3-36 are elevated in people who lose weight following gastric bypass surgery, suggesting a possible role for this peptide in reduced food intake and body weight following this bariatric procedure.

Bombesin-Related Peptides

Discovery of Bombesin and Distribution of Bombesin-Related Peptides

Bombesin (BBS) is a 14-amino-acid peptide first isolated from the skin of the frog, *Bombina bombina*, in 1970 by Erspamer. Injection of exogenous BBS in rat and other mammals reduces food intake, and in the rat it triggers a sequence of behaviors that accompany satiation. While BBS-like immunoreactivity is present in a variety of cells and organs, including the GI tract and the nervous system, BBS itself is not synthesized by mammals. However, several peptides that appear to be structural homologs of BBS have been characterized in mammals. Specifically, gastrin-releasing peptide (GRP), a 27-amino-acid peptide was identified in GI extracts in 1979 by McDonald. It has a 10-amino-acid carboxyl terminal sequence that differs from BBS by just one amino acid. Similarly, neuromedin B (NMB), which exists as a 32-amino-acid peptide as well as a C-terminal 10-amino-acid peptide, is also similar to BBS and related peptides from amphibian skins. Both GRP and NMB reduce food intake when systemically administered. However, neither is as potent as BBS, perhaps because GRP and NMB prefer different and distinct GPCRs, while amphibian BBS binds with high affinity both to GRP- and NMB-preferring receptors. GRP and NMB are found in neurons of the CNS and peripheral nervous system, including enteric neurons of the small and large intestines. Neither peptide appears to be synthesized in enteroendocrine cells, making it unlikely, but not impossible, that BBS-like peptides exert their effects via an endocrine pathway.

Receptors for BBS-Related Peptides

Three GPCRs for BBS-like peptides – BB1, BB2, and BB3 – have been cloned from various mammals, including the human, rat, and monkey. NMB binds with higher affinity to the BB1 receptor than does GRP, whereas GRP has a higher affinity than NMB for the BB2 receptor. The distribution of BB1 and BB2 closely parallel the distribution of NMB and GRP respectively. A third orphan receptor, BB3, appears to be a binding site for BBS-like peptides, but both GRP and NMB bind BB3 with low affinity. While synthetic ligands for BB3 have been synthesized, the endogenous ligand has not been identified.

Sites Where BBS-Related Peptides Act to Control Food Intake

Abdominal visceral BBS receptors

Experiments involving near-arterial infusion of BBS into the celiac or superior mesenteric vascular beds suggest that reduction of food intake by systemic BBS is mediated by receptors in the upper GI tract. Reduction of food intake by systemic BBS depends on both the vagal and spinal innervation of the GI tract, as abolition of BBS-induced reduction of food intake occurs only in rats with bilateral abdominal vagotomy plus transection of the thoracic spinal cord. Furthermore, it appears that some, but not all, of systemic BBS effects on food intake are mediated by small, unmyelinated, primary afferent neurons. Specifically, reductions of food intake by systemically administered BBS or NMB are abolished in capsaicin-treated rats, but reduction of intake by GRP injections is not attenuated. Thus, it appears that the two known mammalian BBS-like peptides act via distinct neural substrates.

Hypothalamic BBS receptors and control of food intake

As with GLP-1, BBS-like peptides reduce food intake when injected into the brain, as well as when they are injected systemically. BBS-like peptides and their receptors are expressed in many brain areas known to participate in control of food intake, including the paraventricular, arcuate, and ventromedial hypothalamic nuclei of the hypothalamus, the lateral parabrachial area in the pons, and the nucleus of the solitary tract in the dorsal medulla. Therefore, it is probable that BBS-like peptides participate in control of food intake at more than one central site. Finally, hypothalamic expression of at least one BBS-like peptide, GRP, has been shown to vary with conditions and treatments that alter food intake. Specifically, GRP expression in the PVN is decreased by food deprivation, and this decrease is reversed by administration of a melanocortin agonist that reduces food intake. Hence, central BBS-like peptides modulate feeding behavior in response to signals of systemic energy deficit and surfeit.

Hindbrain BBS receptors in control of food intake by central and peripheral BBS-related peptides

A role of brain BBS-like peptides in reduction of food intake by systemically administered BBS is uncertain. However, fourth ventricle administration of BBS-receptor agonists reduces food intake. Moreover, the nucleus of the solitary tract adjacent to the area postrema appears to be the most sensitive and selective brain site for BBS-induced reduction of food intake. In this regard, it is interesting that lesions involving the area postrema and nucleus of the solitary tract attenuate reduction of food

intake by both centrally and systemically administered BBS. In addition, hindbrain injection of a GRP antagonist has been shown to attenuate reduction of food intake by systemically administered GRP, indicating that hindbrain GRP receptors are involved in reduction of food intake by systemically injected peptide. Whether these results indicate a role of the hindbrain BBS-like peptide receptors in the communication of other visceral afferent signals to the brain has not been determined.

BBS Receptors and Obesity

Intracranial injection of BB1 and BB2 receptor antagonists results in increases of short-term food intake, but is not reported to increase body weight. Furthermore, rodents that do not express BB1 or BB2 do not become obese. On the other hand, mice in which BB3 had been knocked out are hyperphagic and obese. Because the endogenous ligand for BB3 does not appear to be either GRP or NMB, the contribution of endogenous BBS-like peptides to the pathogenesis of obesity remains an important unresolved issue.

Amylin

Source of Amylin

Amylin is a 37-amino-acid peptide that is co-secreted with insulin from the pancreatic beta cells. Therefore, unlike true enteroendocrine peptides, amylin is secreted primarily in response to postabsorptive stimulation of pancreatic beta cells by glucose. Injection of exogenous amylin reduces food intake in rats, mice, and humans. The effect of exogenous amylin on food intake is achieved by reducing meal size. However, high doses of amylin administered chronically also produce decreased meal frequency. Since endogenous amylin secretion is prandial in nature, it is not yet clear whether reduced meal frequency is a physiological effect of amylin. However, it does seem clear that under conditions of chronic amylin administration both laboratory rodents and the humans exhibit reduced food intake and decreased body-weight gain. Like CCK, GLP-1, and PYY 3-36, amylin inhibits gastric emptying. However, similar to CCK, the effects of amylin on satiation do not depend on inhibition of gastric emptying, since amylin inhibits ingestion when rats are consuming a diet that empties from the stomach via an open gastric cannula (sham feeding).

The Area Postrema and Amylin's Control of Food Intake

Amylin reduces food intake when injected directly into the brain. Furthermore, amylin receptors are expressed in multiple areas of the forebrain and hindbrain.

Nevertheless, reduction of food intake by either acute or chronic systemic administration of amylin is abolished by lesions of the area postrema and adjacent nucleus tractus solitarius (NTS). Moreover, while systemic amylin injection triggers neuronal activation in the parabrachial nucleus, bed nucleus of the stria terminalis, and central nucleus of the amygdala, this activation – along with reduction of food intake – is abolished in area postrema-lesioned rats, indicating that it is synaptically driven and not due to a direct forebrain effect of circulating amylin. Systemic administration of amylin is reported to decrease food-deprivation-induced neuronal activation in the lateral hypothalamus in a manner similar to refeeding. However, experiments designed to determine whether this effect is mediated via the area postrema, or is a direct action of amylin in the brain, have not been reported.

Amylin Agonists and Body-Weight Control in the Human

The amylin agonist, pramlintide, is being used as an adjunct in treating diabetes mellitus in the human, where it contributes to the plasma glucose-lowering effect of insulin through inhibition of glucagon secretion, gastric emptying, and food intake. In addition, pramlintide is reported to produce sustained small-to-moderate reductions of food intake and body weight in obese individuals who are injected the drug on a 2–3-times-daily basis over the course of a year. Therefore, this amylin agonist may turn out to be a useful adjunct for weight control especially in overweight individuals with type 2 diabetes.

Ghrelin

Ghrelin's Discovery and Sources

Ghrelin distribution and secretion from the GI tract

The name ghrelin derives from an Indo-European root word meaning ‘to grow.’ This peptide is the endogenous ligand for the growth hormone-stimulating receptor (GHSR), which was an orphan receptor prior to ghrelin’s isolation from gastric mucosa in 1999. At present, ghrelin is the only peptide secreted by the GI tract that is known to increase food intake. It is a 28-amino-acid peptide cleaved from the protein product of the preproghrelin (PPG) gene. The PPG gene also codes for another peptide, obestatin. Some investigators have reported that obestatin decreases food intake, while others have observed no effect of this peptide on food intake. Although small amounts of ghrelin may be produced in the brain and other organs, virtually all circulating ghrelin comes from the stomach (75%) and upper small intestine (25%), where it is synthesized and secreted by two anatomically distinct populations of mucosal endocrine cells.

So far as is known, ghrelin is unique in that its biological activity depends upon it being octanoylated (octanoylation refers to the formation of an ester or amide with octanoic acid) at the serine-3 amino acid residue.

Ghrelin and meal initiation

Within 2 years of its isolation, several research groups reported that ghrelin increased food intake when injected either systemically or into the brain. Moreover, while peptides such as CCK and GLP-1 reduce food intake primarily by reducing the size of individual meals, exogenous ghrelin increases food intake mainly by shortening the latency to eat. In other words, ghrelin is involved in triggering meal initiation, not in increasing meal size. Consistent with its effect on meal initiation, circulating ghrelin levels rise prior to the onset of meals and fall to lower levels immediately after a meal.

Ghrelin Receptors and the Control of Food Intake

Forebrain ghrelin receptors and food intake

The GHSR or ghrelin receptor is expressed in many areas of the nervous system that are involved in the control of food intake and metabolism. It is abundant in the hypothalamus, especially the ARC and PVN, but it also is expressed in other brain areas, including the ventral tegmental area, amygdala, and the nucleus of the solitary tract. Interestingly, ghrelin injection into any of these areas elicits increases food intake. These observations, and others, suggest that ghrelin may enhance the activity of multiple brain circuits that participate in the reflexive, appetitive, and motivational aspects of feeding behavior. On the other hand, lesion of the ARC or the destruction of NPY-receptor-expressing neurons in the basomedial hypothalamus abolishes increased food intake in response to central ghrelin injection, suggesting that hypothalamic circuitry is necessary for ghrelin-evoked increase in food intake.

Potential for hindbrain and vagal ghrelin receptors in the control of food intake

The ghrelin receptor is expressed in the NTS, and also by primary vagal afferent neurons that terminate there. Furthermore, hindbrain injection of ghrelin increases food intake suggesting that the peptide may interact with viscerosensory signals from the GI tract. In fact, ghrelin-receptor expression is colocalized with the CCK-1 receptor in vagal afferent neurons. Thus, a component of ghrelin action might involve modulating vagally transmitted satiation signals. In this regard, it is interesting that bilateral subdiaphragmatic vagotomy or destruction of the NTS catecholamine neurons has been reported to abolish increased food intake evoked by ghrelin. However, attenuation of ghrelin-evoked feeding in vagotomized rats or rats with selective and verified destruction

of NTS catecholamine neurons has not been replicated by several laboratories that are expert with these lesion preparations. Hence, participation of visceral afferent ghrelin receptors in ghrelin-evoked feeding remains uncertain.

Ghrelin and Control of Body-Weight Gain

Effects of pharmacological and genetic manipulations of ghrelin on food intake and body weight

Chronic central administration of ghrelin results in increased adiposity and reduced oxygen consumption in rodents, indicating that this peptide has effects on energy balance that go beyond its effects on food intake. In addition, mice with targeted deletion of the ghrelin receptor tend to be somewhat leaner than wild-type mice. In contrast to GHSR knockouts, the adiposity of PPG knockout mice does not differ from that of wild-type mice. However, mice that do not express the PPG gene are unable to maintain body temperature when food deprived in moderately cool environment. Therefore, it seems evident that understanding of ghrelin's functions is far from complete, and much remains to be revealed about its role in energy homeostasis.

Circulating ghrelin and response to bariatric surgery

Like other peptides with effects on food intake and adiposity, ghrelin and its receptor have attracted considerable interest as potential points of intervention in treatment of human eating disorders and obesity. Plasma ghrelin levels have been measured by many investigators prior to and following various bariatric surgical procedures for treatment of obesity. Not surprisingly, bariatric procedures in which substantial amounts of the gastric fundus are excised (sleeve gastrectomy) result in reduced plasma ghrelin concentrations. On the other hand, procedures in which weight loss results after gastric banding report mostly increases or no change in plasma ghrelin levels. While some reports suggest that reduction of plasma ghrelin concentration is correlated with weight loss after Roux-en-Y gastric bypass surgery, others find that ghrelin levels are not decreased even though patients experience massive postbypass weight loss. Thus, while altered circulating ghrelin may contribute to decreased appetite and weight loss following some procedures, it is likely that additional factors contribute to the weight-loss efficacy of bariatric procedures, and that decreases in plasma ghrelin are not essential to postsurgical weight loss.

See also: Control of Food Intake; Obesity and Binge Eating Disorder; Stress and Energy Homeostasis.

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Gaze Stabilization and the VOR

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Glossary

Conjugate eye movements – Eye movements of similar amplitude and direction for both eyes.

Disjunctive eye movements – Eye movements of different amplitude for the two eyes. Disjunctive components are typically quantified by measuring the vergence angle, defined as the difference between the right and left eye position.

Nystagmus – A sawtooth-like (zigzag) pattern where slow deviations in eye position are interleaved with oppositely directed fast phases (reflexive saccades) that keep eye position within the oculomotor range.

Optokinetic nystagmus (OKN) – A reflexive visuo-ocular response to motion of a large visual field that matches slow-phase eye velocity to the velocity of the visual surround.

Otolith organs – Inertial sensors that are located in the inner ear and consist of receptor hair cells with different polarization vectors distributed over the utricular (approximately in the horizontal plane) and the saccular (approximately in the sagittal plane) maculae.

Retina – The inner, posterior surface of the eye that houses the photoreceptors responsible for transducing visible light. The region of the fovea with the most densely packed photoreceptors is known as the fovea and provides high visual acuity.

Retinal slip – Difference between the velocity of the movement of a retinal image and the velocity of the eye movement that is picked up by motion detectors in the visual system.

Semicircular canals – Vestibular end organs that measure angular acceleration of the head. In each ear there are three semicircular canals: horizontal, anterior, and posterior. Each canal senses angular motion in its respective plane.

Vergence – The angular difference between right and left eye position. Vergence angle is zero when looking at optical infinity and reaches higher values the closer a target is to the eyes.

Vestibular apparatus – A set of balance receptors in the inner ear consisting of the otolith organs (utricle and saccule) that encode linear acceleration and the semicircular canals (horizontal, anterior, and posterior) that measure angular acceleration.

The vestibulo-ocular reflexes (VORs) are eye movements designed to keep the visual image stable on the retina when the head is in motion in order to facilitate perception. This is largely done by moving the eyes in an equal but opposite direction to the movement of the head. Keeping gaze (i.e., the line of sight) fixed onto objects of interest is a critical task in humans because only a small region of the retina, known as the fovea, has the most densely packed photoreceptors and thus provides high visual acuity. Due to this anatomical feature, light reflecting from important objects must remain on the fovea for one to perceive them accurately. For example, one is quite good at reading the words on this page even when one shakes one's head right and left. In contrast, the world would appear much more unstable without this reflex, as is the case when viewing the world after it has been filmed with a handheld camera. This shaky view is likely what patients with a damaged VOR see. They cannot read while walking or even sitting in bed unless they physically hold their heads perfectly stationary.

Detecting Head Motion with the Vestibular Apparatus

The VOR is activated when movement of the head is detected. This is accomplished by several structures in the inner ear, collectively known as the vestibular apparatus ([Figure 1\(a\)](#)). First, two sets of three nearly orthogonal canals, symmetrically positioned as mirror images of each other on each side of the head, detect head rotations in space ([Figure 1\(b\)](#)). These rotations can be described as movement about three perpendicular axes known as yaw (the longitudinal body axis), pitch (the intra-aural axis), and roll (the naso-occipital axis). Shaking the head is mainly a yaw rotation, whereas nodding the head is mainly a pitch rotation. Bringing the right ear down to the right shoulder constitutes a roll rotation.

Physiologically, head motion is detected because the semicircular canals are filled with fluid. When the head begins to rotate, the bony canals move with it. However, the viscous fluid, known as endolymph, is not physically attached to the body and so it lags behind. Thus, relative to the canals, the endolymph rotates in the opposite direction and consequently displaces a gelatinous

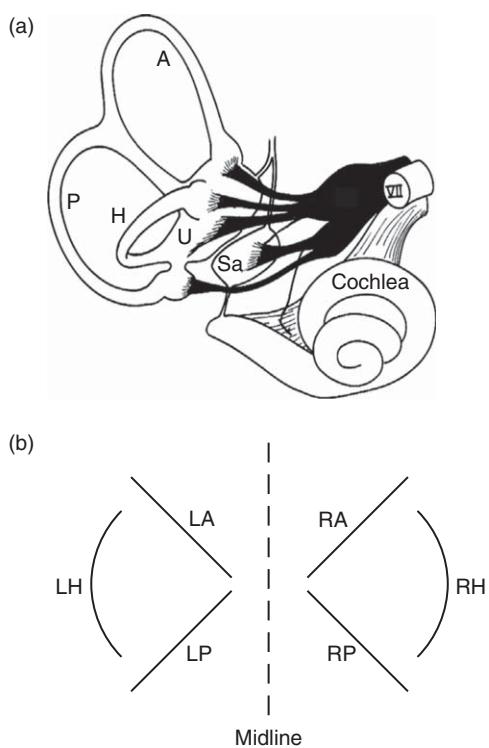


Figure 1 Vestibular apparatus. (a) Structures of the inner ear (side view). The vestibular system consists of three semicircular canals (A, anterior; P, posterior; H, horizontal) that detect rotational motion and two otolith organs (U, utricle; Sa, saccule) that detect translational motion and changes in the body's orientation relative to gravity. Signals from these end organs are transmitted to the vestibular nucleus via the vestibular nerve (cranial nerve VII). These structures are found next to the cochlea that transduces sound waves. (b) The three semicircular canals are bilateral structures (a). RA, right anterior; RP, right posterior; RH, right horizontal; LA, left anterior; LP, left posterior; LH, left horizontal. Pairs of canals (RA-LP; LA-RP; and RH-LH) detect head motion in the same plane. Working like a push-pull mechanism, one canal in the pair elicits excitation, whereas the other canal causes inhibition (depending on the direction of rotation). (a) Reprinted from [Hardy M \(1934\)](#) Observations on the innervation of the macula sacculi in man. *Anatomical Record* 59: 403–418, copyright 1934. With permission of John Wiley & Sons, Inc.

structure located in each canal known as the cupula. Hair cells embedded in the cupula are also displaced by this motion, and their displacement unleashes an electrochemical change (i.e., transduction) that is transmitted to the brain, indicating that the head is rotating. Displacement of the hair cells in one direction causes depolarization (and therefore excitation), whereas displacement in the opposite direction causes hyperpolarization (and therefore inhibition). The VOR associated with head rotations is referred to as the rotational VOR or rVOR.

Two additional structures, the utricle and the saccule, use similar mechanisms to signal that the head is translating or changing its position relative to gravity. Translational movements include standing up from a seated position (i.e., a vertical translation) or accelerating

in a car (i.e., a horizontal translation). Changes relative to gravity include lying down in bed from an upright position and tilting the head forward to look at one's feet. The utricle and saccule are collectively known as the otoliths because their hair cells are weighted with stone-like crystals. The VOR associated with translations of the head is referred to as the translational VOR or tVOR.

Neural Circuitry of the Rotational VOR

Because head movements are extremely fast, the rVOR must be activated very quickly if the image is to be adequately stabilized. Indeed, the associated eye movements follow the head motion by less than 10 ms, making the rVOR one of the fastest reflexes of the body. This is possible because of its relatively simple underlying neural circuitry. The pathway for the rVOR is known as the three-neuron arc because it involves only three sets of neurons between detecting the head motion and moving the eyes. [Figure 2\(a\)](#) (red pathway) describes the rVOR pathway for a rightward head movement that is initially detected by the right horizontal semicircular canal. First, a neuron transmits information from the right canal to the right vestibular nucleus, and subsequently, a second neuron sends this information to the contralateral abducens nucleus (a group of cells that controls the eye muscle that pulls the eye to the left). Finally, a third neuron connects the abducens nucleus to the lateral rectus muscle (the leftward pulling eye muscle), where excitation causes the eye muscle to contract and abduct the left eye, thereby rotating it to the left. In addition, such that both eyes move simultaneously, the left abducens nucleus sends its projection across the brain to the right oculomotor nucleus, which contracts the medial rectus muscle and causes the right eye to move to the left as well. This direct pathway provides the eye muscles with a phasic, velocity-related signal that moves the eyes in an equal and opposite direction to that of the head.

The increase of neural activity caused by fluid rotating in the right horizontal semicircular canal when the head moves to the right is balanced by a decrease of neural activity caused by fluid rotating in the left horizontal semicircular canal. Through the same three-neuron-arc pathway (but mirrored on the opposite sides of the brain), the medial rectus of the left eye and the lateral rectus of the right eye (both of which pull the eyes to the right) are relaxed, thus aiding in the rotation of the eyes to the left ([Figure 2\(a\)](#) – blue pathway).

If the head rotates so far to the right that the eyes reach their orbital limits and cannot rotate any further, then the eyes make a quick movement in the opposite direction and the rVOR begins anew. This occurs, for example, when one is spun in an office chair or rides a merry-go-round (with the eyes closed). The resulting pattern of eye

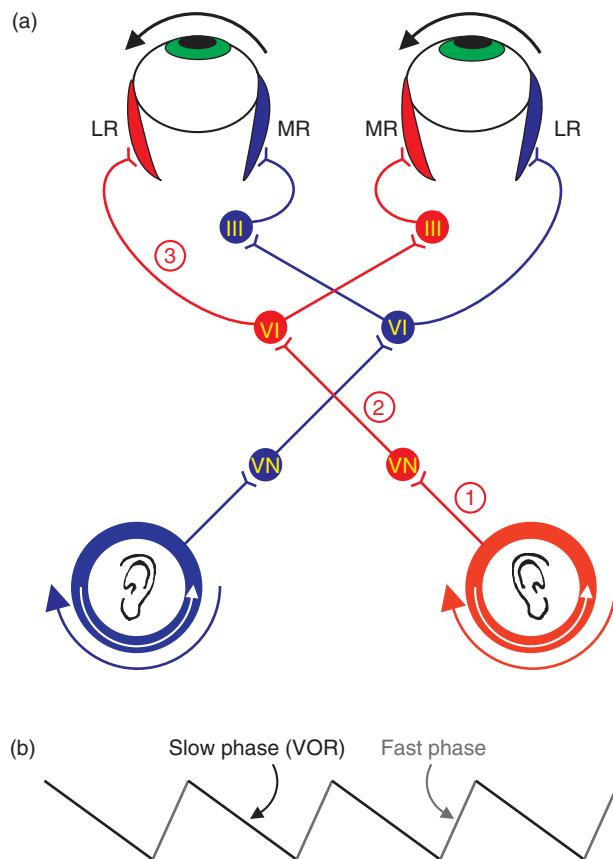


Figure 2 The rotational vestibulo-ocular reflex (rVOR). (a) Excitatory (red) and inhibitory (blue) neural pathways for the rVOR. Head/canal rotation to the right (red and blue arrows) causes endolymph fluid to rotate in the opposite direction (white arrows). This causes excitation signals to reach the right vestibular nucleus (VN) which is then transmitted to the left abducens nucleus (cranial nerve VI), which in turn innervates the lateral rectus muscle (LR) that abducts the left eye to the left. ①, ②, and ③ indicate the cells that comprise the rVOR's three-neuron arc. A signal from VI is also sent to the contralateral oculomotor nucleus (cranial nerve III), which innervates the right medial rectus muscle (MR) that adducts the right eye to the left. The inhibitory pathway is bilaterally symmetric to the excitatory pathway and ultimately causes relaxation of the MR muscle of the left eye and the LR muscle of the right eye. (b) Nystagmus, which alternates between slow-phase and fast-phase eye movements, is seen initially when the head undergoes continuous rotation. This movement pattern dies out over time when head experiences constant velocity rotation. Eye position is plotted as a function of time.

movements is known as nystagmus and is characterized by alternating slow VOR tracking movements and fast resetting eye movements (**Figure 2(b)**).

If the head stops rotating before the eyes reach their limits, then an indirect pathway exists to keep the eyes on target after the head movement is terminated. This indirect pathway is necessary because without it, elastic forces in the eyes and surrounding tissue would draw the eyes back to a more central position. The indirect pathway takes the phasic signal from the vestibular nucleus and sends it to the nucleus prepositus hypoglossi where it is transformed into a tonic, position-related signal (**Figure 3**). The tonic signal is then sent to the abducens nucleus so it too can be transmitted to the eye muscles as it is critical for keeping the image stable on the eye when the head movement is terminated.

The effectiveness of the rVOR is measured by its gain. The gain is simply a ratio of how much the eyes have moved as a function of how much the head has moved.

Thus, a perfect rVOR has a gain of 1 since the eyes move exactly as much as the head moves (but in the opposite direction). In reality, monkeys have been shown to have near-perfect gains of 0.9–1.0, whereas humans have less-than-ideal gains of 0.5–0.8, depending on the speed of the head movement. Likely, cognitive factors influence the gain of the rVOR in humans.

Limitations of the Rotational VOR

The rVOR gains, however, are only this good at certain frequencies of head rotation. In general, the rVOR performs best at relatively high frequencies (from 0.1 to 50 Hz) that are often associated with head movements. This is largely due to the dynamics associated with transducing the semicircular canal signals. At lower frequencies, the optokinetic nystagmus (OKN) takes

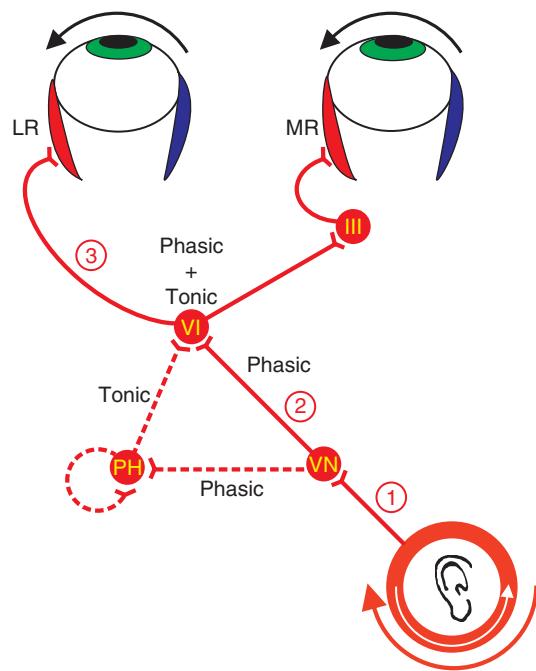


Figure 3 The indirect rVOR pathway (dashed red lines). Phasic signals indicating head velocity branch off from the direct rVOR pathway (solid red lines) at the level of the vestibular nucleus (VN). They are sent to the nucleus prepositus hypoglossi (PH) which performs the mathematical equivalent of integration, resulting in the output of a tonic, position-related command. This command is sent to the abducens and oculomotor nuclei such that the eye muscles receive signals to both move the eye and hold its final position.

over from the VOR. The OKN also maintains a stable image of the world on the retina, but rather than using the vestibular system as an input, it uses the motion of the visual image to initiate the eye movements. For example, the OKN is activated when a stationary observer watches a merry-go-round. As with the VOR, the OKN also produces a nystagmus-like pattern when the eyes reach their oculomotor limits and are quickly moved back in the opposite direction.

The rVOR also fails to respond to head motion during constant-velocity rotations. For example, on a spinning amusement park ride, we can easily detect the initial acceleration of the ride, but if the ride reaches a constant speed and our eyes are closed, we begin to lose the feeling of motion over time. This again is a result of how the semicircular canals detect motion. The endolymph begins to rotate during quick movements, which in turn displaces the hair cells that initiate transduction. However, once constant velocity is reached, the fluid begins to slow down until it no longer displaces the hair bundles and a motion signal is no longer sent to the brain.

Finally, if we are suddenly stopped (i.e., decelerated) from constant-velocity rotation in one direction (with the eyes still closed), we initially misperceive that we are

moving in the opposite direction (although we are really motionless). This is because, while the semicircular canals stop rotating when the body does, the fluid inside the canals circulates in the opposite direction because of its inertia, thus impinging on the cupula and causing a signal to be sent to the brain indicating head motion even though the head is no longer moving.

Interestingly, while rotating at constant velocity or after terminating a rotation, the hair cells physically stop signaling changes in head position after a few seconds. However, the associated nystagmus continues for many tens of seconds. This discrepancy is known as velocity storage because it implies that the signal indicating head motion is kept active somewhere in the brain and extended for reasons that are yet unclear.

The Translational VOR

The function of the tVOR is much like the rVOR in that it rotates the eyes in order to keep the visual image stable during translation. Signals to drive the tVOR arise primarily from the otolith organs. For example, the tVOR is necessary to fixate a telephone pole when one is traveling on a train or to gaze at a rock star while swaying to his or her music. However, there are several notable differences between the two. First, while the rVOR stabilizes the entire visual image, the tVOR only stabilizes one particular object of interest at a time. This is largely due to geometrical issues associated with translations as explained by motion parallax. This phenomenon occurs because (1) objects that are closer to the observer travel a greater distance across the retina than objects that are farther from the observer and (2) during translations, especially along the forward/backward axis, the distance an object travels across the retina depends on where the observer is looking. For (1), a train passenger who fixates on a nearby tree will have to generate a larger compensatory eye movement than if he or she were to fixate on a tree far off in the distance ([Figure 4\(a\)](#)). Since differently sized eye movements are required for objects at different distances, then only one object can be stabilized at any one time. For (2), to a space traveler navigating through space, stars near the line of site appear to move slower (i.e., move a small distance on the retina), while stars located more peripherally move faster (i.e., a greater distance on the retina) ([Figure 4\(b\)](#)). In addition, objects in this optic flow pattern (like the starfield screensaver on your computer) all appear to move in different directions depending on where one is looking, such that different eye movements are required for different gaze direction ([Figure 4\(c\)](#)). Thus again, the tVOR can only produce compensatory movements to one object, moving at one speed, in one direction, at a time.

Second, whereas the rVOR always rotates both eyes in the same direction, the tVOR often needs to move the two

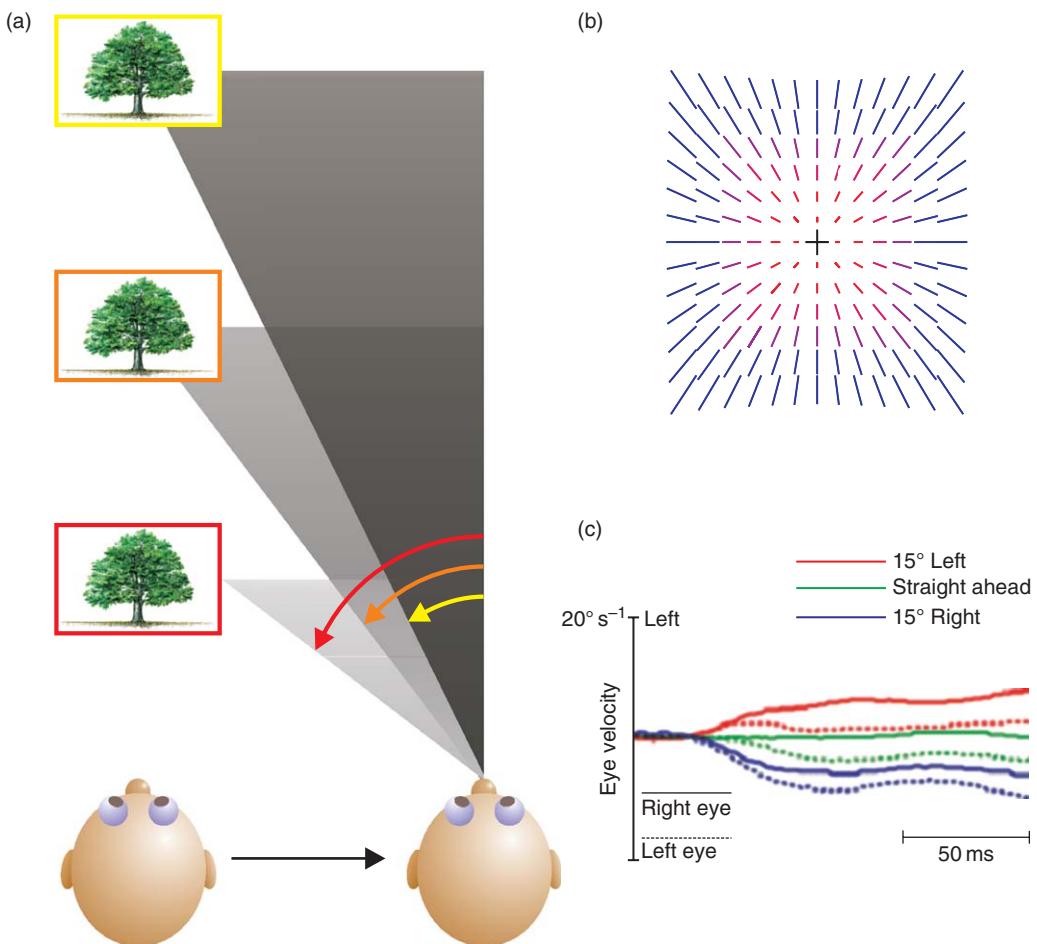


Figure 4 The translational VOR (tVOR). (a) The tVOR must deal with the consequences of motion parallax. Objects (e.g., trees) seen at different distances in depth move different distances on the retina when a person translates. Closer objects (red-boxed tree) move larger distances on the retina (red arrow), whereas farther objects (yellow-boxed tree) move smaller distances (yellow arrow). (b) Optic flow pattern observed during forward translation while looking straight ahead (black cross). Objects in the periphery move more across the retina (purple), while objects closer to the fixation point move less (pink). (c) The amplitude of the tVOR during forward motion depends on the target position and is different for the two eyes. Eye velocities of the right (solid line) and left (dotted) eyes are plotted as a function of time for targets to the left (red), straight ahead (green), and right (blue). (b) Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Neuroscience. Angelaki DE and Hess BJM. Self-motion-induced eye movements: Effects on visual acuity and navigation. 6: 966–976, copyright 2005. (c) Modified with permission from Hess BJM and Angelaki DE (2003) Vestibular contribution to gaze stability during transient forward and backward motion. *Journal of Neurophysiology* 90: 1996–2004. With permission from The American Physiological Society, copyright 2003.

eyes in opposite directions in order for the image of an object to be stabilized. For example, if one fixates on the eraser of a pencil and translates one's head toward it, the eyes must converge toward each other in order to keep the eraser on the retinas of both eyes. The eyes must then diverge, away from each other, as one translates the head back. Lastly, since the tVOR stabilizes only one object at a time on the retina, it is only present in animals, such as primates and humans, which have a fovea. In contrast, animals that do not have a fovea, including all lateral-eyed animals such as rabbits and horses, also do not exhibit a tVOR response. However, all these animals have rVOR responses.

The tVOR successfully stabilizes single images by scaling the incoming vestibular signal by the inverse of target distance and eye position. Because of these extra computations the response time of the tVOR is not as quick as that of the rVOR (the eyes lag the head by 15–30 ms). The tVOR has generally favorable gains for far targets but somewhat low gains (~ 0.5) for near targets. This may be due to the fact that both version (i.e., both eyes moving together) and vergence (i.e., eyes moving in opposite directions) movements must be elicited to follow such close targets and the fact that such eye movements are not typically made in everyday life (i.e., we rarely look at very near objects during navigation).

Tilt VOR

The tilt VOR is the least known of the VOR reflexes mainly due to its extremely low gain (0.1) in humans and primates, and thus its minimal impact on perception. As with the other VORs, the goal of the tilt VOR is to move the eyes in an equal and opposite direction to that of the head and thereby stabilize the visual image, but its range of operations is limited to low frequencies. The signals driving the tilt VOR arise from the otolith organs whenever the head moves relative to gravity. Therefore, the eyes will move up when the head is pitched down and the eyes will roll counterclockwise when the head moves clockwise (i.e., when the right ear moves toward the right shoulder). There is no tilt VOR for yaw rotations since with yaw rotations the head stays upright and does not move relative to gravity. The tilt VOR is much more important and robust in lateral-eyed animals without a well-defined fovea since they only have this one gravity-sensing mechanism to maintain their gaze in line with the horizon. In frontal-eyed animals with well-defined foveas and saccadic systems, the small contribution of the tilt VOR is likely sufficient to assist with the proper maintenance of eye position during eye-head coordination and with the alignment of the two eyes for binocular vision.

Plasticity

The VOR exhibits a remarkable ability to adapt. Because the VOR operates in an open-loop fashion (i.e., the vestibular system is independent of the resultant eye movements), its performance can only be useful if the brain continuously monitors the clarity of the incoming visual scene during head movements. This constant monitoring and the subsequent changes in the VOR that are produced as a result can be observed in everyday life. For example, whenever one dons a new pair of glasses, there is an initial period of discomfort since the VOR is not yet adapted to the new magnification. But over time, the VOR begins to produce larger eye movements with higher magnifications (i.e., an increase in the VOR gain), and smaller eye movements with lower magnifications (i.e., a decrease in the VOR gain). Spectacles that increase or decrease the visual image have been used to demonstrate such gain increases and decreases in the laboratory. As anyone who wears glasses can attest, the brain does not simply exhibit these changes in gain transiently. With enough experience, the brain can store and utilize different gain calibrations for different conditions (e.g., an eyeglass wearer has one gain when their glasses are on and another gain when their glasses are off).

Interestingly, reversing prisms, which change the visual image such that objects in the world located to

the right of an observer are perceived to be to the left, have been shown to actually reverse the VOR over a period of several days. After adaptation, a head movement to the right elicits an eye movement to the right and vice versa. This reversal response disappears almost immediately after the prisms are removed.

Finally, the gain of the VOR can also be adapted when head movements are paired with moving visual stimuli. If the head moves in one direction and the visual world moves in the opposite direction, then the gain of the VOR is increased, whereas if the head and visual world move in the same direction, then the gain of the VOR is decreased (toward a value of zero). Such VOR plasticity is frequency specific and hence the largest changes in gain will be observed around the frequency used for adaptation. In fact, opposite changes in gain (i.e., increasing vs. decreasing) can be made at high versus low frequencies.

Besides changes in gain, the VOR can also modify its direction. If the head is rotated horizontally while the visual world is moved vertically, subsequent horizontal head rotations in the dark elicit both horizontal and vertical eye movements. Aside from such extrinsic changes in the VOR, intrinsic factors such as those brought about by disease or trauma can also be adapted for (e.g., decreases in unilateral vestibular function due to antibiotic-related degeneration and unilateral Ménière's disease).

Neural Pathways for Plasticity

The specific mechanisms responsible for changes in the gain of the VOR are still being scrutinized, but are believed to occur with the help of the cerebellum. A dominant theory states that head movement signals derived from the vestibular organs are compared with visual images from the retina (known as retinal slip) that are a measure of the image's stability (**Figure 5** – yellow cross). Vestibular signals travel to the flocculus and paraflocculus of the cerebellum via mossy fibers which synapse onto the parallel fibers of granule cells. Visual signals reach the cerebellum via the inferior olive nuclei as climbing fibers. Once there, Purkinje cells compare the discrepancy between the visual and vestibular signals and make appropriate changes that are then sent to vestibular nucleus cells that drive the VOR.

Others have proposed that VOR adaptation occurs in the vestibular nucleus itself, while the output of the cerebellum simply provides an error signal for recalibration (**Figure 5** – black double-cross). A subset of vestibular nucleus cells, known as flocculus-target neurons, receives direct inhibition from the flocculus as well as inputs from the ipsilateral vestibular nerve. These cells change their firing pattern quickly and dramatically following changes in the VOR. Importantly, their firing-rate

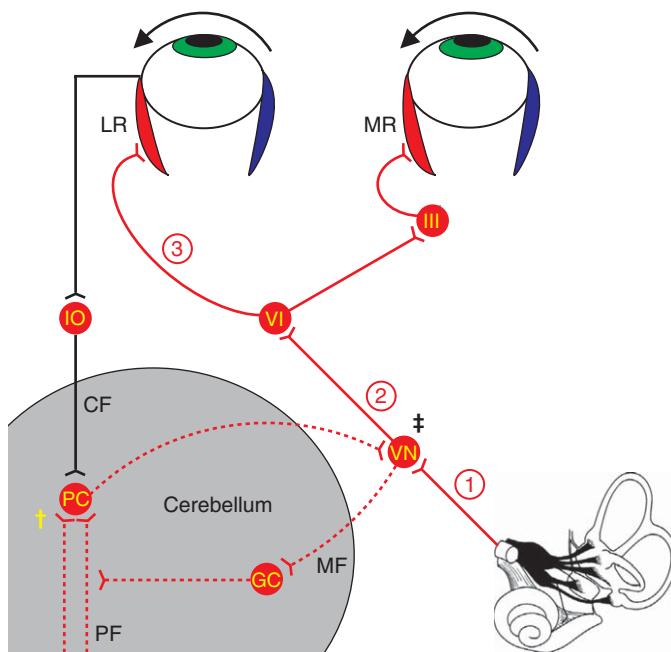


Figure 5 Two possible sites for VOR adaptation. Vestibular signals from the vestibular nucleus (VN) are taken to Purkinje cells (PC) of the cerebellum via mossy fibers (MF), granule cells (GC), and parallel fibers. Retinal slip signals are brought to the same Purkinje cells via the inferior olive (IO) and climbing fibers (CF). Once the two are compared, adjustments in gain can then be made (yellow cross) and the resultant signals are sent back to the VN. Alternatively, modifications may be made in the VN itself (black double-cross).

modifications are in the appropriate direction for the plasticity to take place. Currently, none of these theories can account for all the experimental findings. Likely, VOR plasticity occurs at multiple locations, both inside and outside the cerebellum. These different sites might account for the different gain changes observed at different head-oscillation frequencies, directions of motion, and timescales.

See also: Cardiovascular Conditioning: Neural Substrates; Orientation and Navigation.

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Hemispheric Specialization: Language, Space, and Sexual Differentiation

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Glossary

Broca's aphasia – Also known as expressive aphasia or nonfluent aphasia, this type of aphasia constitutes language difficulties associated with damage to a specific region within the inferior frontal lobe of the left hemisphere (Broca's area). Broca's aphasia is characterized by labored speech consisting largely of content words and devoid of syntax and by difficulty using syntax and grammar to understand language.

Categorical spatial relations – Spatial relations that group locations into equivalence classes so as to delineate regions and boundaries or limits separating or connecting patches of space (e.g., object A is below object B; or, object C is touching object D).

Coordinate spatial relations – Spatial relations that occur within a 'metric' space and that allow perception and expression of space in a quantitative sense (e.g., object A and object B are about 1 m apart).

Hemispheric specialization – The fact that the left and right cortical hemispheres are functionally asymmetric, with each side having its own processing abilities and biases.

Sexual differentiation – The process of development of differences between males and females from an undifferentiated zygote; differences in behavior, neurophysiology, or brain-behavior relationships as a function of biological sex.

Split-brain patients – The individuals whose left and right cortical hemispheres have been surgically disconnected by severing the corpus callosum and other connecting fibers; usually performed as a treatment for severe epilepsy when less-invasive forms of treatment have failed.

Wernicke's aphasia – Also known as receptive or fluent aphasia, this type of aphasia constitutes language difficulties associated with damage to a specific region within the posterior part of the superior temporal gyrus of the left hemisphere (Wernicke's area). Wernicke's aphasia is characterized by difficulty in the understanding and comprehension of words; speech often sounds natural, with syntax and grammar being relatively normal, but is typically meaningless, often containing natural-sounding real words and nonwords in a context that does not make sense.

Introduction

The left and right cerebral hemispheres of the human brain are functionally asymmetric, with each side having its own abilities and biases. Functional left-right brain differences are ubiquitous across information-processing domains in humans and across contemporary animal species. This article illustrates characteristics of hemispheric asymmetry in humans by focusing on three well-studied topics: language, processing locations in space, and sexual differentiation. For each of these topics, hemispheric asymmetry in humans is emphasized, while also considering the relationship to asymmetries in other contemporary species that may shed light on the evolutionary origins of hemispheric specialization.

Language

The most well-established processing asymmetry in humans deals with various aspects of language. It has been observed for well over 2000 years that various problems in the generation and understanding of language are typically more severe after damage to the left side of the brain than after damage to the right side of the brain. For over 150 years, these aspects of hemispheric asymmetry have been studied in a formal, scientific manner. For example, in the latter half of the nineteenth century, a French neurologist named Paul Broca reported that certain characteristic difficulties in producing fluent speech were associated with damage to a region within the frontal lobe of the left hemisphere. Characteristics of what has come to be called 'Broca's aphasia' (also known as 'expressive aphasia' or 'nonfluent aphasia') include labored speech consisting largely of content words and devoid of syntax or grammar (sometimes referred to as 'telegraphic speech'). Comprehension may be relatively normal, although there is often great difficulty when syntax or grammar is essential for determining meaning. A few years later, a German neurologist named Karl Wernicke reported that receptive language problems were associated with damage to a region further back in the left hemisphere, within the temporal lobe. Characteristics of what has come to be called 'Wernicke's aphasia' (also known as 'receptive aphasia' or 'fluent aphasia') include

difficulty in the understanding and comprehension of language, both spoken and written. Although speech sounds natural and syntax and grammar are relatively normal, it is typically meaningless, often containing natural-sounding nonwords as well as real words in a context that does not make any sense. From the perspective of hemispheric asymmetry, it is important to understand that damage to corresponding regions of the right brain hemisphere is far less likely to produce these various forms of aphasia. Broca's and Wernicke's areas are connected through a neural pathway known as the 'arcuate fasciculus' so that those areas along with others constitute a left-hemisphere network that underlies much of our language ability.

In the years since the pioneering work of Broca and Wernicke, neurologists and cognitive neuroscientists have used a variety of techniques to refine and extend our understanding of hemispheric specialization for language. The study of language disorders after brain damage has continued to provide a rich source of information, especially as modern brain imaging techniques permit damage to be localized precisely within the living brain. Our understanding of hemispheric specialization was greatly enhanced in the latter part of the twentieth century by the study of the so-called split-brain patients, work for which neurobiologist Roger Sperry received the Nobel prize in 1981. In the normal brain, the left and right cerebral hemispheres are interconnected by a vast network of neurons, with the largest and most prominent fiber tract being the corpus callosum. As a last resort for the treatment of severe epilepsy, the corpus callosum (and sometimes smaller fiber tracts as well) is surgically severed, thereby disconnecting the left and right hemispheres or splitting the brain. Using a clever set of methods to present information, it is possible to test these split-brain patients to examine the competence of each cerebral hemisphere in the absence of its partner. The latter half of the twentieth century also saw the emergence of behavioral techniques to study hemispheric specialization in neurologically intact individuals, leading to an explosion of research on various aspects of functional asymmetry as well as on the ways in which the two hemispheres interact in the normal brain. This time period also saw the development and refinement of techniques that permit us to observe which brain areas are most activated as individuals perform tasks that are carefully chosen to involve specific language-related processes. These techniques include things such as measuring the electrical activity of the brain (e.g., event-related potentials or ERPs) and functional neuroimaging (e.g., positron emission tomography (PET); functional magnetic resonance imaging (fMRI); and magnetoencephalography (MEG)). Along with this, techniques have been developed to temporarily interfere with processing in specific brain areas (the Wada test; transcranial magnetic stimulation

(TMS)) so as to study the behavioral consequences of what might be thought of as temporary cortical lesions. One advantage of having so many techniques to study brain-behavior relationships is that, while each technique has shortcomings that sometimes make inferences difficult, together the techniques provide a powerful set of converging operations that paint the following picture of hemispheric specialization for language.

It is estimated that speaking is controlled by the left hemisphere in 95% of right-handed individuals and 65–70% of left-handed individuals, with the remaining left-handers being evenly divided between those for whom speaking is controlled by the right hemisphere and those for whom speaking is controlled bilaterally. In understanding speech, the left hemisphere is superior to the right in processing phonetic information, in processing syntax and grammar, and for processing many aspects of word meaning. These aspects of left-hemisphere superiority extend to the derivation of meaning from printed material and, perhaps, to other forms of communication such as American sign language. With respect to reading, an area of the left hemisphere in the vicinity of the angular gyrus seems particularly important for processing visual word form.

Though the left hemisphere is regarded as being specialized for language, the right hemisphere is not completely without ability in this regard and may even be superior to the left for certain language-related functions. For example, though the left hemisphere is superior for identifying words and phonemes, the right hemisphere is superior for identifying the prosody or rhythm of speech and for identifying the emotional tone conveyed by speech. The right hemisphere is also involved in processing narrative-level linguistic information of the sort that develops over several sentences and creates a context that is necessary for appreciating things such as the plot line of stories and the punch line of jokes. Although there is clear evidence of left-hemisphere superiority for generating propositional speech, the right hemisphere is capable of producing what is termed as 'formulaic' or 'automatic speech' (e.g., reciting a well-learned nursery rhyme, swearing, and so forth). Furthermore, if the left hemisphere is extensively damaged or removed at a sufficiently young age, the intact right hemisphere is capable of developing normal speech.

In language, as in other domains, many hemispheric asymmetries in humans appear to be complementary, with each side being superior for different and sometimes contradictory aspects of a task. In addition to those functions already noted, there are other, more subtle, instances of complementary specialization. For example, when a word is presented (e.g., bank), the left hemisphere quickly restricts activation to the dominant meaning (a monetary institution) or the meaning most consistent with the preceding words, whereas the right hemisphere maintains activation of multiple meanings or remotely associated

words (both a monetary institution and a river bank). Note that both processing biases are useful for understanding normal discourse. The left-hemisphere strategy would generally be more effective for quickly honing in on the intended meaning, whereas the right-hemisphere strategy would be more effective for reinterpreting subsequent words indicate that a different, usually subordinate meaning was intended (e.g., she put all her money in her purse, took it to the bank of the mighty Mississippi, and threw it in). Another example concerns the processing of letters within a printed word, with the left hemisphere treating the word as a linguistic unit and processing the letters in parallel and the right hemisphere distributing attention sequentially over the letters. Thus, after left-hemisphere damage, reading may occur in a laborious letter-by-letter manner mediated largely by the intact right hemisphere.

Hemispheric specialization for language is, for the most part, universal and extends across the world's languages. However, there is informative variation. For example, the left hemisphere is superior for the identification of pitch for speakers of languages, such as Mandarin Chinese, Thai, and Vietnamese for which phonemic and lexical discriminations are made on the basis of pitch, but not for speakers of languages such as English for which pitch has no linguistic relevance (in fact, for speakers of English there is even some indication of right-hemisphere superiority for pitch discrimination). Another example concerns hemispheric specialization for two kinds of Japanese script: Kana, a syllabic script, versus Kanji, a visually elaborate logographic script borrowed from Chinese characters. While there is clear evidence of left-hemisphere superiority for identifying Kana characters, the evidence is less clear for identification of Kanji characters, for which there may even be right-hemisphere superiority. In general, right-hemisphere involvement in reading increases with things such as the visual complexity of printed characters.

Although no other contemporary species possesses anything approaching the language capabilities of humans, the left hemisphere is dominant for producing vocalizations in a number of other vertebrates, including some species of songbirds, rats, and amphibians. Though there is disagreement about whether these vocalizations are, in fact, direct precursors of human speech, the existence of so much asymmetry in other species raises the possibility that when language did develop, it was built disproportionately on pre-existing left-hemisphere skills. It has also been hypothesized that human language first evolved from manual and facial gestures rather than from vocalizations. Contemporary research with a number of species indicates a sufficient amount of left-hemisphere superiority for such gestures to suggest that, from this perspective as well, the asymmetric substrate for language may be quite old in evolutionary terms.

Space

Just as the left hemisphere was historically recognized as being specialized for language, the right hemisphere was thought to be specialized for a variety of nonverbal, perceptual functions, including the localization of stimuli in space. However, even more than in the case of language, the two hemispheres work in a complementary manner to process spatial relationships. More specifically, the right hemisphere is specialized for using a coordinate representation of spatial relationships to extract information about such things as distance and precise location. In contrast, the left hemisphere is specialized for using what has been called a categorical representation of spatial relationships in order to assign a spatial relation to a category such as 'connected to' or 'above.' As an illustration, consider an experiment in which a horizontal line is presented along with a single dot, with the dot appearing in one of the 12 locations, six above the line and six below. When the task is to indicate whether the dot falls within 1 cm of the line regardless of whether the dot is above or below the line (a coordinate judgment), there is a right-hemisphere advantage. However, when the task is to indicate whether the dot is above or below the line regardless of distance from the line (a categorical judgment), there is a left-hemisphere advantage. Such effects occur for other stimuli and tasks as well. For example, the right hemisphere is better able than the left to recognize that the distance between two objects or pictures has been changed, whereas the left hemisphere is better able than the right to recognize that the relative position of the two objects or pictures has been reversed (**Figure 1**).

The potential advantage of complementary specialization for categorical and coordinate spatial processing is illustrated by neural network computer simulations. A particularly interesting finding is that networks which are split in order that some of their hidden units

Examples of categorical questions

- Is the dot above the line?
- Is the arrow pointing left?
- ↑ Is the triangle touching the oval?

Examples of coordinate questions

- Is the dot within 1 cm of the line?
- Is the arrow longer than 1 cm?
- ↑ Is the triangle taller than the oval?

Figure 1 Examples of questions about categorical and coordinate spatial relationships. The left and right brain hemispheres are specialized for processing categorical and coordinate spatial relationships, respectively.

(simulated, neuron-like elements) contributed only to a categorical judgment and others contributed only to a coordinate judgment, performed better than unsplit networks in which all the hidden units contributed to both types of spatial judgments. These results suggest that there are computational advantages to segregate these two types of spatial processes. Other findings indicate that two tasks interfere with each other more when they require resources from the same cerebral hemisphere than when they can be performed by the opposite hemisphere. Thus, hemispheric specialization for complementary processes may permit those processes to be sufficiently segregated from each other so as to permit each process to proceed with little interference from its complementary counterpart. Consistent with this idea, greater hemispheric specialization for two complementary tasks in domestic chickens is associated with enhanced ability to perform those tasks at the same time: finding food (depending on a type of local-level visual discrimination for which the left hemisphere of the chicken is typically specialized) and being vigilant for predators (which depends on a more global type of visual discrimination and motion perception for which the right hemisphere of the chicken is typically localized).

Hemispheric specialization for spatial processing may be related to complementary hemispheric specialization for the identification of visual patterns. Specifically, the right hemisphere is specialized for extracting what are called the global aspects of visual stimuli (e.g., the outer contour of a face, or the distance relationships among facial features), whereas the left hemisphere is specialized for extracting what are the called local aspects of visual stimuli (e.g., details about the small, individual features of the face). It is interesting, in this regard, that both processing of global perceptual characteristics and of coordinate spatial relationships may depend on analysis of relatively low visual spatial frequencies (for which right-hemisphere superiority has been hypothesized), whereas processing of local detail and categorical spatial relationships may depend on analysis of relatively high visual spatial frequencies (for which left-hemisphere superiority has been hypothesized; **Figure 2**).

Studies with contemporary animal species suggest that complementary hemispheric specialization for these different aspects of visual processing may have a long evolutionary history. In fact, it has even been hypothesized that all contemporary vertebrate groups have inherited a basic pattern of asymmetry from a common chordate ancestor. Whether or not the various forms of asymmetry are homologous in this manner is not clear, but the parallels among species are provocative; consider, for example, some of the asymmetries in the domestic chicken that were described earlier.

The existence of animal models has made it possible to expand the methods for testing hypotheses about



Figure 2 A large letter H (the global level) made up of small letter J's (the local level). The right and left brain hemispheres are specialized for processing global and local information, respectively.

hemispheric specialization by using techniques and manipulations that are not possible with humans. To illustrate this, consider what has been learned about the ontogeny of hemispheric specialization from studies of the domestic chicken, in which the left hemisphere is typically specialized for visual pattern discrimination and the right hemisphere is typically specialized for detecting predators and for attack and copulation behaviors. During the last 5 days before hatching, when the visual system is undergoing rapid development, the head of the chick embryo is positioned in the egg such that the left eye (which projects to the right side of the brain) is pressed against the body and the right eye (which projects to the left side of the brain) is turned outward toward the translucent shell. If the egg is exposed to light during this critical period, the chick shows the typical asymmetries when it hatches. If there is no light during this critical period, the direction of asymmetry is random.

Furthermore, the typical asymmetries can be reversed by manipulating the embryo during this critical period so that only the left eye receives light. These results indicate that hemispheric specialization may be determined by the interaction of several biological and environmental variables, leading to speculation about similar interactions in humans. Furthermore, the results indicate how valuable animal studies are likely to be in sorting out the contributions of various factors.

Sexual Differentiation

The foregoing summary of hemispheric specialization for language and spatial processing was based on research with a typical right-handed population. Although there is sufficient commonality to make such a summary useful, it glosses over the fact that there is individual variation in the magnitude and even the direction of hemispheric asymmetry. We have already noted that the incidence of left-hemisphere dominance for producing speech is lower among left-handers than among right-handers, although most left-handers show the same asymmetry as right-handers. In general, this same pattern with respect to

handedness characterizes other forms of hemispheric specialization as well. That is, on the average, left-handers show asymmetries in the same direction as right-handers (with, of course, the exception of handedness), but with smaller average differences that can be attributable to more variability among left-handers in the direction of asymmetry.

After handedness, the most-studied dimension of individual variation is biological sex, or sexual differentiation. To be sure, there are reliable sex differences in cognition. For example, women tend to outperform men on a number of verbal, language-related tasks, whereas men tend to outperform women on a number of visuospatial tasks. Not surprisingly, this has led to a search for neurological correlates. With respect to language, an early hypothesis was that women have more bilateral representation of language, giving them an advantage for verbal processes (because both hemispheres are involved) but putting them at a disadvantage for various processes for which the right hemisphere is specialized (because some of the right hemisphere's functional space is devoted to language). There are, in fact, findings consistent with at least part of this hypothesis, even though the evidence is not uniform. For example, the incidence and seriousness of various aphasias after left-hemisphere brain damage tend to be less in women than in men, consistent with the possibility that the right hemisphere is more involved in language for women than for men. Some functional imaging studies provide converging support for this idea. For example, it has been reported that during phonological tasks (e.g., a rhyming task), brain activation in men is largely lateralized to the left inferior frontal gyrus, whereas brain activation in women is more diffuse and involves the inferior frontal gyrus in both hemispheres. Such results are not always found, however, and a recent meta-analysis of several brain imaging studies showed no consistent differences in asymmetric activation patterns between men and women.

It is instructive to consider possible reasons for the discrepant findings that have been reported. Something as broad as language consists of a number of specific processes, ranging from auditory (the early stages of speech perception), visual-orthographic (the early stages of reading), phonological, syntactic, semantic, and so on. There are already some indications that sex differences vary across these different aspects of language. In addition, there is evidence that functional asymmetry in women varies to some extent across phases of the menstrual cycle. For example, both behavioral and functional brain imaging studies suggest increased asymmetry during menstruation, when estradiol and progesterone plasma concentrations are at their lowest, and greater symmetry in the mid-luteal phase, when estradiol and progesterone plasma concentrations are high. Thus, the pattern of sex differences in a particular study may

depend on the hormone state of the women tested. Another complicating factor is that, even when sex differences are found, they do not necessarily indicate a fundamental difference in neural organization. For example, men and women may use different cognitive or emotional strategies to perform a task or bring different levels of motivation to a task, all of which can affect the pattern of brain activation. These kinds of strategy differences could arise for many reasons other than a difference in neural organization, including things such as socialization and a host of other environmental experiences. Such strategy differences could also be sensitive to subtle variations in a task and occur differentially across studies that, at first glance, seem to involve the same demands.

There are no consistent findings with respect to sex differences in hemispheric asymmetry for localizing stimuli in space. In addition to the complicating factors already noted, the study of spatial localization is complicated further by the fact that hemispheric specialization is different for processing categorical and coordinate spatial relations. To be sure, many behavioral and functional imaging studies of spatial processing have included both men and women, providing ample opportunity to observe consistent sex differences. The fact that none have emerged suggests that, if there are any, they are small relative to the overlap between men and women.

Although many aspects of sexual differentiation have been studied in other species, and there is ample evidence of hormonal effects on the developing brain, not much is known about the differences between males and females in functional hemispheric asymmetry. To some extent, this reflects a paucity of studies that were designed specifically to detect sex differences. At the same time, however, as the number of studies with other species has grown exponentially, so has the number of studies that include both males and females thereby providing an opportunity to observe differences between them. As we formulate more precise hypotheses regarding the manner in which hemispheric asymmetry in humans is related to biological sex and influenced by circulating hormones, the existence of animal models will provide fertile ground for testing them.

Putting the Brain Back Together

In this article, characteristics of hemispheric asymmetry in humans have been illustrated by focusing on language and the processing of spatial relationships. In each domain, hemispheric asymmetry for specific processes can be thought of as complementary, with each side being superior for different, and sometimes contradictory, aspects of a task. Given the ubiquity of functional asymmetry across processing domains and across species, it seems reasonable to view such asymmetry as having evolutionary

advantages. A plausible view is that hemispheric specialization for complementary processes permits those processes to be sufficiently segregated so as to reduce maladaptive interference between them. While this would have important advantages, this arrangement would require that the results of those complementary processes be shared efficiently across the brain hemispheres, so as to create an integrated, unitary view of the world and plan of action. More generally, cognitive neuroscience has done an increasingly good job of taking the brain apart, by studying hemispheric specialization and by identifying specific processing modules within each hemisphere. Indeed, the rate of progress has accelerated with the use of more sophisticated measures of brain activation and increasingly clever behavioral manipulations. In our enthusiasm for taking the brain apart, we should not overlook the fact that modern functional imaging techniques demonstrate that even simple tasks involve neural networks dispersed across both brain hemispheres. As we go forward, a significant challenge will be to understand the manner in which the different elements of these neural networks and the activities of the two hemispheres fit together and coordinate their activity, at both biological and behavioral levels.

See also: Agnosia; Birdsong and Vocal Learning during Development; Cognition: Learning and Memory: Spatial; Contribution of Split-Brain Studies to the Evolution of the Concept of Hemispheric Specialization; Development and Language; Disorders of Face Processing; Dyslexia (Developmental); Language and Communication – Brain Substrate; Orientation and Navigation; Sex Hormones, Mood, and Cognition.

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Relevant Websites

- <http://www.youtube.com> – Examples of interviews of patients with Broca's and Wernicke's aphasias can be found on You Tube.
- <http://www.nidcd.nih.gov> – Illustrations of the brain areas associated with Broca's and Wernicke's aphasias can be found on many websites.

Implicit Learning and Memory: Psychological and Neural Aspects

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Glossary

Explicit/declarative memory – Memories that one is consciously aware of and uses deliberate thought to store and use. This type of memory encompasses both memories for life events, called episodic memories, and general knowledge for facts, called semantic memories. **Huntington's disease** – Degenerative neurological disease that leads to loss of neurons within the striatum itself, and leads to motor and cognitive deficits on tasks requiring the striatum.

Implicit/nondeclarative memory – Memories that can be acquired and used without conscious awareness or intention to learn or apply information.

Parkinson's disease – Degenerative neurological disease affecting a specific group of neurons in the midbrain that produce the neurotransmitter dopamine. The progressive loss of neurons causes diminished dopamine transmission in the striatum and leads to motor and cognitive deficits on tasks requiring the striatum.

Priming – A type of implicit memory where previous experience with some material makes future processing of the same material easier.

Procedural memory – Memories for 'how' to do something, such as riding a bike.

discovered that some learning and memory abilities could develop normally, and these types of learning and memory have been termed 'implicit.'

Implicit learning and memory refer to a heterogeneous set of abilities that, as a whole, mainly are characterized in opposition to explicit (or declarative) memory, which involves awareness for how a memory was acquired and the contents of memory. Implicit (or nondeclarative) learning and memory, on the other hand, refer to situations where there is a lack of awareness of what has been learned, a lack of intention to learn, or difficulty describing what has been learned. Rather, evidence of implicit learning and memory is provided through changes in performance on a task as a result of experience with the task.

Priming and skill learning are two major branches of implicit learning and memory, and some forms of classical conditioning additionally belong under this umbrella term. Priming refers to a change in processing of a stimulus as a result of a previous encounter with the same or a related stimulus, and facilitated processing may occur as a result of a single exposure to a stimulus. In contrast, skill learning refers to the gradual improvement in performance of some task with practice. Despite a range of differences between these two categories of implicit learning and memory, there are several commonalities. Both priming and skill learning may be demonstrated in the absence of awareness of what is being learned, both may be characterized by some degree of automaticity, and both may be relatively long lasting. Classical conditioning involves learning an association between an initially neutral stimulus and a significant stimulus that elicits reflexive behavior such that the neutral stimulus comes to elicit the automatic response with training.

Just as the psychological characterization of implicit learning and memory relies on its contrasts with explicit memory, the neural characterization has emphasized independence from the MTLs that are damaged in amnesics. Studies across different neurological patient populations and increased use of neuroimaging methods

Implicit Learning and Memory

Studies of patients with neurological damage have resulted in taxonomy of memory that emphasizes separable memory functions that rely on distinct brain regions. Five decades ago, it was discovered that patients with damage to their medial temporal lobe (MTL) or related diencephalic structures suffered from a severe inability to remember new facts and events. The deficit was selectively mnemonic and not related to attention, language, perception, or reasoning deficits. However, it was also

have contributed to a better understanding of how the brain supports the wide range of abilities covered by the term implicit learning and memory.

Priming

Perceptual and Conceptual Priming

Priming is commonly divided into perceptual and conceptual priming. Perceptual priming involves facilitated processing of the perceptual features of stimuli and involves words and pictures, and visual, auditory, and tactile information (see **Figures 1(a)–1(d)**). A common task involves studying a list of words and a later indirect test that allows influence of the studied words. For example, a subject may be asked to complete a word stem or fragment (ASSA____ or_SS_SI_) or to identify briefly presented words as words or nonwords. Previously studied words are more likely to be used to complete fragments than other possible words and are more quickly identified. In conceptual priming, previous experience with materials can be expressed through use of information to answer general knowledge questions without necessarily intentionally using the studied information.

Perceptual priming is modality specific, that is, priming across modalities (e.g., visual to auditory) is ineffective. Manipulations that alter perceptual features of studied items, such as font, alter perceptual priming, and priming is greatest when there is perceptual similarity between study and test. Conversely, manipulating the depth of processing of semantic or conceptual information about stimuli does not affect perceptual priming. Conceptual priming, by contrast, occurs across modalities and is enhanced when deeper semantic processing is emphasized at study, but is unaffected by perceptual manipulations.

Increased processing fluency observed in priming could be supported through tuning of neural regions involved in processing the stimuli themselves. Studies using fMRI to study brain activity during exposure to stimuli, show decreased neural responses to repeated stimuli, suggesting a more efficient response as a result of experience. This decreased response to repeated stimuli, as measured with functional magnetic resonance imaging (fMRI), is referred to as neural priming. Decreases in response to stimulus repetitions have been observed in cortical regions that would be expected to be involved in initial processing of stimuli, such as prefrontal regions for conceptual priming and posterior visual cortices for perceptual priming (**Figure 1(e)**). These decreases may be lateralized according to spatial presentation of basic visual shapes and localized to specific cortical regions depending on whether the materials are faces or houses. Damage to lateral occipital regions can selectively impair perceptual and not conceptual priming,

whereas patients with damage to the MTL, the striatum, or cerebellum perform normally on priming tasks. These findings are consistent with a general proposal that priming involves greater fluency in the processing of the stimuli *per se* that depends on neocortical regions and that the specific region involved depends on modality of the study and test materials.

Associative Priming

Associative priming generally uses the types of materials described above, but combines words into pairs (e.g., ELEPHANT-MOTEL). Subsequent tests may then require completing word stems when words are presented in the studied combinations or in combination with novel words or studied words that were part of a different pairing (e.g., ELEPHANT-GRA__). The results from these types of priming studies in patients with MTL damage are mixed, but there is some evidence from fMRI studies that MTL regions are engaged during associative priming.

Stimulus–Response Mappings

An alternative to the view that repeated experience with a stimulus makes stimulus processing more efficient is that what is learned is a proper response mapping. By this view, repetition benefits derive from redeploying a previous response instead of relying on a more efficient perceptual analysis. This idea was tested by repeating perceptually identical stimuli while changing response requirements (see **Figure 1 (f)**). Changing response requirements decreased neural priming, which returned when stimulus–response mappings returned to those initially primed. Furthermore, across subjects, behavioral priming and response time costs, resulting from switching response requirements, were associated with prefrontal cortical activity and not with activity in visual cortical regions.

The paradigms used in studies employing neuromaging have deviated from those used in neuropsychological studies owing to the need for simplified responses in neuroimaging. The results described above, obtained with a simple yes/no button press decision, may differ from the paradigms commonly used in neurologically impaired populations. However, the contribution of stimulus response learning to priming is one aspect of how repeated encounters with stimuli influence behavior.

Skill Learning

Skill learning is also referred to as procedural learning, which captures the idea that learning is expressed as facilitation of the procedures involved in task

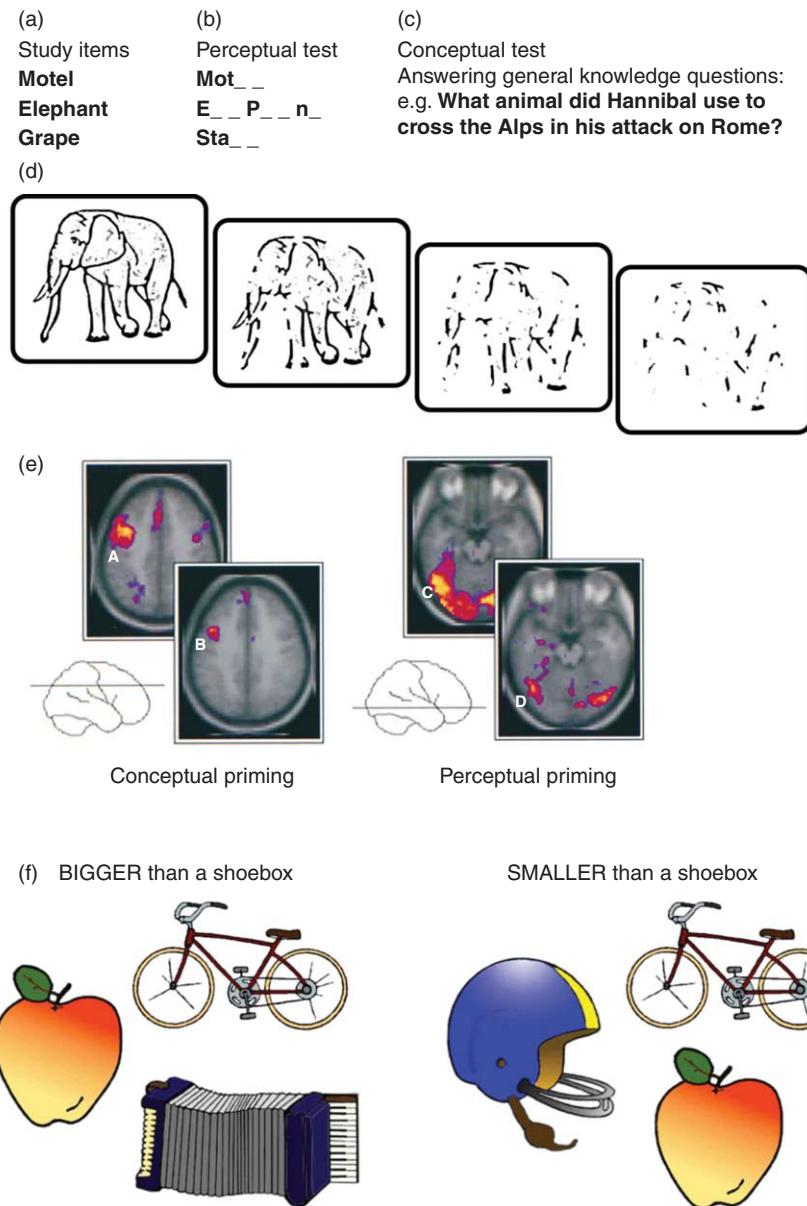


Figure 1 (a) Example of study items on a priming task. (b) In perceptual priming tests, participants are asked to complete word stems or fragments. (c) Perceptual priming can also use pictures, and previous study of pictures will allow identification of earlier, more degraded visual presentations. (d) In a conceptual priming test, the semantic properties of the studied items will facilitate answering general knowledge questions. Changing the font of items will affect the perceptual test (b) but have no effect on measures of conceptual priming (d). (e) Example of fMRI data from studies of perceptual and conceptual priming: (A) shows a comparison of brain activity when processing novel items and (B) shows a comparison of novel and repeated items in a conceptual priming task. The difference between novel and repeated seen in (B) indicates that within the same regions that are engaged when processing the novel items, there is a decrease in activity when previously encountered items are presented. In (C) and (D), the same comparisons and patterns are shown for a perceptual priming task. The line drawings indicate from where in the brain the displayed horizontal slice was taken. (f) Example stimuli from a priming task where participants first indicate with a yes/no response whether each item is BIGGER than a shoebox. Next they must instead indicate whether the item is SMALLER than a shoebox. In this way, only the response cue changes while the items that must be processed in order to make the decision remain constant. The response time slowing incurred when the response mapping is switched, suggests that, in some cases, priming is related to learning a particular response mapping rather than a representation of item information being more accessible. (e) Reprinted from Schacter DL and Buckner RL (1998) Priming and the brain. *Neuron* 20(2): 185–195.

performance. As such, skills differ from other types of memory because memory must be assessed by performance of the task itself. In some cases, the implicit nature of learning is because it is difficult to express what is being learned. A distinction is often made between knowing how and knowing that. For example, when learning to ride a bicycle, one is aware of learning, but the knowledge one can verbalize is not supporting performance or the how. In other cases, the structure to be learned is not explicitly signaled, but embedded incidentally in the task being performed.

The overview of skill learning is grouped into motor, perceptual, and cognitive skills, reflecting how the tasks used to assess skill learning have been classified rather than a true division of psychological or neural processes supporting performance.

Motor Skill Learning

Mirror tracing

On a mirror-tracing task, participants trace the outline of geometric figures while viewing the figures reflected in a mirror. The ability to trace becomes more accurate and rapid with practice, and the ability may be transferred to

novel figures not seen during practice. Patients with damage to the MTL, as in amnesia, or to the striatum, as in Parkinson's or Huntington's disease, can perform this task normally. In contrast, damage to the cerebellum leads to impaired mirror tracing. Evidence from positron emission tomography (PET) imaging has shown activation in a frontal-parietal-cerebellar network when performing the task, consistent with the pattern seen in neurological populations.

Motor adaptation

In motor adaptation tasks, an external perturbation is introduced such that motor behavior must be adjusted to perform a task. A common task requires participants to manually track a visual target while a manipulandum exerts a predictable force (Figure 2(a)). The ability to compensate for the forces and accurately track the target is learned gradually. The cerebellum has been implicated in learning both through study of neurologically impaired populations and neuroimaging in healthy populations and is thought to be critical in updating internal models of motor output from trial to trial (Figure 2 (b)). This process of updating behavior in response to error is distinct from that attributed to the striatum, which has been

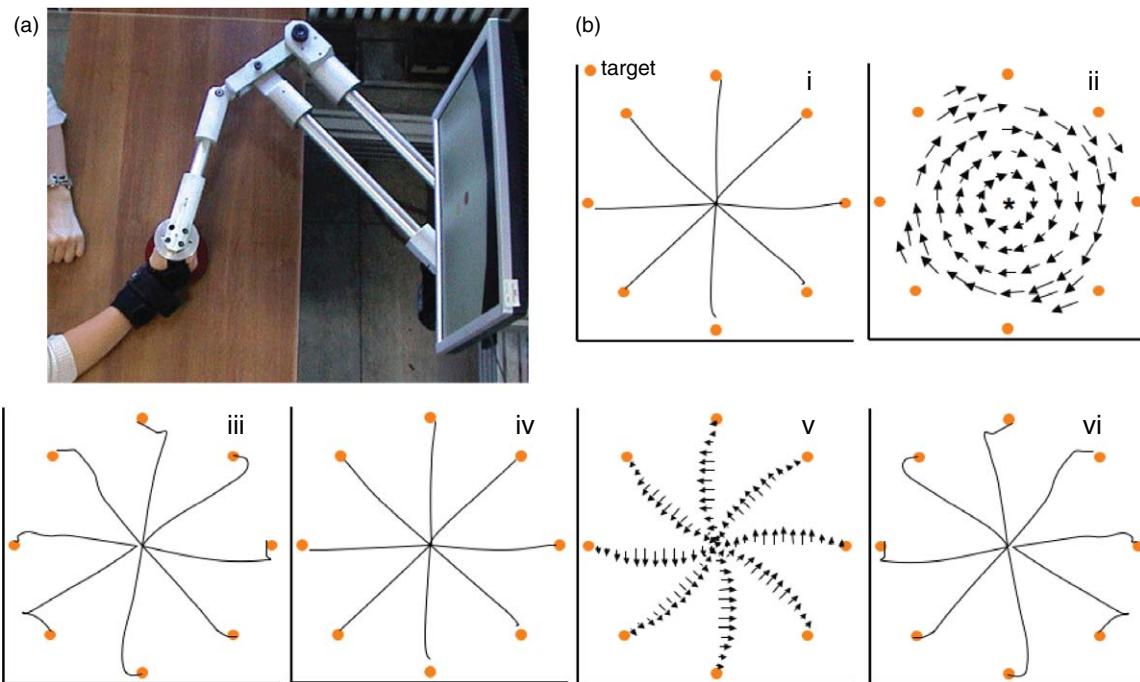


Figure 2 (a) Experimental setup for a motor adaptation task. The subject grips the handle of the manipulandum. A cursor corresponding to the position of the handle is displayed on the video monitor facing the subject. A target position for the subjects reaching movement is also displayed. With practice, the subject learns to compensate for the forces produced by the robot arm. (b) Cartoon of a subject's hand paths across stages of training: (i) Typical hand path in the null field. (ii) Example of a force field produced by a robot arm. (iii) Subject's hand path in the field before training. (iv) Hand path after extended training in the field similar to paths in null field. (v) Forces applied by a subject to counter the force field effects after training. (vi) After-effects seen when targets presented with null field conditions are intermixed during training.

linked to errors attributable to changes in the target on this type of task. The parietal cortex is thought to play an important role in transforming visuo-motor representations during online visually guided reaching consistent with its general role in spatial processing, whereas the cerebellum may not be critical for the within-trial online corrections.

Rotary pursuit

Rotary pursuit tasks require participants to maintain contact between a handheld stylus and a small target area on a rotating disk. The ability to maintain contact improves with practice. Amnesics can perform normally on this task, whereas patients with Huntington's disease are impaired and studies of patients with Parkinson's disease have yielded mixed results. Neuroimaging evidence suggests involvement of both the striatum and cerebellum in addition to cortical regions while executing the motor task, whereas activity in the supplementary motor area (SMA) and posterior parietal cortex has been linked to learning on the task. It has also been suggested that learning may be associated with increased activity in motor preparation areas and decreased activity visual processing areas, reflecting a shift from reliance on continuous external visual feedback to reliance on internally generated models allowing movement preparation and smooth execution.

Sequence learning

Motor sequence learning has been studied extensively using many task variations. In general, participants press a button in response to the spatial location of a visual stimulus (**Figures 3(a)** and **3(b)**). Unbeknownst to the participant, the sequence of visual stimuli sometimes follows a repeated pattern. The latency to push the buttons decreases with practice, in particular for the repeated pattern.

Patients with damage to the MTL can learn these tasks normally, whereas damage to the striatum impairs sequence specific learning. Although neurologically healthy participants often do not report awareness of the embedded pattern or display substantial knowledge when

probed, explicit knowledge can develop. Even in such cases, parallel implicit learning occurs as a result of the motor actions, and awareness of the sequence does not necessarily translate into ability to rapidly perform the motor skill. Patients with damage to the striatum or cerebellum may report sequence awareness and still be impaired on the performance of the motor skill. The striatum has also been implicated in sequence learning in neuroimaging studies. In addition, involvement of the SMA is commonly seen, consistent with the importance of motor planning, and the parietal cortex is consistently implicated and has been linked to learning of the spatial sequence. Damage to the cerebellum leads to impaired implicit sequence learning, as does damage to the striatum, but the particular role of the cerebellum is not clear. Evidence from neuroimaging has suggested that the cerebellum may be critical in the execution of the learned skill.

Perceptual Skills

Mirror reading

Learning to read mirror-reversed text has commonly been used to assess perceptual skill development. Amnesics can improve their mirror reading skill without recognizing previously studied specific words and can retain the skill for several months. The role of the striatum is not entirely clear. Parkinson's patients often show intact mirror-reading skill, whereas Huntington's patients with intact memory for specific words show a mirror-reading impairment, perhaps due to some visuo-spatial deficit in Huntington's disease. Consistent with the importance of visuo-spatial transformations in mirror reading, neuroimaging has implicated parietal cortex in addition to the striatum.

Visual prototype learning

Prototype learning commonly takes the form of observing dot patterns that are deviations from a single prototype. After studying multiple prototype distortions, subjects are told that the patterns were from a

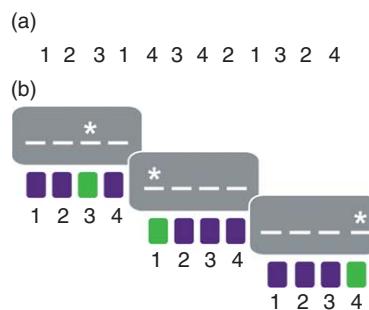


Figure 3 (a) Repeating sequence used in motor sequence learning task. (b) Participants press a button corresponding to the spatial location of the asterisk onscreen.

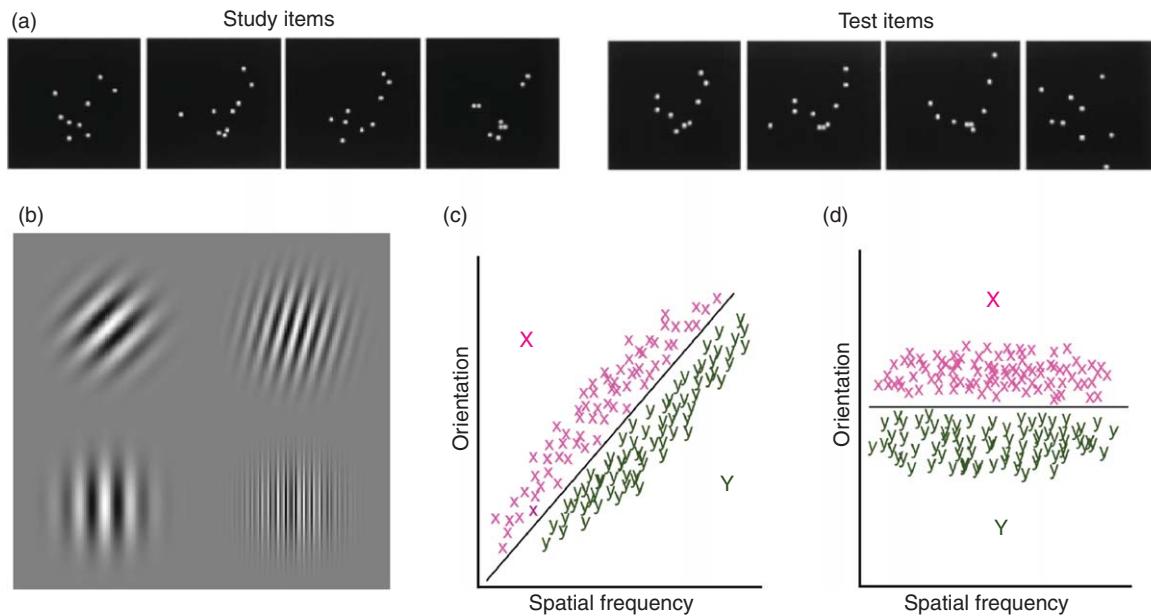


Figure 4 (a) Examples of dot patterns used as study and test items used to assess visual prototype learning. Study items were high distortions of a prototype dot pattern. From left to right are shown the training prototype, low and high distortions of the training prototype, and random dot patterns. (b) Example Gabor patches that vary in spatial frequency and orientation from trial to trial are used in perceptual categorization experiments. (c) Plot of stimuli that might be used in an information-integration category-learning task. X's denote the spatial frequency and orientation of exemplars from category X and Y's denote category Y exemplars. An optimal decision bound requires integrating information about orientation and spatial frequency prior to making a decision and is indicated by the diagonal line. (d) In a rule-based category learning task, the optimal decision boundary follows a rule that can easily be verbalized (indicated by the horizontal line). (a) Reproduced from Reber PJ, Stark CEL, and Squire LR (1998) Cortical areas supporting category learning identified using functional MRI. *Proceedings of the National Academy of Sciences of United States of America* 95: 747–750.

single category and that they must classify subsequently presented patterns as category members or not (**Figure 4(a)**). Amnesics can perform such categorization like healthy controls, but unlike healthy individuals, they are impaired when asked to explicitly recognize previously seen items. Patients with striatal damage also perform normally on this type of learning. Neuroimaging studies have implicated early visual areas, which show decreased activity when category members are classified. Interestingly, when healthy subjects are asked to intentionally learn the category, subsequent classification involves increased activity in MTL and prefrontal regions, in contrast to the pattern of activity seen after incidental learning.

Perceptual categorization

In perceptual categorization, subjects are asked to intentionally learn to classify visual patterns by trial and error. However, the complexity of the rules governing category membership can make it difficult to verbalize a strategy for performing categorizations. For example, Gabor patches that vary in frequency and orientation may be used as stimuli and nonlinear combinations of the perceptual dimensions govern category membership (see **Figure 4(b)**). No two stimuli need be alike because the perceptual dimensions can be varied

continuously. Therefore, a general perceptual rule has to be applied to each new stimulus. Amnesics can learn such categorization tasks. Damage to the striatum impairs learning of complex nonlinear category boundaries, but sometimes leaves learning of simple linear boundaries intact.

Contextual cueing

The implicit learning abilities discussed here all share the characteristic that they appear not to depend on the integrity of the MTL. However, implicit learning impairment has been demonstrated in patients with MTL damage. Contextual cueing is a task where visual displays with repeated spatial configurations allow facilitated detection of a target. The representation of the spatial configuration is thought to depend on the MTL regardless of the fact that subjects are not informed of the regularity, do not report awareness of the repetition, or necessarily show recognition of repeated configurations. Amnesics are not able to take advantage of the repetition, and neuroimaging studies have shown involvement of the MTL. Thus, implicit learning is not necessarily independent of the MTL. This depends on the task domain and specific capacities needed to perform a task.

Cognitive Skills

Category learning

Several category-learning tasks are similar to the perceptual categorization or prototype learning tasks. However, rather than involving continuous stimulus dimensions that are difficult to verbalize, they involve multiple discrete features. The combination of some features may be diagnostic of category membership while other features may be irrelevant. These types of categories can be learned by patients with damage to the MTL, but although the exact category rules can be difficult to verbalize, healthy participants often explicitly generate hypotheses and may thus achieve enhanced performance relative to patients with brain damage (Figure 5(a)).

Probabilistic category learning

In probabilistic classification tasks, stimuli are probabilistically associated with categories, and subjects learn based on trial-by-trial feedback. Single features and combinations of these features each predict the category

outcome with a certain probability. A frequently used cover story is that subjects must predict whether it will be sunny or rainy based on a deck of four cards (Figure 5(b)) Based on feedback, categorization should eventually improve, but, because the category association is probabilistic, feedback on any single trial may be misleading. Subjects are deemed accurate when they select the more likely outcome and will perform best if they predict the more frequent outcome across many trials. This structure is thought to make memory for cue–outcome relations on individual trials unhelpful, and although subjects routinely learn to perform with 70–80% accuracy they report a feeling of that they have not learned.

Amnesics, in some cases, can learn this task despite poor memory for details about testing. However, healthy subjects can exhibit explicit knowledge about the task structure when probed more specifically, and there is neuroimaging evidence for engagement of the MTL in such cases, suggesting that healthy subjects may use different or additional knowledge when possible. The necessity for integrating across multiple trials

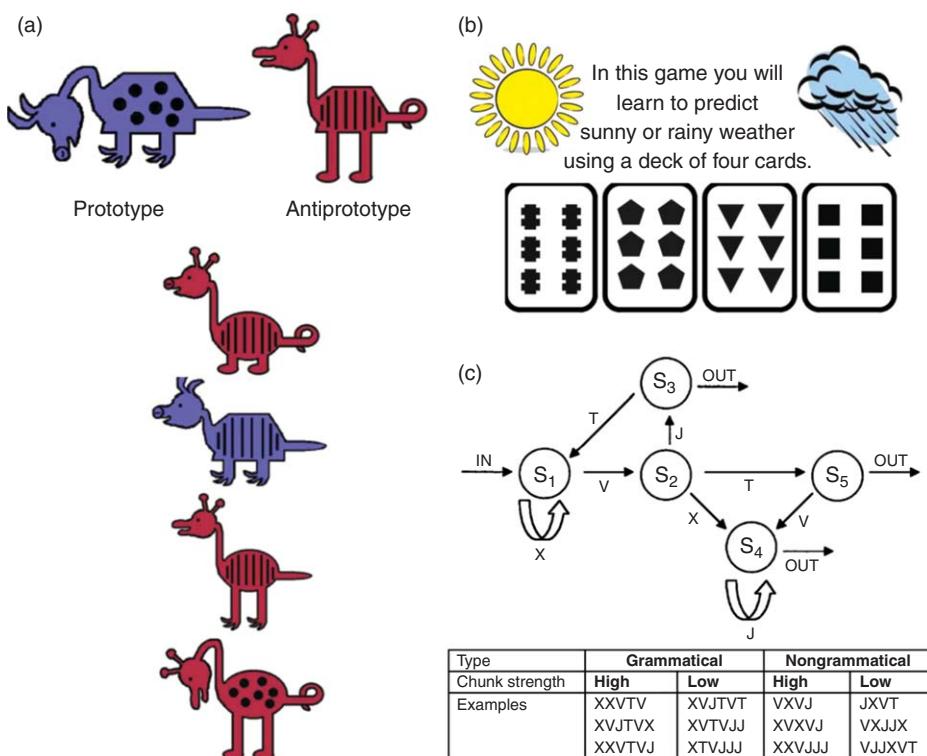


Figure 5 (a) Example stimuli from a categorization task. Subjects study examples of cartoon animals. Subsequently, they are told that they saw animals called ‘Peggles’ and now they must decide whether or not the following animals are Peggles. (b) Example of stimuli used in probabilistic classification tasks. One or more of the cards are presented on each trial and a prediction of sunny or rainy outcome is made. (c) Finite-state grammar used to generate letter strings. (d) Examples of training and test strings that are either grammatical or not and have high or low chunk strength. (a) Adapted from Bozoki A, Grossman M, and Smith EE (2006). Can patients with Alzheimer’s disease learn a category implicitly? *Neuropsychologia* 44(5): 816–827. (c and d) Adapted from Chang GY and Knowlton BJ (2004). Visual feature learning in artificial grammar classification. *Journal of Experimental Psychology: Learning, Memory, and Cognition* 30(3): 714–722.

on this task has been suggested to parallel gradual learning of stimulus-response learning, which in animals depends on the striatum. In humans, patients with damage to the striatum show impaired learning. However, it appears that the critical involvement of the striatum is tied to learning from trial-by-trial feedback. When the same task is learned through observation of multiple trials where cues and outcomes are presented simultaneously, patients with Parkinson's disease can show that they have learned to make accurate category predictions. Consistent with this, neuroimaging studies show that the MTL rather than the striatum is engaged when learning by observation. Thus, it appears that the task can be learned in an implicit or explicit way and that this engages different neural systems, as is the case in visual prototype learning.

Artificial grammar

Artificial grammars consist of letter strings generated based on an underlying set of rules. Multiple letter-string exemplars are presented to subjects. Subsequently, subjects are told that they saw exemplars generated according to a specific set of rules and that they now must distinguish between new letter strings that either are or are not generated according to the same rules (see **Figure 5(c)**).

Multiple types of knowledge may be acquired. For example, abstract rules about generating grammatical strings or more specific knowledge about string features such as similarity between chunks of letters within grammatical strings (chunk strength) can be learned. Evidence from neuroimaging is mixed, with one study finding MTL activity related to chunk strength and caudate activity related to rule adherence. Some studies have found prefrontal, occipital, and parietal cortex activity and others have found decreases in occipital cortex activity as a function of string fragment priming. Similarly to the prototype distortion task, damage to the MTL, striatum, or cerebellum does not impair grammar learning, which may rely on number of implicit processes, such as rule abstraction and priming, to support various aspects of performance.

Classical Conditioning

Eyeblink conditioning

Eyeblink conditioning is perhaps the most extensively studied form of implicit learning. In this paradigm, a conditioned stimulus (e.g., a tone or a light) predicts the occurrence of an air puff to the eye. Repeated pairings result in the acquisition of an eyeblink timed to prevent the air puff on the eyeball. The neural circuitry underlying acquisition, execution, and retention of this behavior is primarily localized to the cerebellum and has been outlined in exquisite detail. Studies in animals,

using a range of techniques, have outlined the specific regions of the cerebellum critical for the unconditioned eyeblink response, the conditioned response, and for the representation of conditioned stimuli, thus providing a detailed model system for studying memory formation in general.

Neuropsychological studies of patients with cerebellar damage and fMRI studies in healthy adults have confirmed the necessity of the cerebellum for this form of learning in humans as well. As with the other forms of procedural learning described herein, awareness of contingencies does not appear to be critical for learning, and amnesics with damage to the MTL acquire a conditioned eyeblink.

However, two forms of eyeblink conditioning, delay and trace conditioning, exist, and map on to procedural and declarative memory, respectively. When a temporal gap is inserted between the conditioned stimulus offset and the air puff onset (trace conditioning), additional neural processes are necessary. Acquisition of trace eyeblink conditioning requires the hippocampus, as has been shown in animals and in humans with damage to the MTLs. Moreover, it is suggested that trace eyeblink conditioning in humans depends on awareness of the contingency between the conditioned stimulus and the air puff. Such awareness does not aid learning for delay conditioning, consistent with a distinction between procedural and declarative forms of eyeblink conditioning.

Characteristic of Implicit Learning and Memory

Awareness

A central challenge inherent to the study of implicit learning and memory relates to the role of awareness in learning. In patients with amnesia, it is relatively easy to demonstrate that memory is not accompanied by awareness of previous study. By contrast, while healthy subjects may use implicitly learned information without knowingly retrieving it, the possibility of contamination from explicit memory makes it important to carefully control or measure such contributions. On some tasks, being aware of what is learned will not necessarily help. For example, the ability to verbally characterize what you are doing when riding a bike is not likely to critically contribute to the bike riding. However, on the other tasks, the role of awareness and whether learning can truly be called implicit remain the most controversial issues related to implicit learning and memory. Nonetheless, the ability to show evidence of learning in amnesics who have no explicit memory for the material suggests that awareness need not accompany learning.

Automaticity

Implicit learning and memory are often characterized by automaticity relative to explicit memory. A common focus when studying skill learning is on the development of automaticity. Skill acquisition often requires executive control in its initial phases, and measuring whether performance becomes insensitive to interference from concurrent performance of an additional task is a common way to operationalize automaticity as it develops with practice. Most people recognize such a change in automaticity when riding a bike or driving a car. In the case of priming, automaticity can refer to a similar ability to exhibit intact learning and memory in the face of interference with executive control resources. In addition, automaticity captures the idea that previously studied information is used automatically without intentional efforts to access previously studied information.

Longevity

For both priming and skill learning, retention of memory has been demonstrated after months and years, which is impressive considering the limited practice afforded and that further practice outside the laboratory is unlikely. However, those may also be the factors that allow implicit memories to remain undisturbed. If interference is a critical factor in forgetting, as is thought to be the case for explicit memory, then the idiosyncratic materials used in the laboratory to measure implicit learning may be a benefit. How implicit memory is supported long term will become better understood as more is learned about how such memories are consolidated and how factors such as sleep and amount of practice affect them.

Implicit Learning and Memory: Neural Aspects

Cerebral Cortex

Across both priming and skill learning, distinct neocortical regions are involved depending on task modality and the specific requirements of a task. Frontal and occipital cortical regions are involved in priming depending on whether conceptual or perceptual processing is necessary. When skill learning depends on visual processing, occipital regions are engaged, parietal cortex is involved in tasks requiring spatial processing, and motor and supplementary motor cortex is involved when motor skills are acquired.

Striatum

The striatum appears to play a more general role in skill learning, across task domains. The striatum is a complex, heterogeneous structure and may contribute to skill learning in several key ways. The anatomy of the striatum has been described as consisting of distinct fronto-striatal loops with distinct striatal territories connected to discrete cortical regions (e.g., caudate with dorsolateral prefrontal cortex and putamen with premotor cortex). In addition, these partially segregated loops can communicate through the striatum. As such, the striatum can support multiple aspects of skill learning.

Tasks that rely on incremental trial-and-error learning, such as stimulus-response learning, engage the striatum as measured with fMRI and are generally impaired in populations with striatal damage or when dopaminergic signaling is manipulated. This is consistent with demonstrations of dopamine-modulated plasticity at fronto-striatal synapses that can support stimulus-response learning and with a general role for the striatum in processing feedback. In particular, the striatum is thought to be critical when feedback is received in response to internally generated plans that subsequently are updated.

Another role proposed for the striatum is chunking action sequences into units. By chunking a series of actions into a single unit, execution becomes more reliable and rapid. On the other hand, chunking leads to more stereotyped and less flexible behavior. Evidence for unitizing comes from recordings in rodents where repeated performance results in neural activity signaling the onset and offset of an action sequence instead of activity at every step of the sequence. Such a mechanism would be relevant for sequencing tasks.

Feedback processing is most commonly associated with anterior aspects of the striatum involving the caudate. The putamen has instead been associated more specifically with motor learning. In some cases, acquisition of motor sequences has been associated with anterior striatum and execution of well-learned sequences with the posterior striatum. Future research is needed to understand how distinct striatal regions support distinct aspect of implicit learning and how these contributions change as learning progresses.

Cerebellum

The role for the cerebellum in skill learning is similar to that of the striatum, in that it involves adjusting an internal model in response to feedback. However, the cerebellum has been associated with tasks where continuous sensory feedback is provided, and novel mappings between

visual information and motor behavior are developed (e.g., mirror-tracing and motor adaptation tasks).

When it comes to learning a discrete response, such as a conditioned eyeblink, the role of specific regions within the cerebellar system has been identified, and it has been detailed how various aspects of conditioned stimuli and responses depend on specific regions to support the resulting implicitly learned behavior, which is a specific well-timed motor action. Interestingly, although timing is critical, the cerebellum alone is unable to support learning when a temporal gap is inserted between a predictive cue and the air puff.

Thus, the cerebellum is critical for several types of implicit motor learning. However, the detailed knowledge about the neural substrates of classically conditioned responses developed primarily in animal stands in contrast to the more limited knowledge of the cerebellum's role in other types of motor learning frequently studied in humans. Future research may bridge this gap and leverage the extensive knowledge developed in animal models.

Medial Temporal Lobe

Involvement of the MTL is often observed when explicit knowledge plays a role. However, this is not uniformly the case. There are examples of MTL damage leading to impaired implicit learning and, using neuroimaging, demonstrations of MTL activity on implicit learning task such as contextual cueing. In addition, MTL activity has been linked to learning of higher-order relations on sequence learning tasks or item associations in priming tasks, suggesting that the MTL may be involved when relations among items are critical regardless of explicit knowledge or awareness of what is being learned. Additionally, evidence from eyeblink conditioning has shown that the MTL becomes critical when associations need to bridge across time.

Conclusion

The brain exhibits an impressive breadth of ways to learn about the environment. Multiple mechanisms are available to process novel and repeated information regardless of any intention to learn. In general, the psychological distinction between different learning and memory processes has been supported by finding dissociable neural signatures, by using neuroimaging techniques in healthy populations and by finding differences between patient populations with brain injuries. As suggested by the descriptions above, without the presence of neurological damage, multiple types of memory may contribute to performing a task. Both implicit and explicit processes

may contribute or both priming and skill learning may play a role. Although the use of different experimental paradigms aims at testing specific skills, most tasks do not yield pure measures of the targeted mechanisms. However, some principles have emerged suggesting that the striatum and cerebellum play general roles and that changes occur in the cortical regions that initially process stimuli and that these regions vary depending on the task modality. It remains a challenge to dissect further the cognitive and neural processes that support implicit learning and memory and, in particular, to further understand how these processes interact and change with practice.

See also: Amnesia; Basal Ganglia; Behavioral Planning: Neurophysiological Approach of the Frontal Lobe Function in Primates; Brain Imaging; Cerebellum: Associative Learning; Conscious and the Unconscious; Declarative Memory; Episodic and Autobiographical Memory: Psychological and Neural Aspects; Parkinson's Disease; Sleep: Learning and Memory.

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Language and Communication – Brain Substrate

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Glossary

Apraxia of speech – A communicative disorder involving the voluntary production of speech.

Articulatory code – The representation of speech sounds derived by internally simulating movements needed to produce those sounds.

Paralinguistic – The nonverbal elements of communication.

Paraphasic error – A speech error involving the substitution of an incorrect but related sound or word, as in ‘tree’ for ‘free,’ or ‘chair’ for ‘bed.’

Phoneme – One of a small set of speech sounds used in a particular language; it is the smallest unit of sound that leads to a difference in meaning.

Pragmatics – The field of linguistics addressing the way that meaning changes as a function of context.

Semantics – The study of language meaning.

Suprasegmental – A vocal effect that extends over more than a single speech sound.

analysis, which determines structure–function relationships by noting deficits associated with damage to particular brain regions, and neuroimaging methodologies, which identify brain activity modulated by demands on the processes presumed to underlie language and communication. These two approaches are somewhat complementary, as lesion analysis can be used to identify brain regions necessary for a particular language or communicative function, while neuroimaging is useful for identifying a complete set of brain regions that participate in the underlying computational processes.

Patient Studies

Aphasias

Many historians of science argue that the modern era of neuropsychology begins with the work of Paul Broca, a nineteenth-century neurologist. Broca prompted scientific discussion as to whether language ability could be localized in the brain with his classic report of two patients with profound language-production deficits following large left frontal lobe lesions. Localization received further support from Broca’s contemporary, Carl Wernicke, who reported two patients with severe language-comprehension deficits, apparently due to the presence of a lesion in the posterior portion of the left temporal lobe. Although Wernicke’s aphasics can speak fluently, their speech includes made-up words known as ‘paraphasias’ (e.g., ‘treen’ for ‘train’), and their sentences are often incoherent. In contrast, the incidence of aphasic deficits in patients with lesions in the right hemisphere is far less common.

Even today, cognitive neuroscientists’ understanding of the relationship between brain activity and language ability derives largely from the study of brain-injured patients. The logic of these studies is that the damaged area plays a critical role in the compromised function. Consequently, the left hemisphere is considered to be the language hemisphere, while the right hemisphere is the

Language is a system for evoking cognitive models through the systematic use of sounds, gestural signs, or printed symbols. Language involves several different kinds of representations, each governed by rules and regularities in the way in which they can be combined. Speech, for example, can be understood as being structured at the level of phonemes, or speech sounds, lexemes, or words, and grammatical constructions that allow speakers to combine a finite array of words into a potentially infinite set of sentence meanings. Moreover, communication involves more than language, including paralinguistic and inferential components, such as tone of voice, or prosody, facial expressions, co-speech gestures, and the utilization of background and contextual knowledge.

Study of the neural substrate of language and communication has primarily involved two methodologies: lesion

'minor' hemisphere. Since damage to the front portion of the brain is associated with difficulty in speaking, it is assumed that left frontal areas play a crucial role in language production. Similarly, since damage to the posterior portion of the brain is associated with difficulty in understanding language, it is assumed that left posterior temporal areas play a crucial role in language comprehension.

Although the modern model of the neural basis of language is considerably more complex than that outlined by nineteenth-century neurologists, such as Broca and Wernicke, a good deal of contact with its historical predecessor remains. Consequently, we begin with a brief account of each of the seven basic aphasic syndromes, or common clusters of symptoms, and follow with a synopsis of localization claims agreed to by the twenty-first-century neurologists and neuropsychologists.

Broca's aphasia

Common features in the speech of Broca's aphasics include effortful speech replete with pauses, false starts, and unclear words. Their conversational speech is not grammatical, consisting mainly of nouns, with a few verbs. The so-called 'function words' (determiners, prepositions, and conjunctions) and 'bound morphemes' (e.g., '-s' to denote the plural, or '-ly' to indicate an adverb) are often omitted from the speech of Broca's aphasics. These patients experience word-finding difficulty, both in conversational speech, and in picture-naming tasks. When asked to repeat a word or a phrase, Broca's aphasics are often unable to do so, producing the same sort of halting utterances characteristic of their conversational speech. Although comprehension in Broca's aphasics is generally good; careful testing suggests that they have difficulty when adequate understanding of a sentence depends critically on grammatical information, without the support of background knowledge and contextual information.

As classically defined, Broca's area is the third frontal convolution of the inferior frontal gyrus. The proximity of this area to primary and premotor cortices coupled with the speech-production deficits associated with this syndrome, led to an early characterization of Broca's aphasia as 'expressive' aphasia, to be contrasted with the fluent 'receptive' aphasia described by Wernicke, which in turn was supported by the proximity of Wernicke's area to primary auditory cortex. However, extensive study of the relationship between lesion sites and particular deficits has led to the conclusion that it is the existence of lesions to the anterior portion of the insula that is crucial for the prediction of whether a given patient will suffer from the speech-production deficit known as 'apraxia of speech.'

Another characterization of Broca's aphasia is as 'agrammatic' aphasia, due to the putative involvement

of Broca's area in knowledge of grammar. This characterization derives both from the ungrammatical nature of their output, and the demonstration that comprehension deficits in Broca's aphasia seemed to depend specifically on knowledge of grammar. However, the tight linkage between Broca's area and grammatical knowledge has also fallen out of favor due to reports of grammatical processing deficits in patients with other aphasia syndromes, as well as the demonstration that similar grammatical deficits could be induced in neurotypical adults by various methods of stimulus degradation, or through the imposition of stressful conditions.

Wernicke's aphasia

Language production in Wernicke's aphasia is fluent, though it often contains paraphasic errors. These patients' primary deficit, however, is in language comprehension. They experience moderate-to-severe difficulty on repetition and naming tasks, and while their comprehension of written words is often better than their auditory word comprehension, they are impaired both in their ability to read and to write.

As classically defined, Wernicke's area is the posterior region of the left superior temporal sulcus. However, systematic study has failed to uphold a strict one-to-one relationship between Wernicke's aphasia and lesions to this region. Some investigators have reported Wernicke's aphasia in patients with lesions in frontal regions, post-Rolandic temporo-parietal areas, and other temporal lobe regions, especially the middle temporal gyrus.

Conduction aphasia

As in Wernicke's aphasia, conduction aphasics are characterized by fluent language production containing frequent paraphasic errors. Moreover, conduction aphasics are unable to repeat words or phrases that are read to them. Unlike Wernicke's aphasics, however, conduction aphasics' ability to understand language is largely preserved. Similar to most aphasics, these patients occasionally experience difficulty in naming tasks, and their reading and writing ability is sometimes impaired.

Conduction aphasia is classically associated with lesions to the arcuate fasciculus, the white matter tract that connects Broca's area in frontal cortex to Wernicke's area in the temporal lobe. This aphasic syndrome – intact comprehension accompanied by the inability to repeat spoken words – was actually predicted by Wernicke based on his model in which the arcuate fasciculus connected Wernicke's area, where the sound images of words were stored, to Broca's area, where the motor images of words were stored.

Wernicke argued that speech production was normal in conduction aphasics because the connection between conceptual centers and Broca's area was intact; speech

comprehension was normal because the connection between Wernicke's area and the brain's conceptual centers was intact. Repetition was compromised because this task required a connection between Wernicke's area and Broca's area, without the conceptual centers as an intervening step. Indeed, Wernicke's account is consistent with the report that conduction aphasics' behavior on repetition tasks often suggests that they understand the phrase to be repeated, but are unable to produce a verbatim reproduction.

Global aphasia

Global aphasia is characterized by the complete inability either to speak or to understand language, and is typically associated with large lesions affecting up to 75% of the left hemisphere. Lesioned areas include the Broca's area, the insula, the arcuate fasciculus, Wernicke's area, the anterior superior temporal gyrus, the middle temporal gyrus, and the underlying white matter, along with motor, somatosensory, and auditory cortices.

Anomic aphasia

Anomic aphasia is characterized by severe word-finding difficulties, while fluency, comprehension, and repetition are all relatively intact. Many more severe aphasic syndromes resolve over time to anomic aphasia, and thus, this syndrome is associated with a wide variety of left hemisphere lesions.

Transcortical motor aphasia

Lesions to the superior aspect of the anterior frontal lobe often give rise to speech production difficulties akin to those in Broca's aphasia. While their spontaneous output is often telegraphic in character, transcortical motor aphasics show a remarkable ability to repeat even very long sentences. Over time, these patients' speech-production ability typically improves, and their condition resolves to anomic aphasia.

Transcortical sensory aphasia

Relatively small lesions in the posterior middle temporal gyrus, or occasionally in the posterior parietal region, have been associated with transcortical sensory aphasia. Larger lesions that include the superior temporal cortex are often associated with Wernicke's aphasia. Similar to their counterparts with Wernicke's aphasia, transcortical sensory aphasics have fluent output, impaired comprehension, and experience difficulties in naming tasks. Unlike Wernicke's aphasics, however, patients with transcortical sensory aphasia have preserved repetition, as they are able to repeat words, sentences of considerable length and complexity, and even words in languages they do not speak. Transcortical sensory aphasia is an acute condition, resolving eventually to mild anomic aphasia.

Aphasia and the neural substrate of language

While aphasic syndromes discussed above provide a useful heuristic for describing the communicative deficits of left hemisphere stroke patients, they have proven less useful as guides for identifying the neural substrate of language. That is, classification of a patient as a Broca's aphasic, for example, need not imply the patient's lesion site includes the inferior frontal gyrus. Conversely, a patient with damage to the inferior frontal gyrus will not necessarily present with Broca's aphasia. However, neurologists have enjoyed more success in localization efforts by restricting their attention to the relationship between lesion sites and specific language deficits, irrespective of the general aphasia type of the patients who experience those deficits.

For example, the repetition problems experienced by conduction aphasics have been linked to the ability to keep recently encountered auditory information active for a few seconds, in order to facilitate the processing of stimuli that unfold in time – as in the case of spoken language. Cognitive psychologists refer to these processes alternately as echoic memory, or the phonological store. Besides lesions to the arcuate fasciculus, conduction aphasics typically have lesions in the posterior superior temporal gyrus. Moreover, patients with other aphasic syndromes who present with repetition deficits also have lesions in the posterior superior temporal gyrus. Consequently, this brain region has been implicated as an essential component of the phonological store.

Similarly, the impaired semantic comprehension associated with Wernicke's aphasia has been linked specifically to damage to the middle temporal gyrus. As patients with damage to the middle temporal gyrus and the underlying white matter are impaired on word comprehension irrespective of their aphasia syndrome, this brain region has been implicated as an essential component of the lexical–semantic network. Analogous reasoning has implicated the anterior superior temporal gyrus in the comprehension of complex sentence structures, and the arcuate fasciculus for transmitting information from the temporal lobe language areas to motor speech areas in the frontal lobe. Patient studies suggest that the precentral gyrus of the insula is crucial for articulatory planning, while Broca's area proper (the third frontal convolution of the inferior frontal gyrus) is implicated in end-stage articulatory processes.

Right Hemisphere Damage

Although patients with right hemisphere damage show preservation of core language abilities, such as naming, fluency, and the ability to understand individual sentences, they often present with other, more subtle communicative deficits. For example, their speech, while including words that are well formed phonemically,

often has a flat quality sometimes described as ‘robotic.’ Moreover, their conversation is frequently marked by diversions from the topic at hand, tangential remarks, and offensive comments. In experimental studies of their comprehension, these patients have also been shown to have deficits in the comprehension of various pragmatic language phenomena.

Aprosodia

Prosody is suprasegmental information in the speech signal that can be used to convey affective or grammatical information. Aprosodia is thus impaired ability to produce or comprehend prosodic contours on speech. Prosodic information is conveyed by variations in pitch, loudness, duration, voice quality, and rhythm. As noted above, one classic aspect of right hemisphere syndrome is aprosodic output, conveying a flat affect. Although aprosodic output is sometimes accompanied by deficits in affective processing, such as inappropriate emotional responses to events, it is clearly dissociable from the latter. Affective processing deficits can occur with or without aprosodic speech, and aprosodic speech can occur in patients whose emotional responses are entirely normal.

Regarding the comprehension of prosody, some evidence suggests greater left hemisphere involvement in processing prosody that signals linguistic distinctions, and greater right hemisphere involvement in processing affective prosody. More recent studies have attempted to relate these patterns of lateralization to the acoustic underpinnings of linguistic versus affective prosody. Patient studies suggest that right-hemisphere-damaged patients’ preserved ability to appreciate linguistic prosody can be traced to their reliance on rhythm cues thought to be preferentially processed in the (intact) left hemisphere. Their deficits in the appreciation of emotional prosody may be linked to an impaired ability to detect pitch differences preferentially processed in the right hemisphere.

High-level communicative deficits

Right hemisphere damage has also been associated with deficits in the comprehension of a number of pragmatic language phenomena, or language whose meaning depends on extra-linguistic knowledge about the physical and social context as well as background knowledge about the way the world works. These pragmatic language-comprehension deficits include difficulty in appreciating humor, sarcasm, discourse appropriateness, the communicative intentions of one’s interlocutor, and knowledge of what one’s interlocutor does and does not know (i.e., theory of mind).

Neuroimaging: Language Production

The advent of twentieth-century neuroimaging techniques ushered in a new era of the cognitive neuroscience of language by enabling researchers to discover brain areas underlying various language and communicative processes in healthy adults. These studies typically involve monitoring local changes in various kinds of metabolic activity caused by experimental manipulation of the cognitive demands on the language system. Positron emission tomography (PET), for example, is typically used to measure cerebral blood flow in particular brain regions. Functional magnetic resonance imaging (fMRI) is used to monitor changes in the oxygenation value of hemoglobin in the blood and, besides being a less-invasive method than PET scanning, is superior to PET in both spatial and temporal resolution.

One major advantage of noninvasive imaging techniques is that they allow the investigator to see an entire network of brain regions that participate in the cognitive task of interest, rather than being restricted to the identification of a few essential nodes in that network. As such, neuroimaging has led to the understanding of a language area in the brain that had largely escaped the attention of neurologists, due to the fact that it is typically spared in left hemisphere strokes. The so-called ‘basal temporal language area’ is comprised of a region in the posterior inferior temporal cortex, and is activated in a variety of language neuroimaging paradigms.

Findings from neuroimaging studies of language production are briefly discussed in this section, while studies of language comprehension are covered in the section entitled ‘Neuroimaging: Language comprehension.’

Speech

As motion is inherent to the act of speaking, neuroimaging techniques are not well suited for the investigation of speech-production processes. However, studies to date have implicated the left basal temporal language area, the left anterior insula, the left frontal operculum, and the right cerebellum. Activity in the left anterior insula and the left frontal operculum have been specifically related to articulatory planning, consistent with the neuropsychological literature on apraxia of speech. Semantically driven speech output, as in a naming task, involves enhanced activity in left posterior inferior frontal cortex. Finally, motor control of speech activates bilateral sensorimotor cortices.

Writing

If speech production is a somewhat understudied area in the cognitive neuroscience of language, the study of the neural correlates of writing is virtually uncharted

territory. A handful of studies on the production of written words, however, point to the importance of the left superior parietal lobule and the left inferior posterior frontal cortex.

Neuroimaging: Language Comprehension

Sound

Speech perception and phonological processing

Hearing spoken words activate bilateral superior temporal gyri. Neuroimaging thus implicates right hemisphere brain regions in speech perception, a fact difficult to detect with the lesion localization technique. The dominant role of the left hemisphere in the processing of speech may relate to hemispheric specialization in tuning for different sorts of auditory information. Recent investigations support a left hemisphere bias for processing rapid acoustic transitions versus a right hemisphere bias for spectral variations. The former are important for discriminating between different consonants, while the latter is important for tone languages such as Mandarin.

Many details concerning the anatomy and physiology of the human auditory system are unknown. Based on knowledge of primates and other mammals, however, a number of investigators have suggested the existence of multiple parallel processing streams in the human auditory system. A ‘what’ stream (sometimes referred to as ‘ventral’ by analogy to the visual system) in the posterior central parietal occipital junction, primarily in the left hemisphere, serves as an interface between sound and meaning representations. A ‘where’ or ‘how’ stream (sometimes referred to as ‘dorsal’ by analogy to the visual system) involves the inferior parietal and frontal cortices and is thought to be important for auditory–motor integration important in some accounts of speech perception.

The import of motor areas for speech production is motivated by the fact that phonemes are defined by articulatory features, and that reference to these motor features might facilitate the recognition of speech sounds whose acoustic properties are highly variable across speakers. Recent studies of phoneme perception report the activation of both auditory areas in the temporal lobe, including Heschl’s gyrus and the planum temporale, and motor areas in the frontal lobe, including the mouth motor area and inferior precentral cortex.

Phonological store

Neuroimaging studies to date vary somewhat in their localization of the phonological store, or ‘loop’ as it is sometimes called. This variability may be attributable to the extent to which the experimental task requires participants to employ an articulatory code to activate phonemes. In tasks that do not promote the use of an articulatory code, the activation involves the left superior

temporal sulcus – consistent with the observations of repetition deficits in patients with damage to this area. In tasks that do promote the use of an articulatory code, the activation is more dorsal, at the junction between the posterior superior temporal cortex and the inferior part of the supramarginal gyrus. Some data also implicate the pars triangularis (in Broca’s area) as part of a network of brain areas supporting the transient store of phonological information.

Prosody

Prosody conveys suprarexical meaning, that is, meaning above the level of an individual word. Linguistically, prosody can cue whether a statement is a question or an assertion. It can also convey affective information such as the mood of the speaker, or the correct affective response to the message being communicated. Although the neural substrate of prosody appreciation has received little attention, a few studies report activation in the right inferior frontal gyrus in response to spoken language with affective prosody compared to neutral intonation.

Word Meaning

Spoken words

fMRI indicates that accessing the meaning of a spoken word activates the left inferior frontal gyrus, left posterior middle temporal cortex, posterior temporo-parietal cortex, and anterior inferior temporal cortex. Meta-analysis of multiple studies points to a number of brain areas specifically involved in accessing the meaning of spoken words, and distinct from cortical areas implicated in the processing of phonological properties. In the frontal lobe, semantic areas include the upper portion of the inferior frontal gyrus, the dorsal part of the pars opercularis, the ventral part of the pars triangularis, and down to pars orbitalis. In the temporal lobe, semantic areas included the middle and inferior temporal gyri, the temporal pole, and the angular gyrus. Regions of the posterior superior temporal sulcus (Wernicke’s area) and the middle temporal sulcus have been activated both by tasks manipulating demands on phonological processing and those manipulating semantic processing.

Written words

Reading words activates much the same brain areas as does hearing spoken words, including the left posterior middle temporal cortex, posterior temporo-parietal cortex, and anterior inferior temporal cortex. Moreover, visually presented words also activate the posterior fusiform and lingual gyri. Interestingly, activation of the latter two areas is also observed in picture-naming paradigms, suggesting their role in reading may be related to a more general function of connecting visual images to their associated meanings.

Real-time neuroimaging measures

Owing in part to the limited temporal resolution of functional neuroimaging based on hemodynamic measures (i.e., blood flow in PET and blood oxygenation levels in fMRI), the portrait of the brain regions involved in word comprehension is rather static. Reading a word, for example, involves cognitive steps, including visual encoding of the stimulus, potentially matching it to its phonological instantiation, and retrieving associated semantic information, all through a dynamic series of neural processes unfolding at the millisecond level. Although the image of multiple brain regions activated over the course of seconds is an inadequate picture of the underlying processing stream, information from functional neuroimaging can be supplemented with complementary data from methods, such as electroencephalography (EEG) and magnetoencephalography (MEG), with less accurate spatial resolution, but millisecond-level temporal resolution.

Event-related potentials (ERPs) derived from the EEG reveal a series of components sensitive to manipulations of the perceptual demands of word recognition (the P1, N1, and P2 components), the semantic demands of word recognition (the N400), and the demands on memory (the N400, whose amplitude is reduced by repetition) as well as the late positive complex (LPC), whose amplitude is typically enhanced by the recognition of a previously presented stimulus. Evoked magnetic fields (EMFs) derived from the MEG reveal a similar series of components, and the greater spatial resolution of the MEG affords more accurate localization of the neural sources of these components.

MEG studies of word comprehension suggest that words are processed through repeated activation cycles in an extended network of brain areas. When the stimulus is a spoken word, the initial activation is in bilateral superior temporal cortices; when it is a written word, the initial activation is in striate and extra-striate cortices. Activity then spreads anteriorly – along the ventral aspect of the inferior temporal cortex for visual stimuli, and along the middle temporal gyrus for auditory stimuli – involving the left superior temporal sulcus, left inferior prefrontal regions, and bilateral medial prefrontal areas for both written and spoken words. Visually presented words continue to elicit activity in inferotemporal and posteromedial areas not activated for spoken words; likewise, spoken words elicit sustained modality-specific activity in the perisylvian area.

Importantly, following the initial feed-forward sweep of activity along the visual and auditory ‘ventral’ streams, distributed source models show waves of activity spreading back and forth between activated temporal and frontal lobe areas. The computational role of cells in these areas likely changes as a function of time and the nature of the input (feedforward vs. feedback), making the attribution

of a single function to a particular cortical area difficult, and potentially misleading.

Understanding Sentences

Functional neuroimaging studies that have contrasted people’s comprehension of increasingly complex linguistic stimuli – for example, their comprehension of individual words versus the same words embedded in sentences – have revealed a concomitant increase in the volume of cortex activated by these stimuli, along with increasing activation of the right hemisphere. Presumably, the recruitment of additional brain areas reflects the added computational demands of understanding sentences. Besides activating the relevant meanings of individual words, understanding sentences involves combining those meanings using one’s knowledge of grammatical rules and regularities. The relationship between semantics and syntax is a matter of some debate in linguistics, with traditional accounts arguing for two qualitatively distinct and independent processing modules, and more recent accounts that suggest individual words and grammatical constructions both involve associations between form and meaning, but at varying levels of abstraction.

Investigation of the neural substrate of sentence comprehension has thus centered on the possible dissociation between the processing of meaning from that of grammar. Early support for the traditional view came from lesion studies, with the apparent dissociation between deficits in grammar in Broca’s aphasics from deficits in semantics in Wernicke’s aphasics. However, the importance of Wernicke’s area (and surrounding regions of the temporal lobes) for semantics has been upheld, whereas the importance of traditionally defined Broca’s area as a dedicated grammar module has not.

Other data argued to support the traditional distinction between meaning and grammar derives from the apparent dissociation of ERP responses to violations at the level of meaning (such as the N400 component) from violations at the level of grammar (such as the early left anterior negativity, or ELAN, the left anterior negativity, or LAN, and the P600). At present, the validity of these dissociations is questionable, owing to the presence of N400 modulation in response to grammatical violations, and the report of P600 modulations in response to particular sorts of semantic violations. While the difference between knowledge of meaning and knowledge of grammar is indisputable, current data leave open the issue of whether this difference is best construed as a difference in degree or a difference in kind.

Grammar

Manipulating the grammatical complexity of language stimuli modulates activity in classical Broca’s area, as well as the left posterior inferior frontal gyrus, and the

anterior aspect of the left superior temporal gyrus. In addition, some studies have implicated the basal ganglia and the insula. Moreover, while left hemisphere brain areas have shown greater sensitivity to manipulations of grammatical complexity, such manipulations also result in modulation of activity in the right hemisphere homologues of the regions mentioned above.

Meaning

Neuroimaging researchers have had difficulty in identifying brain regions as specifically sensitive to grammatical processing, as these manipulations invariably affect the difficulty of semantic processing, or the processing of meaning. Left frontal lobe areas implicated in the processing of grammatical regularities also seem to be activated in paradigms designed to manipulate semantic processing difficulty, and both sorts of activations may reflect the operation of working memory in sentence processing. One suggestion, however, is that a ventral region of inferior frontal cortex (pars orbitalis) underlies semantic judgments, while a dorsal region (pars opercularis) is related to judgments about grammaticality.

The influence of various cognitive and linguistic factors on deriving sentence meaning has perhaps been studied more fruitfully with scalp-recorded ERP measures such as the N400. First noted in experiments contrasting sentences that ended sensibly and predictably with others that ended with an incongruous word, the N400 has proven to be extremely sensitive to semantic context. For example, N400 amplitude shows a strong inverse correlation with the predictability of the eliciting word within a given sentence context. N400 amplitude also declines across the course of a congruent sentence, starting large and becoming smaller with each additional open-class word. Similar amplitude decreases do not occur for words in grammatical but meaningless word strings, suggesting N400 amplitude reflects the buildup of contextual constraints as a sentence proceeds.

Neural generators of the N400 sentence congruity effect likely include the majority of the left temporal lobe, with additional activity in the right anterior temporal lobe. The amplitude of this ERP component is greatly reduced in patients with lesions in the left temporal lobe or the temporoparietal junction, and slightly reduced in patients with lesions in the perisylvian region of the right hemisphere. Dipole modeling of the MEG counterpart, the N400m, consistently points to sources in the left superior and middle temporal gyri, and individually variable activity in homologous regions of the right hemisphere. Intracranial recordings in epileptic patients indicate the contribution of ventral temporal and medial temporal lobe areas to the scalp-recorded N400 effect. Neuroimaging studies manipulating sentence congruity also point to the importance of activation in the left inferior frontal lobe, although this activity may be more

closely related to a late positive-going effect in the ERP likely related to memory retrieval processes.

Understanding Text and Discourse

Just as neuroimaging studies have registered an increase in the extent of activation to sentences relative to words, the task of understanding sentences in the context of connected texts, such as narratives, or in discourse, also results in greater areas of activation than does the comprehension of individual sentences. This is because the comprehension of a text or a discourse goes beyond the individual meanings of its constitutive sentences. The connection between two successive utterances, for example, is often left implicit, thus requiring the reader to infer the ‘bridge’ between them. Understanding a particular sentence as being coherent with what has come before often involves the application of real-world background knowledge about the topic of discussion, as distinct from the particular meanings of the words employed. Similarly, comprehending remarks in discourse involves an understanding of the communicative situation, including the social relationships between the participants, as well as conversational norms known as ‘communicative maxims.’

The goal in text comprehension is typically the construction of a situation model, a cognitive model of the object, activity, or event described in the text. The construction of this model requires the integration of linguistic information provided in the text with background and local contextual information. Perhaps not surprisingly, neuroimaging studies of text and discourse comprehension implicate brain regions extending beyond those implicated in the comprehension of individual words and sentences. Text comprehension has been observed to activate the anterior temporal lobes bilaterally, extending into the temporal poles, the full extent of the left superior temporal sulcus, the inferior frontal gyri, and left hemisphere frontomedial and parietomedial regions.

In contrast to the patient literature linking right hemisphere damage to higher-level semantic and pragmatic processes, data from neuroimaging studies do not indicate a privileged role for the right hemisphere in text and discourse comprehension. The right hemisphere frontal activations have previously been observed for spoken materials independent of content and context, suggesting they relate to auditory processing of speech. The most consistent right hemisphere activation to date has been that of the anterior temporal lobe at coordinates very similar to those of its left hemisphere counterpart. The anterior temporal lobes have been suggested to play a role in autobiographical, emotional, and episodic memory, as well as category-specific retrieval processes, leading to the suggestion that their role in narrative text comprehension is related to their role in the encoding of episodic

memories. Further study is required to ascertain the functional significance of text-related activations.

In fact, the study of language and communication in naturalistic contexts as in text and discourse comprehension highlights their many different components. The above review highlights brain regions involved in the transformation of acoustic and visual information to meaning in comprehension, as well as the transformation of meaning to motoric output in production. However, besides the processes specific to language, text comprehension involves both long-term and working-memory processes, executive function and attention shifts, emotional processing, inferential reasoning, the attribution of human intentions, and presumably, many other processes as well. In many ways, then, we can consider the neural substrate of language to be the entire brain.

See also: Brain Imaging; Brain Mapping of Language and Memory in Epilepsy; Development and Language; Dyslexia (Developmental); Evolutionary and Developmental Issues in Cognitive Neuroscience; Hemispheric Specialization: Language, Space, and Sexual Differentiation.

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Memory Consolidation

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Glossary

Amygdala – A nuclear complex in the dorsomedial portion of the temporal lobe that forms part of the limbic system. The amygdala comprises several functionally and anatomically distinct nuclei: the central nucleus, the medial nucleus, the cortical portions, and the basolateral complex or basolateral amygdala (BLA; basal, lateral, and accessory basal nuclei). The amygdala has many connections to and from subcortical and cortical brain areas.

Basolateral amygdala (BLA) – The BLA comprises the basal, lateral, and accessory basal nuclei of the amygdala. Extensive evidence indicates that the BLA is the portion of the amygdala which is critical in mediating modulatory influences on memory consolidation.

Calcium-calmodulin-dependent protein kinase II (CaMKII) – A protein kinase activated by calcium. In neurons, CaMKII activation in response to increases in cellular calcium is a key biochemical event downstream of glutamate receptor activation associated with synaptic plasticity.

Caudate nucleus – A major component of the basal ganglia, together with the putamen and globus pallidus. The caudate nucleus has been shown to play a crucial role in formation of some types of memory, such as response memories.

Consolidation hypothesis – Originally proposed by Georg E Müller and Alfons Pilzecker in 1900, the consolidation hypothesis refers to the stabilization of a new memory over time by neural processes activated by recently learned information.

Corticosterone – The main glucocorticoid hormone in rodents. Glucocorticoids are secreted by the adrenal cortex and regulate several aspects of the brain function, including consolidation of emotional memory.

Cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) – A protein activated by cAMP that increases gene transcription by binding to specific regions of DNA. CREB has been shown to play a major role in stimulating gene transcription associated with synaptic plasticity and memory consolidation.

Declarative memory – Declarative or explicit memory is defined as the conscious memory for facts and events.

Entorhinal cortex (EC) – Cortical area located in the anterior part of the parahippocampal gyrus. The EC provides major inputs to both the hippocampus and the

amygdala and is importantly involved in memory consolidation. The EC is one of the first areas to be affected in patients with Alzheimer's disease.

Epinephrine – Usually referred to as adrenaline, epinephrine is a catecholamine hormone released by the adrenal medulla. Epinephrine modulates memory consolidation, probably by activating vagal afferents to the brain.

Extracellular signal-regulated protein kinase (ERK)

– ERKs are a subset of the mitogen-activated protein kinase (MAPK) family of protein kinases involved in intracellular signaling. ERK-mediated signaling downstream of receptor activation in neurons plays a major role in synaptic plasticity and memory formation.

Hebbian synapse – A concept describing memory formation at the synaptic level. In his book published in 1949, the psychologist DO Hebb proposed a hypothesis of memory based on the assumption that when two neural cells are activated simultaneously, structural or metabolic changes would increase the efficiency of communication between the cells. Such a mechanism would be the cellular basis for the consolidation and storage of long-term memory.

Hippocampus – The hippocampus is part of the limbic system, located in the medial temporal lobe. It plays an important role in the formation of new memories, particularly declarative memory involving spatial or contextual information.

Long-term potentiation (LTP) – It is a cellular phenomenon by which synapse efficiency is persistently increased. Described by Terje Lømo and Tim Bliss in 1973, LTP is operationally defined as a long-lasting increase in the amplitude of synaptic response following high-frequency stimulation of afferent fibers.

Considered by many as an example of Hebb's rule at work, LTP is the leading model for the synaptic changes that might be involved in underlying memory formation.

Magnetic resonance imaging (MRI) – An imaging technique that provides detailed images of portions of the body in any plane. MRI is especially useful for brain imaging because it provides high levels of contrast between different soft tissues. MRI is based on the use of a powerful magnetic field that aligns the nuclear magnetization of hydrogen atoms in the tissues.

Functional MRI (fMRI) is a specialized application of MRI used to measure changing neural activity in brain areas.

Mitogen-activated protein kinase (MAPK) – The MAPK family of protein kinases consists of serine-/threonine-specific protein kinases that mediate cellular responses to extracellular chemical messengers such as neurotransmitters and growth factors. MAPKs regulate a number of cellular functions, including gene expression, mitosis, cell proliferation, differentiation, and survival. In neurons, the MAPK signaling pathway has been shown to play a major role in synaptic plasticity and memory formation.

Norepinephrine – Norepinephrine or noradrenaline is a catecholamine that acts as a neurotransmitter in both the brain and peripheral nervous system upon release by noradrenergic neurons. Norepinephrine in brain areas, including the amygdala, has been shown to mediate modulatory influences on consolidation of emotional memory.

Nucleus accumbens (NAcc) – It is a brain structure that is part of the basal ganglia. The NAcc is connected to the BLA and plays a role in modulating memory consolidation.

Nucleus basalis magnocellularis (NBM) – It is a region of the rat brain that is homologous to Meynert's basal nucleus of primates. The NBM is located in the ventromedial region of the globus pallidus and contains mostly cholinergic neurons.

Nucleus of the solitary tract (NST) – The NST, located in the brainstem, is the site of the termination of afferent fibers running in the facial, glossopharyngeal, and vagus nerves. The NST receives projections from vagal afferents, and noradrenergic projections from the NST in turn influence other brain regions including the BLA, regulating memory consolidation.

Positron emission tomography (PET) – An imaging technique that produces a three-dimensional image of functional processes in body tissues. PET is based on the detection of gamma rays emitted indirectly by a radionuclide introduced into the body bound to a biologically active molecule (for instance, a glucose analog).

Procedural memory – Procedural memory, sometimes referred to as motor skill memory, is the long-term memory of skills and procedures. Unlike declarative memory, procedural memory seems not to depend on the functional integrity of the temporal lobe structures.

Protein kinase A (PKA) – PKA or cAMP-dependent protein kinase is a protein kinase activated by the second messenger cAMP. The cAMP/PKA pathway leads to stimulation of gene transcription and is considered one of the main cellular signaling mechanisms involved in mediating and regulating synaptic plasticity and memory formation.

Protein kinase C (PKC) – A family of protein kinases consisting of several isozymes activated through the

same signal transduction pathway as phospholipase C. Thus, the activation of most PKC isoforms depends on calcium and diacylglycerol. PKC activation downstream of glutamate receptor activation in neurons is associated with synaptic plasticity and memory formation.

Retrograde amnesia – Amnesia can be defined as an impairment of one or more aspects of memory function. Retrograde amnesia refers to the impairment in the ability to retrieve memories acquired prior to the trauma, lesion, or neuropathology that produced the impairment.

Stria terminalis (ST) – A brain structure that serves as a major output pathway of the amygdala. Lesions of the ST prevent the modulatory influence of the amygdala in other brain regions.

Early History: The Perseveration–Consolidation Hypothesis of Müller and Pilzecker

The concept of memory consolidation refers to the stabilization of a new memory over time by neural processes activated by recently learned information. The ‘perseveration–consolidation hypothesis’ was originally proposed by Georg E Müller and Alfons Pilzecker in 1900. In a series of studies with human subjects conducted between 1892 and 1900 at the University of Göttingen, Germany, Müller and Pilzecker showed that the retention of recently learned information was disrupted by the presentation of novel information shortly after the original learning. The consolidation hypothesis offered an explanation for Théodule Ribot’s observation (1890) that with brain injury or disease, more recent memories are lost, whereas remote memories are preserved.

Although the consolidation hypothesis provided a conceptual framework for explaining retrograde amnesia seen after head injury, as noted by William McDougall as early as 1901, about half a century passed before it became a major influence on experimental studies of learning and memory. In 1946, Russell and Nathan published a study summarizing a large number of cases of retrograde amnesia induced by head injury. The patients could not recall events occurring minutes, hours, or days prior to the injury. According to the consolidation hypothesis, recent memories are selectively lost after head injury because they were not consolidated prior to the injury, and were thus not stored.

In 1949, Donald O Hebb published his seminal book, *The Organization of Behaviour*, which provided a foundation for the contemporary concept of synaptic plasticity as

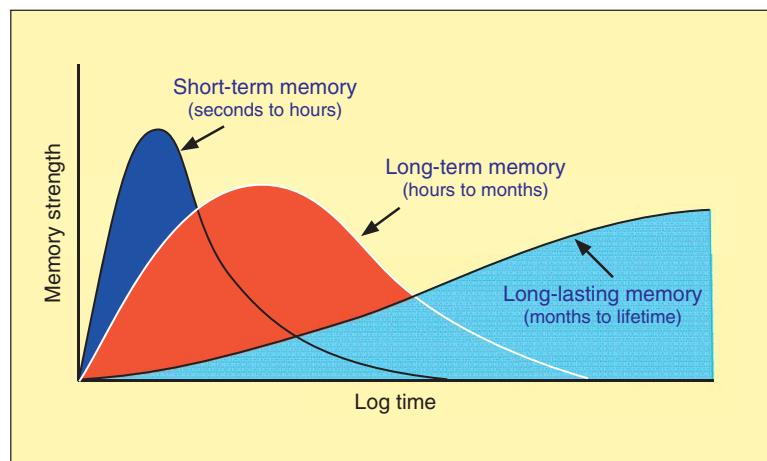


Figure 1 Stages of memory formation. Evidence indicates that short-term and different phases of long-term memory are processed as temporally independent parallel stages. Reproduced from McGaugh JL (2000) Memory: A century of consolidation. *Science* 287: 248–251.

the cellular basis of memory formation. Hebb's dual-trace hypothesis of memory proposed that an initial short-term trace based on neural reverberation induces neural alterations (based on the now famous 'Hebbian synapse', a concept which proposed a process of associative learning at the synaptic level), resulting in the storage of long-term memory. Although Hebb did not discuss the consolidation hypothesis or cite Müller and Pilzecker, his proposal that reverberating neural activity leads to the stabilization of neural circuits is clearly a more explicit version of the perseveration–consolidation hypothesis. The consolidation hypothesis as proposed by Müller and Pilzecker and modified by Hebb and others has, for over a century, guided neurobiological research on memory formation and is now supported by extensive evidence (Figure 1).

Experimental Manipulation of Memory Consolidation

The first evidence of experimentally induced retrograde amnesia was provided by the independent studies of both Duncan and Gerard in 1949, showing that the amount of memory impairment induced by electroconvulsive shock (ECS) after training varied inversely with the interval between training and ECS administration. In the 1960s, a number of findings were instrumental in increasing our understanding of the neural mechanisms involved in experimental retrograde amnesia. The demonstration that electrical stimulation of the amygdala after training induced retrograde memory loss stimulated the investigation of brain systems mediating consolidation. Studies showing that the administration of protein synthesis inhibitors after training blocks memory consolidation in animals provided a stepping stone for the investigation

of molecular mechanisms mediating memory formation. Importantly, the finding that protein synthesis inhibitors impaired long-term memory without blocking short-term memory provided strong support for the view that recent and remote memories are based on different neural processes. The application of protein synthesis inhibitors continues to this day as a widely used tool for the investigation of the molecular basis of memory, despite increasing evidence that protein synthesis inhibitors can induce nonspecific effects, such as stimulation of neurotransmitter release, which might confound the interpretation of the findings. Finally, the finding by McGaugh that memory retention in rats is enhanced by the posttraining administration of stimulant drugs showed that memory consolidation can be experimentally enhanced as well as impaired, strongly indicating that memory consolidation is sensitive to different types of posttraining manipulations. Together, these early studies provided the basic framework upon which subsequent experimental work on memory consolidation has relied up to the present day.

The introduction of posttraining manipulations contributed a powerful approach for the experimental investigation of memory consolidation. When an animal is given a treatment (e.g., a brain lesion or an injection of an inhibitory or stimulant drug) before being trained in a memory task, or prior to memory retention testing, any resulting alteration in behavioral performance might be due to the treatment's effects on sensory and motor functioning in place of or in addition to effects on learning and memory. Such treatments thus confound the interpretation of the findings, making it difficult to assess the treatment-induced influence on mechanisms mediating memory formation and expression. On the other hand, the use of posttraining injections of drugs that transiently

affect neurobiological substrates of memory consolidation without producing permanent damage or long-term functional impairment enables the investigation of memory consolidation without affecting behavior during either the training or retention testing.

Stages of Memory Consolidation

Most experimental research on memory consolidation has focused on a time window of several hours after learning. However, evidence that memory consolidation may continue for weeks, months, and perhaps even years in humans, suggests that there are different stages of memory consolidation. The early stage is very likely to be that suggested by Müller and Pilzecker, and Hebb. Evidence that different stages of consolidation rely on different cellular mechanisms and brain systems has been provided by human and animal studies showing that lesions of the hippocampus generally impair memory of recently learned information (i.e., within days or weeks prior to the lesion), whereas the ability to recall older memories is preserved. Thus, although the hippocampus and related structures are crucially involved in mediating the consolidation of several types of memory, other brain areas appear to play a more prominent role as loci of consolidation and storage at later stages.

The time-limited role of the hippocampus for the storage of some types of memory has led to the widely accepted view of systems consolidation, in which neural alterations associated with memory consolidation and storage occur first in the hippocampus followed by the gradual consolidation of a more distributed memory trace in neocortical areas. This view is supported by findings from animal studies where the effects of pharmacological manipulations of different brain areas on memory consolidation depend on the time interval between training and intervention. Thus, memory consolidation and storage would involve the sequential activity of the hippocampus followed by cortical areas such as the entorhinal and posterior parietal cortices. More recently, evidence suggesting that the memory-related engagement of cellular mechanisms involved in synaptic plasticity occurs in the hippocampus and cortical areas with a similar time course has indicated the need for a more complex model in which long-term consolidation in humans depends on a complex and integrated interplay between the hippocampus and cortical areas rather than on a simple sequential activation of brain structures.

Multiple Memory Systems

An important aspect of memory formation is the evidence that different types of memory are mediated by relatively

independent brain systems. Considerable evidence indicates that the early consolidation of cognitive or declarative memories relies mostly upon the medial temporal lobe (e.g., hippocampus), whereas the formation of procedural and habit memories depends on the basal ganglia (e.g., caudate putamen and striatum). Converging evidence from animal and human studies suggests that multiple memory systems can be activated simultaneously and in parallel by learning, and interact and compete with each other in influencing the behavioral responses after learning. For instance, a rat trained to find a reward (such as food) in the right arm of a maze might simultaneously learn two types of memory: where to go to find food (a memory based on spatial location) and what behavioral response to make (i.e., turn right to get into the arm where the food is). Experimental evidence using different amounts of training and functional inactivation of the hippocampus and caudate nucleus prior to testing indicates that formation of the place memory relies primarily on the hippocampus and the response memory involves the caudate nucleus. In addition, evidence indicates that both hippocampus- and basal-ganglia-based types of memory receive neuromodulatory influences from the amygdala.

Molecular Basis of Memory Consolidation

In addition to the investigations of the neural basis of memory consolidation at the systems level, extensive research has investigated the challenging issue of understanding the cellular modifications triggered by learning that enable the long-term storage of memories. Early researchers, including Ramon y Cajal and Hebb, have hypothesized that the storage of information relies on activity-dependent modifications of synaptic efficiency in neurons stimulated by learning. The concept of synaptic plasticity, that is, neural stimulation can induce changes in synaptic efficiency that permit the strengthening of associations between neurons, has become the guiding principle of research investigating the basic cellular mechanisms mediating memory consolidation.

The seminal demonstration by Bliss and Lomo in 1973 that high-frequency stimulation of the rabbit hippocampal perforant path produced a long-lasting increase in synaptic response (a phenomenon called long-term potentiation, LTP) provided the first consistent experimental evidence that a mechanism based on synaptic plasticity might be the key molecular process mediating memory formation. Subsequent work provided insight into the biochemical machinery underlying LTP. A major finding was that hippocampal LTP induction is blocked by the antagonism of the *N*-methyl-D-aspartate (NMDA) type of glutamate receptor channel. The observation by Morris and colleagues that NMDA receptor

blockade could also block spatial learning in rats marked the beginning of a long series of studies using pharmacological and genetic manipulations of specific brain areas, which together indicated that many of the basic molecular mechanisms underlying LTP induction and maintenance are also required for learning and memory consolidation. In addition to rodent models, the investigation of the molecular basis of memory has greatly benefited from work with other model organisms such as *Drosophila*, and notably the work of Eric Kandel with the sea snail *Aplysia*.

Currently, the most widely accepted view of the molecular basis of long-lasting declarative memory formation proposes that the synapses activated by learning in the hippocampus and other areas undergo long-lasting facilitation triggered by the activation of NMDA receptors and a vast repertoire of other neuronal receptors, such as α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and metabotropic (mGluR) glutamate receptors, as well as protein kinase signaling pathways, leading to *de novo* protein synthesis, alterations in gene expression, structural reinforcement of activated synapses, and the growth of new synaptic connections. LTP, or a cellular process similar to LTP, appears to be involved in mediating at least some components of some types of memory. Extensive genetic and pharmacological evidence indicates that early molecular events downstream of excitatory receptor activation that are likely to mediate both synaptic plasticity and memory consolidation include activation of calcium-calmodulin-dependent protein kinase II (CaMKII), the phospholipase C (PLC)/protein kinase C (PKC), the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA), the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated protein kinase (ERK), and the phosphatidylinositol 3-kinase (PI3K) signaling pathways. Late-phase LTP and memory consolidation depend on PKA-induced activation of the cAMP response element-binding protein (CREB) transcription factor, *de novo* protein synthesis, and expression of specific immediate-early genes, such as c-fos, Arc, and zif268, as well as the synthesis of neurotrophins such as brain-derived neurotrophic factor (BDNF). More recently, studies have focused on epigenetic mechanisms regulating gene expression, such as processes involving chromatin remodeling, histone modification, and DNA methylation, which have been shown to play an important role in the consolidation of long-term memory. This complex sequence of biochemical events is proposed to produce stable changes in synaptic strength. It is worth noting that the widely accepted view of long-term memory consolidation as an LTP-like cascade of biochemical events leading to increased efficacy of specific synapses has been challenged by several findings (e.g., those of the nonspecific and complex effects of protein synthesis inhibitors and lack of effect of NMDA receptor antagonists under various learning circumstances), as well as by other hypotheses such as

those based on posttranslational modification and impermanent redundant neural circuits.

Short- and Long-Term Memory

As noted above, at least two components of memory can be discerned based on both their duration and cellular processes: short-term memory, which endures for a few hours, and long-term memory, which can last for days and even years. Although the prevailing view for many years has been that the early and late stages of memory are serially linked, current evidence suggests that short- and long-term memory are based on parallel and independent processes (Figure 1). Studies of memory and synaptic plasticity in different species and experimental models (e.g., short- and long-term facilitation in *Aplysia*) indicate that treatments can block short-term plasticity without affecting long-term plasticity. In rats, the evidence that some drugs infused in brain areas, including the hippocampus and entorhinal cortex (EC), after training block short-term memory but not long-term memory is critical as it indicates that short- and long-term memory can be dissociated into independent processes.

Regulating Memory Strength: Endogenous Modulation of Consolidation

The findings that long-term memories consolidate slowly in humans as well as in a wide variety of animal species, and that memory formation can be enhanced by post-training administration of a number of chemical agents, raise the question of why our brains are designed in such a way as to enable facilitatory influences on memory formation by chemical mediators within a relatively wide time window after learning. Extensive evidence indicates that hormones normally released by novel, stressful, and arousing experiences (Figure 2), are among the chemical agents capable of influencing consolidation. These hormones include epinephrine, corticosterone adrenocorticotropic hormone, corticotrophin-releasing hormone, corticosterone, substance P, cholecystokinin, vasopressin, and β -endorphin. Such evidence strongly suggests that the slow memory consolidation process enables hormonal responses and other neuromodulatory systems activated by the learned experience to regulate the strength of memory formation and storage after learning. This mechanism of the endogenous modulation of consolidation may thus serve as an adaptive function by which emotionally arousing experiences (e.g., events perceived as potential danger and threats to one's survival) are generally better remembered.

Among endogenous hormonal systems modulating memory consolidation, stress hormones released by the

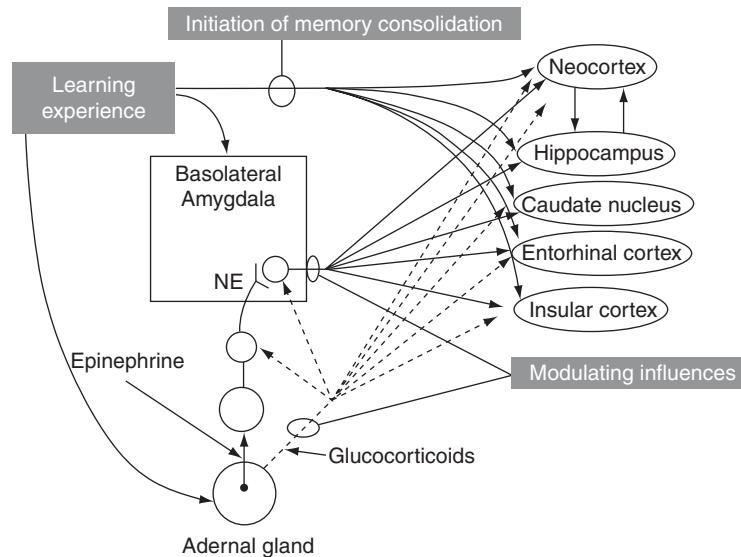


Figure 2 Neurobiological systems regulating memory consolidation. Shown in the figure is the release of peripheral stress hormones after learning activates the noradrenergic system in the basolateral amygdala, which in turn influences neural plasticity in other brain regions including the hippocampus, entorhinal cortex, insular cortex, and neocortical areas. Adapted from McGaugh JL (2000) Memory: A century of consolidation. *Science* 287: 248–251.

adrenal medulla (epinephrine) and adrenal cortex (corticosterone in the rat and cortisol in humans) have been studied most extensively. Both epinephrine and corticosterone produce dose- and time-dependent enhancement of memory consolidation. Epinephrine does not freely pass the blood-brain barrier and is likely to act on consolidation by activating peripheral β -adrenergic receptors on vagal afferents projecting to the nucleus of the solitary tract (NST) in the brainstem. Noradrenergic projections from the NST in turn influence other brain regions, including the basolateral nucleus of the amygdala (BLA; **Figure 2**). Glucocorticoids such as corticosterone readily enter the brain and activate neuronal glucocorticoid receptors.

Extensive evidence indicates that the activation of β -adrenergic receptors within the BLA is critical for mediating the modulatory influences of both epinephrine and glucocorticoids on consolidation. For instance, both lesions of the BLA and intra-BLA infusion of β -adrenergic receptor antagonists block the enhancing effect of posttraining systemic administration of the synthetic glucocorticoid dexamethasone on memory retention in rats. Conversely, infusions of β -adrenergic agonists into the BLA enhance memory consolidation. In addition, the findings that either footshock stimulation or administration of memory-enhancing drugs produces an increase in norepinephrine (NE) release in the BLA, whereas memory-impairing drugs decrease NE release, supports the view that activation of the noradrenergic system in the BLA is an endogenous system crucial in regulating memory consolidation.

In addition to preventing the effects of systemic injections of NE and glucocorticoids on consolidation, BLA lesions can block the effects of other drugs on consolidation, including memory-impairing drugs such as diazepam and the general anesthetic propofol. Also, in addition to β -adrenergic receptors, a variety of neuronal receptors and cell signaling pathways within the BLA are involved in mediating the modulatory influences of the BLA on consolidation. Thus, memory consolidation can be modulated by the pharmacological manipulation of neuronal receptors, including α -adrenergic, dopaminergic, serotonergic, opioid, cholinergic muscarinic, γ -aminobutyric acid (GABA)ergic, bombesin, and glutamate metabotropic and ionotropic receptors, as well as by agents acting directly on protein kinase signaling cascades downstream of receptor activation, such as the cAMP/PKA and MAPK/ERK pathways.

Although most of the research investigating the neural mechanisms involved in regulating consolidation has been carried out in animal models, human studies have supported the view that stress hormones and amygdala activation influence memory. In studies examining the effects of β -adrenergic receptor antagonists or a placebo on the memory of pictures accompanied by an emotionally arousing story, human subjects given a placebo showed better retention of the pictures presented during the most emotionally arousing part of the story, whereas the enhancing effect of emotional arousal was prevented in subjects given a β -adrenergic receptor antagonist. In addition, emotional arousal fails to enhance memory of the arousing material in human subjects with selective

lesions of the amygdala. Imaging studies using positron emission tomography (PET) and magnetic resonance imaging (MRI) scans have found that long-term memory retention of emotionally arousing stimuli is highly correlated with the degree of amygdala activation during learning.

Regarding the glucocorticoid modulation of memory consolidation in humans, most studies show impaired cognitive performance after glucocorticoid exposure. These findings are probably related to the pronounced impairing effects of glucocorticoids on memory retrieval (as opposed to their enhancing effect on consolidation), as well as to the long-term detrimental effects of glucocorticoids on hippocampal function. However, some studies have shown a memory-enhancing effect of glucocorticoids in human subjects. For instance, cortisol has been shown to enhance memory of emotionally arousing pictures in human subjects, and dexamethasone enhanced declarative memory in patients with major depression. The important regulatory action of glucocorticoids on memory consolidation and retrieval has led to the investigation of glucocorticoid receptor agonists and antagonists as potential therapeutic agents for the treatment of cognitive deficits associated with neuropsychiatric disorders as well as for the treatment of disorders associated with traumatic memories such as posttraumatic stress disorder (PTSD).

Interaction of Brain Systems in Modulating Memory Consolidation

The evidence reviewed above indicates that the BLA is a critical site in mediating and integrating neuromodulatory influences on memory consolidation. The BLA may be a locus for learning-induced synaptic plasticity leading to long-term storage of emotionally motivated memory within the BLA. However, extensive evidence indicates that the primary role of the BLA is to regulate memory storage in other brain areas activated by it after learning. The BLA projects to many brain regions, including the hippocampus, basal forebrain, nucleus accumbens (NAcc), striatum, and various cortical regions.

A major pathway connecting the BLA to other brain areas (including the NAcc and dorsal striatum) is the stria terminalis (ST), and the BLA-ST pathway provides a major efferent projection enabling BLA influences on memory consolidation in other brain regions. Lesions of the ST block the effect of electrical stimulation of the amygdala on memory for aversively motivated training without affecting acquisition. ST lesions also prevent the memory-enhancing effects of posttraining intra-amygdala infusions of NE; systemic injections of epinephrine and drugs affecting the glucocorticoid, opioid, and cholinergic systems; and infusions of the muscarinic cholinergic

agonist oxotremorine directly into the striatum. As found with ST lesions, NAcc lesions block the memory-enhancing effect of posttraining systemic administration of the synthetic glucocorticoid dexamethasone, suggesting that BLA projections to the NAcc via the ST are crucial for mediating BLA influences on memory consolidation. As the dorsal hippocampus has a high density of glucocorticoid receptors, the hippocampus is a likely locus of the glucocorticoid modulation of memory consolidation. In fact, BLA or NAcc lesions block the enhancing effect of infusion of a glucocorticoid receptor agonist directly into the hippocampus after training. As with lesions, the infusion of a β -adrenergic receptor antagonist into the BLA prevents the memory-enhancing effect of intra-hippocampal infusion of a glucocorticoid receptor agonist.

In addition, several electrophysiological studies indicate that the amygdala is crucial in modulating hippocampal LTP. For instance, either lesions or functional inactivation of the amygdala can block the impairing effects of stress on hippocampal LTP and spatial memory, and lesions of the BLA, but not central amygdala, attenuate LTP induction at perforant path-dentate gyrus synapses. Conversely, BLA stimulation facilitates dentate gyrus LTP. Both NE and corticosterone are involved in the influence of BLA activity on hippocampal LTP.

Several cortical areas are also major loci of memory consolidation. The rhinal cortices (perirhinal and ECs) constitute an interface between the hippocampus and the neocortex. Extensive evidence indicates that the EC plays a major role in memory consolidation in a way that involves interplay with the hippocampus and cortical areas such as the posterior parietal cortex. The EC receives direct projections from the BLA, and the firing of BLA neurons produces neuronal activation in the EC. The finding that excitotoxic lesions of the BLA prevent memory enhancement induced by posttraining infusions of the cAMP analog 8-Br-cAMP (an agent that stimulates the cAMP/PKA signaling pathway) into the EC indicates that the BLA is crucial in mediating the modulatory influences of the EC on consolidation. Importantly, a recent study has shown that BLA activity after associative learning increases transmission from perirhinal to entorhinal neurons and indicated that BLA-mediated facilitation of rhinal interactions may be a major mechanism for the formation of emotional memory.

Other cortical areas have been shown to depend on BLA activity to influence memory consolidation. Infusions of a β -adrenergic receptor antagonist into the BLA prevented the memory-enhancing effect of infusions of 8-Br-cAMP into the insular cortex, and BLA lesions blocked the enhancing effects of infusions of oxotremorine into the rostral anterior cingulate cortex. The nucleus basalis magnocellularis (NBM) provides cholinergic projections to the cortex. The finding that functional

inactivation of the NBM impairs aversive learning suggests that ACh-mediated activation of the cortex is crucial for emotional memory. The release of ACh in the auditory cortex is essential for the consolidation of plasticity-related changes in cortical function after Pavlovian conditioning. The BLA is a major source of afferents to the NBM, and evidence indicates that the BLA-ST pathway modulates cortical activity via projections to the NBM. Importantly, the enhancement of consolidation induced by infusions of NE into the BLA was prevented in rats with selective lesions of the NBM induced by the p57 nerve growth factor 192 IgC-saporin. These and other lines of evidence strongly suggest that BLA-cortical cholinergic projections mediated by the NBM are crucial in enabling the modulatory influence of the amygdala noradrenergic system on consolidation.

Together, the evidence reviewed above indicates that the BLA modulates memory consolidation by regulating neuronal activation and synaptic plasticity in brain areas involved in long-lasting consolidation and storage, such as the hippocampus and several cortical areas (**Figure 2**).

Consolidation and Reconsolidation

The traditional consolidation theory has been challenged in recent years by evidence that reactivation of a previously consolidated memory during retrieval might again render this memory susceptible to disruption by amnesic treatments, a process generally referred to as reconsolidation. Although the concept of memory reconsolidation was initially proposed in the 1960s, it was only in 2000 that a new wave of studies showing that administration of drugs to animals after retrieval could impair memory assessed at subsequent retention tests sparked considerable interest in reconsolidation. Although reconsolidation has been defined by experiments using intracerebral infusions of protein synthesis inhibitors, several studies have extended the candidate mechanisms involved in reconsolidation-like processes to a number of neuronal receptors and signal transduction pathways. Thus, experiments using systemic or intra-cerebral injections in rodents have shown that memory for emotionally motivated tasks can be impaired by postretrieval administration of a variety of pharmacological agents, including benzodiazepines, antagonists of glutamatergic, noradrenergic, and glucocorticoid receptors, and inhibitors of protein kinases such as PKA and ERK/MAPK. These studies argue in favor of the so-called ‘reconsolidation hypothesis’ based on findings of amnesia produced by postretrieval interventions. As with consolidation and extinction, brain areas mediating the effects of postretrieval amnesic treatments include the dorsal hippocampus and basolateral amygdala.

Other studies, however, have provided evidence against reconsolidation, showing negative results (i.e., lack of the effect of postretrieval treatments) or opposite effects related to memory extinction. Another major caveat for the reconsolidation hypothesis is that many of the findings interpreted as possible reconsolidation impairments have been shown to be transient or reversible. The recovery of the deficits induced by postretrieval interventions can be either spontaneous or triggered by reminders. This has led to the interpretation of many findings as temporary retrieval deficits rather than reconsolidation blockades. Other studies, however, have provided evidence for reconsolidation by showing long-lasting memory impairment after postretrieval administration of amnesic treatments, with no detectable recovery. Evidence has been also provided suggesting that, as with consolidation, memories can undergo reconsolidation at both the cellular and systems level.

Taken together, the evidence suggests that the occurrence of reconsolidation-like processes in animals in an experimental setting depends on several factors including duration of exposure to the original context, absence of significant extinction, time interval between learning and retrieval, and encoding of new information at the time of retrieval. At this point, it seems clear that under specific conditions, either temporary or long-lasting labialization of recently learned memory traces can occur. It may be possible to reconcile the apparent discrepant findings regarding the reconsolidation phenomenon by viewing consolidation and reconsolidation as different but integrated components of the long-term processes underlying memory storage.

See also: Active Avoidance and Escape Learning; Aging and Cognition; Amnesia; Analysis of Learning in Invertebrates; Animal Models of Learning and Memory; Blocking, Neural Basis of; Brain Aging: Structural Imaging Biomarkers of Risk of Alzheimer’s Disease; Cerebellum: Associative Learning; Cholinergic Systems in Aging and Alzheimer’s Disease: Neurotrophic Molecular Analysis; Cognition: Learning and Memory: Pavlovian; Cognition: Learning and Memory: Spatial; Cognitive Decline in Laboratory Animals: Models, Measures, and Validity; Declarative Memory; Emotion–Cognition Interactions; Episodic and Autobiographical Memory: Psychological and Neural Aspects; Fear Conditioning; Habituation; Hormones and Memory; Implicit Learning and Memory: Psychological and Neural Aspects; Knock-Outs: Learning and Memory; Learning and Memory: Computational Models; Memory and Aging, Neural Basis of; Neural Basis of Classical Conditioning; Neural Basis of Recognition Memory in Nonhuman Primates; Neural Basis of Working Memory; Neural Substrates of Conditioned Fear and Anxiety; Neurogenesis and Memory; Neuron Excitability

and Learning; Neurotransmitters and Neuromodulators Regulating Sleep and Wakefulness; Protein Synthesis and Memory; Role of Gene Transcription in Long-Term Memory Storage; Short-Term Memory: Psychological and Neural Aspects; Sleep: Learning and Memory; Stress and Energy Homeostasis; Synapse Formation and Memory; Synaptic Mechanisms for Encoding Memory; Temporal Lobe and Object Recognition; Transgenic Technologies and Their Application to the Study of Senile Dementia; Transient Global Amnesia: Neuropsychology, Psychopathology, and Neuroimaging.

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Mirror Neuron Mechanism

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Glossary

Mirror neurons – A distinct class of neurons found in the premotor cortex and inferior parietal lobule of a monkey. These neurons are discharged when the monkey executes a given motor act or observes the same motor act performed by another individual.

Motor act – Unlike the term movement that describes a displacement of body parts, motor act indicates movement with a goal.

Motor cortex – Defined as that part of the frontal lobe that lacks the layer containing granular cells. Its electrical stimulation, at low threshold, elicits discrete movements of contralateral body parts.

Voluntary movement – Manifestations of a centrally generated intention to act. The way in which intention is generated is irrelevant for the notion of voluntary movement. It may result from bodily needs such as hunger or thirst, or from higher-order deliberations based on one's own beliefs and desires. What counts for voluntary movements is that the individual has a goal and that this goal determines movements leading to its achievement. Unlike for reflexes, in voluntary behavior stimuli do not determine a motor response, they only set the occasion for it. According to their needs, animals may or may not respond to the same stimulus.

mirror mechanism has been demonstrated in humans using noninvasive techniques. Here we discuss the main functions in which mirror mechanism is involved.

Action and Intention Understanding

When we see a person acting upon an object, we are able, typically, to extract two main information: what this person is doing and why. If, for example, Mary' hand enters in contact with a cup of coffee, John immediately knows whether she is grasping it or not, and, according to how she grasped it, he can also understand why she is doing it (e.g., for drinking coffee). How can John understand the goal of the motor act and the intention behind it? A possibility is that he is using an inferential reasoning elaborating, through some cognitive mechanism, the acquired visual information. The alternative possibility is that, in most cases of everyday life, this is not necessary and these cognitive operations are performed by a specific mechanism that directly transforms visual information into a motor format. The discovery of mirror neurons supports the existence of this second mechanism.

The Mirror Mechanism: Monkey Data

Figure 1 shows a lateral view of the monkey motor cortex. The areas where mirror neurons are located are areas PFG, AIP, and F5. This figure (upper part) illustrates as well the defining characteristic of these neurons. They discharge both when the monkey performs a motor act and when it observes another individual (a human being or another monkey) doing a similar motor act. The degree of similarity between the effective executed and observed motor acts varies from one neuron to another. In general, however, the identity of goals is sufficient. Mirror neurons do not discharge in response to the presentation of food or other interesting objects.

Convincing demonstration that mirror neurons play a role in the capacity to understand other's motor act is based on experiments in which monkeys could not see what an agent was doing, but had clues to understand it. In one of these experiments, the monkey heard the sounds of a motor act (such as ripping a piece of paper) without seeing it; in another series, the monkeys knew that behind

Introduction

One of the most exciting events in neurosciences over the past years has been the discovery of a mechanism that directly ties action and action perception. This mechanism – the mirror mechanism – is of great interest because it enables to give a neurophysiological explanation to a large number of cognitive abilities ranging from action to intention understanding, and from imitation to some aspects of social cognition.

The mirror mechanism has been originally found in monkeys by using single-neuron recordings. These studies showed that there are neurons (i.e., mirror neurons), in the premotor cortex and in the inferior parietal lobule, that discharge both when the monkey executes a specific motor act (e.g., grasping) and when it observes another individual doing a similar motor act. Subsequently, the

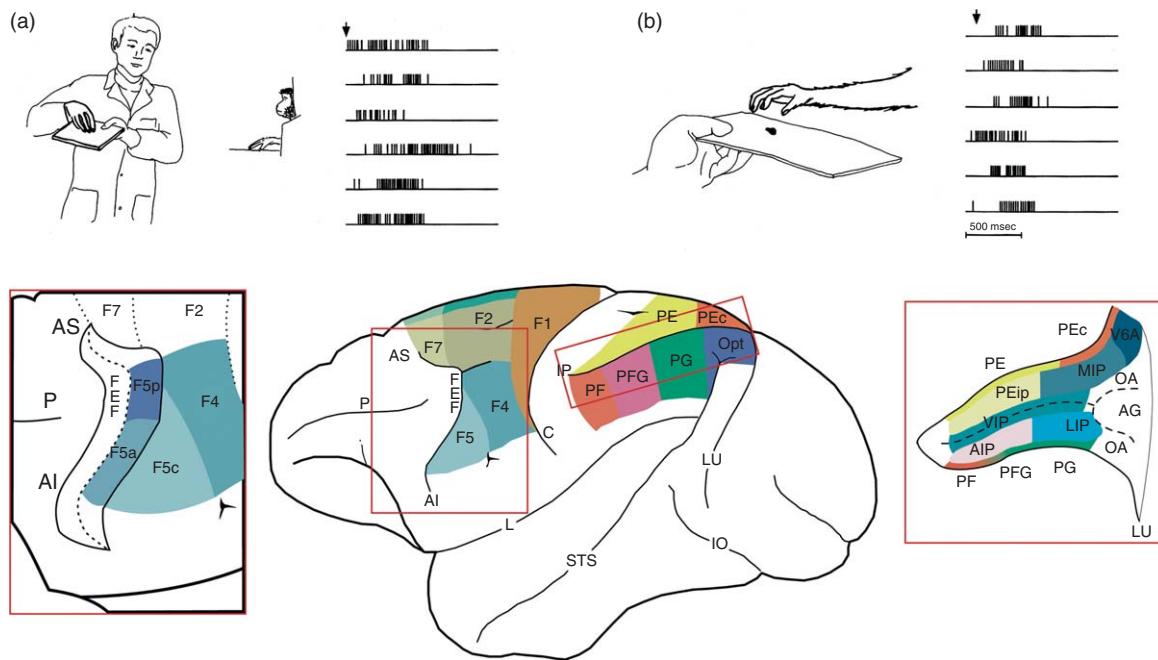


Figure 1 Example of a mirror neuron and anatomy of agranular frontal and posterior parietal cortex of the macaque monkey. Upper part: F5 mirror neuron. The neuron discharges when the monkey grasps an object (a) and when it observes another individual grasping it (b). Lower part: The central part of the figure shows the cytoarchitectonic parcellation of the agranular frontal cortex (areas indicated with F and Arabic numbers) and of the parietal lobe (areas indicated with P and progressive letters). The enlargement of the frontal region (rectangle on the left) shows the parcellation of area F5. The rectangle on the right shows the areas buried within the intraparietal sulcus. AI, inferior arcuate sulcus; AIP, anterior intraparietal area; AS, superior arcuate sulcus; C, central sulcus; CA, calcarine fissure; CG, cingulate cortex; FEF, frontal eye field; IP, intraparietal sulcus; L, lateral sulcus; LIP, lateral intraparietal area; LU, lunate sulcus; MIP, medial intraparietal area; POs, parieto-occipital sulcus; P, principal sulcus; STS, superior temporal sulcus.

a screen there was an object, but could see the hand/object interaction that represents the typical trigger feature for most mirror neurons. The monkey saw the hand of the experimenter disappearing behind the screen and could only image mentally the action goal. The results showed that in both conditions the mirror neurons fired providing that the set of external contingencies allowed the monkey to understand the action done by another individual. In other words, what counted was the meaning of the motor act seen, heard, or even imagined.

Some recent experiments on parietal cortex revealed a new aspect of mirror neuron functions. Single neurons were recorded from ventral intraparietal area (VIP) and from area PFG. Peripersonal neurons were selected. These neurons respond to tactile stimuli on specific monkey body part and to visual stimuli moved near the tactile receptive fields (peripersonal space). There were no visual responses to stimuli presented far from the monkey. However, when the experimenter stood in front of the monkey, neurons began to respond to visual stimuli presented in the experimenter's peripersonal space in a spatial position congruent to that of the monkey's visual field (**Figure 2**). These results indicate that, besides the action of others, mirror neurons also provide information on peripersonal space and body of others.

Originally, mirror neuron responses were described as essentially independent of the distance between the monkey and the location of the motor act. The distance issue has been recently reexamined more formally in a larger sample of neurons. The results showed that 50% of mirror neurons responded with the strength regardless of the position of the stimulus. For the remaining 50% of neurons, half of them were selective for stimuli presented within the monkey's peripersonal space, while the other half discharged only for stimuli presented far from the monkey. These findings show that mirror neurons are not only important for understanding what the others are doing, but also prime the motor behavior most adequate for stimulus location.

The Mirror Mechanism in Humans

The mirror mechanism is present in humans as well. Majority of the brain imaging studies showed that, in healthy volunteers, observation of other's actions determines the activation of the ventral premotor cortex, the posterior part of the inferior frontal gyrus (a sector of Broca's area), besides the visual areas, and of the inferior parietal lobule that is of the same cortical regions where mirror neurons are located in the monkey. Further fMRI experiments showed

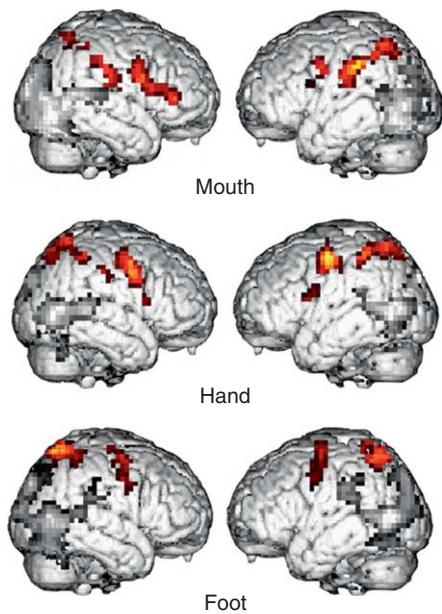


Figure 2 Somatotopy of premotor and parietal cortices as revealed by action observation. Upper panel: Cortical areas activated during the observation of object-related mouth actions (biting an apple). Middle panel: Cortical areas activated during the observation of object-related hand actions (grasping a cup or a ball). Lower panel: Cortical areas activated during the observation of object-related foot actions (kicking a ball or pushing a brake). Projections of the activations foci on the lateral surface of a standard brain (Montreal Neurological Institute (MNI)). Reproduced with permission from Buccino G, Fink GR, et al. (2001) Action observation activates premotor and parietal areas in a somatotopic manner: An fMRI study. European Journal of Neuroscience 13: 400–404.

that the mirror network is somatotopically organized. The somatotopic organization is present both in the premotor cortex and in the parietal lobe (**Figure 3**).

Evidence proving the existence of the mirror mechanism in humans comes from transcranial magnetic stimulation studies. Observation of other's actions increases the excitability of the motor cortex of the observer. Most interestingly, the facilitation concerns exactly those muscles involved in movement execution. Finally, magnetoencephalography (MEG) and electroencephalography (EEG) studies showed that mere observation of others' actions determines the desynchronization of cortical motor rhythms, similar, although of less intensity of that observed during active movement.

In recent years, a possibility was raised that the activation of the motor areas during action observation and action execution could result not from the activations of mirror neurons, but from the activation of two different neural populations located in the same areas: one active during action observation, the other during action execution. Recently, a functional magnetic resonance imaging (fMRI) study tested this hypothesis using the adaptation technique. The results showed that the responses in the right inferior parietal lobe (IPL) were attenuated when

the participants observed a recently executed action relative to one that had not been previously performed. This adaptation across action and perception convincingly demonstrates that the IPL responds selectively to the motor and perceptual representations of actions.

Intention Understanding in Monkey

The problem of intentionality has been traditionally considered a philosophical problem. However, recent neurophysiological experiments appear to provide a neural basis for motor intention. This is true both for the intention of the person who is acting and for understanding the intentions of others.

To approach the problem of the neural substrate of intention, it was investigated whether the discharge of neurons coding motor acts of a given action reflect the intention of that action. Monkeys were trained to grasp objects for two different goals: to place them into a container or to bring them to their mouth. The initial motor acts, reaching and grasping, were identical in the two conditions, whereas the goals of the two actions were different. After training, grasping neurons were recorded from IPL and their discharge studied in the two conditions mentioned above. The results showed that the majority of IPL grasping neurons discharged with a different intensity according to the final goal of the action in which grasping was embedded (action-constrained neurons). Grasping in order to bring food to the mouth was the most represented motor act. Examples of action-constrained grasping are shown in **Figure 4**.

A very interesting finding of these studies was that many action-constrained neurons have mirror properties as well, discharging in response to the observation of motor acts done by others. To find out whether the visual responses of these neurons were influenced by the actions in which the motor acts were embedded, action-constrained mirror neurons were tested in the same two conditions used for studying their motor properties. Monkeys, instead of grasping objects, observed the experimenter performing the two actions. The results showed that the majority of IPL mirror neurons were differently activated when the observed motor act belonged to one action or another. Examples are shown in **Figure 4**.

What could explain this behavior? If one examines the motor behavior of action-constrained grasping neurons, it is frequently found that the neuron's discharge continues if grasping is performed within the appropriate action, while it stops abruptly in the inappropriate action. This prolonged discharge suggests activation of neurons coding the next step of the executed action. In agreement with this interpretation are data on the receptive field properties of parietal neurons showing that motor acts performed actively, or even passive movements, activate neurons that code the next motor act in

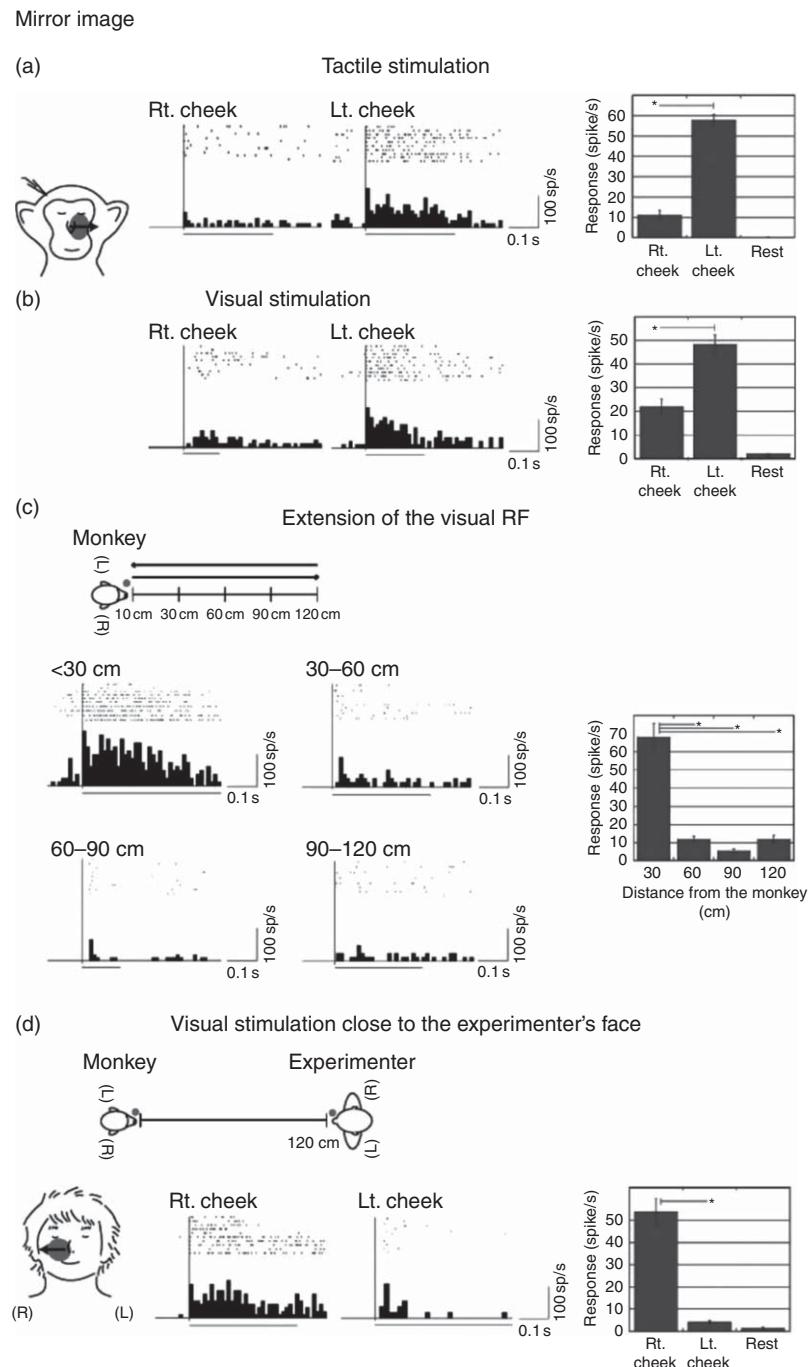


Figure 3 Example of a mirror peripersonal matching neuron. (a) and (b) Responses to tactile and visual stimulation. Receptive field (RF) on the left cheek; visual RF located close to the same part of the face. Rasters and responses histograms of neuron activity are aligned on stimulus movement onset (long vertical line). (c) Visual response in the peripersonal space in the monkey. Note that the responses are present only when the stimulus is near the left cheek (<30 cm). (d) Visual responses evoked by moving stimuli close to the experimenter's right cheek. Reproduced with permission from Ishida H, Nakajima K, Inase M, and Murata A (2009) Shared mapping of own and other's bodies in visuo-tactile bimodal area of the monkey parietal cortex. Journal of Cognitive Neuroscience (doi:10.1162/jocn.2009.21185).

an action sequence. Thus, it is very likely that when an action-constrained grasping neuron is activated by the observation of a grasping motor act, it triggers the whole motor chain in the observer, who, in this way, has an internal representation of the action that the agent intends to do. Thanks to this mechanism, the observer may understand the agent's intention.

Intention Understanding in Humans

In humans as well, there is evidence from an fMRI study that the mirror mechanism is involved in understanding the intention behind the observed motor acts. This study had three conditions. In the first condition

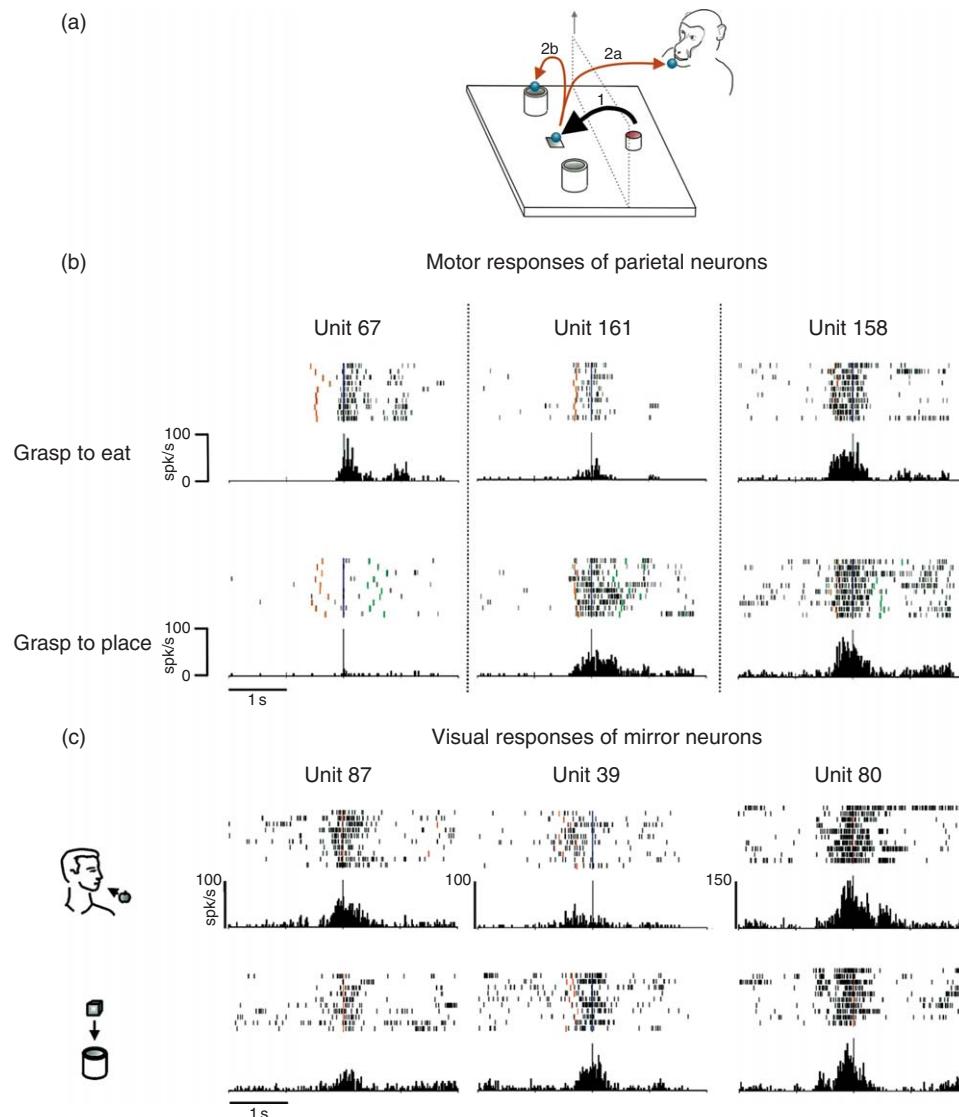


Figure 4 Motor and visual responses of mirror neurons in IPL. (a) The apparatus and the paradigm used for the motor task. (b) Motor responses of three IPL neurons during grasping in conditions grasp to eat and grasp to place. Rasters and histograms are synchronized with the moment when the monkey touched the object to be grasped. Red bars, monkey releases the hand from the starting position; green bars, monkey touches the container; x axis, time, bin 0 20 ms; y axis, discharge frequency. (c) Visual responses of three IPL mirror neurons during the observation of grasping to eat and grasping to place done by an experimenter. Conventions as in the motor task. Reproduced with permission from Fogassi L, Ferrari PF, Gesierich B, Rozzi S, Chersi F, and Rizzolatti G (2005) Parietal lobe: From action organization to intention understanding. *Science* 308: 662–667.

(context), the volunteers saw some objects (e.g., a teapot, a mug, and a plate with some food on it) arranged as if a person was ready to drink the tea or the same objects arranged as if a person had just finished having his/her breakfast; in the second condition (action), the volunteers were shown a hand that grasped a mug without any context; in the third condition (intention), the volunteers saw the same hand action within the two contexts. The context suggested the intention of the agent, that is, grasping the cup for drinking or grasping it for cleaning the table.

The results showed that in both action and intention conditions there was an activation of the areas endowed with a mirror mechanism. Crucial was the comparison between intention and action conditions. This comparison showed that the understanding of the intention of the doer determined a marked increase in activity of the right frontal node of the mirror network.

These data clearly show that the mirror neuron mechanism play an important role in intention understanding. This does not imply, of course, that other more cognitive ways of reading minds do not exist.

Indeed, recent fMRI studies showed that, in some specific conditions, the understanding of motor acts by others require, besides the mirror mechanism, the activation of areas outside the mirror network. For example, when tasks require a top-down inference either to assess the meaning of a motor act in an implausible context or to judge whether the intention of the observed action was ordinary or unusual, there is an increase of activity in the posterior superior temporal sulcus (STS) region, posterior cingulate cortex, and the medial prefrontal cortex.

Imitation

The data reviewed so far show that mirror system is involved in action and intention understanding. From the very beginning of mirror neuron studies it was clear, however, that, in humans, the motor system, unlike in monkeys, also resonates in response to the observation of intransitive movements, including those without any obvious meaning. What could be the reason of this resonance?

The most likely interpretation relates to the capacity of humans, unlike monkeys, to imitate the actions done by others. Note that the term imitation has several meanings. There are, however, two main senses in which it is most commonly used. One defines imitation as the capacity of an individual to replicate an observed motor act; the other defines imitation as the capacity to acquire, by observation, a new motor behavior and to repeat it using the same movements employed by the agent. In both cases, imitation requires the capacity to transform sensory information into a motor activation.

Evidence that the mirror mechanism is involved in imitation as an immediate replica of the observed motor act was demonstrated by an fMRI study. In this study, volunteers were tested in two main conditions: observation and observation-execution. In the observation condition, participants were shown a moving finger, a cross on a stationary finger, or a cross on empty background. The instruction was to observe the stimuli. In the observation-execution condition, the same stimuli were presented, but, this time, the instruction was to lift the right finger, as fast as possible, in response to them. The crucial contrast was between the trials in which the volunteers made the movement in response to an observed action (imitation) and the trials in which the movement was triggered by the cross (a nonimitative behavior). The results showed that the activation of the mirror system and, in particular, of the posterior part of inferior frontal gyrus (IFG) was stronger during imitation.

Further evidence that the mirror system plays a fundamental role in this type of imitation was provided by repetitive TMS (rTMS), a technique that provokes a transient depression of the stimulated region. In a group

of volunteers, the caudal part of the left frontal gyrus (Broca's area) was stimulated while they (1) pressed keys on a keyboard, (2) pressed the keys in response to a point of red light indicating which key to press, or (3) imitated a key pressing movement done by another individual. The data showed that rTMS lowered the participants' performance during imitation, but not during the other two tasks.

More complex is the mechanism involved in imitations learning. In this case, imitation appears to result from the interaction of two distinct processes: (1) segmentations of the action to be imitated into its individual elements, and their transformation into the corresponding potential movements of the observer; and (2) organization of these potential movements into a temporal and spatial pattern that replicates that shown by the demonstrator. There is evidence that the first step is achieved through the mirror mechanism, while the second step is mostly due to the activity of the prefrontal lobe and, in particular, of area 46 that memorizes and recombines the motor elements in the new pattern.

Empathy

So far we discussed the mirror mechanism underlying the understanding of cold actions, that is, of actions devoid of clear emotion content. Does the mirror mechanism also mediate the understanding of emotions of others?

Before answering this question, it is important to remind that emotions are mental states accompanied by motor and vegetative reactions. Furthermore, electrical stimulation of emotional centers (e.g., anterior insula) elicits viscero-motor responses in both monkeys and in humans. This finding indicates that the emotional structures, and the anterior insula in particular, are endowed with a motor machinery similar to that of the cortical centers controlling the cold actions but, with in addition, a control on vegetative responses.

Do these emotional centers respond, besides their specific (e.g., pain) stimuli, to the observation of the emotions in others? A series of studies showed that this is the case. The strongest evidence in favor of it came from brain imaging studies of two basic emotions: disgust and pain.

Studies of disgust showed that when an individual is exposed to an unpleasant odor, there is an intense activation of the amygdala, the insula, and the anterior cingulate. Prompted by these data, an fMRI study was carried out that investigated whether the insula sites that show activation during the experience of disgust also show activation during the observation of faces expressing disgust. The study consisted of two sessions. In the first, the participants were exposed to unpleasant and pleasant odorants; in the second, they watched a video showing the

face expression of people sniffing an unpleasant, a pleasant, or a neutral odor. In accord with previous data, three main structures became active during the exposure to smells: the amygdala, the insula, and the anterior cingulate. The most interesting result of the study was that the same foci within the insula and the anterior cingulate that were activated by the exposure to disgusting odorants were also activated by the observation of disgust. This indicates that the same viscero-motor structures that control emotions are also activated by the vision of that emotion. "Your disgust becomes my disgust."

In addition to disgust, activations in the insula and in the anterior cingulate cortex were also observed in studies in which emotional reactions to pain were investigated using an event-related fMRI paradigm. In this study there were two conditions. In one the participants were subjected to a mildly painful electric shock from electrodes placed on their hand, in the second they were asked to watch the same electrodes positioned to the hand of a loved one. They were told that the loved person would receive the same shock to which they had been subjected earlier. The results showed that the same sites of the insula and of the cingulate cortex became active in both conditions. This result shows that both direct pain experience and its evocation are mediated by a mirroring similar to that found for disgust.

One should stress, however, that the notion that the activation of viscero-motor structures provides the basis for the recognition of emotion, does not exclude that emotions may also be recognized indirectly, using cognition. Some particular visual features representing the basic features of an emotion, like in emoticon, allows emotion recognition. This emotion recognition is, however, different from that mediated by the insula and the anterior cingulate, because only the latter creates a shared feeling between the observer and the person actually feeling the emotion.

Language

Although there is strong disagreement on the evolutionary roots of human speech, nobody denies that speech is something more than a mere collection of curious sounds. This point was clearly demonstrated by Alvin Liberman who showed that an efficient communication system could not be built by simply using tone combinations. According to him, the unique communicative property of speech derives from the capacity of speech sounds to elicit a motor representation of the heard sounds in the listener. There is evidence that this capacity has a precise neural correlate.

In a TMS experiment, the left hemisphere speech motor centers were stimulated and motor evoked potentials (MEPs) were recorded from the tongue muscles in volunteers instructed to listen carefully to acoustically presented verbal and nonverbal material. The stimuli were words and bitonal sounds. In the middle of words,

there was either a double 'f' or a double 'r.' 'F' is a consonant that, when pronounced, requires virtually no tongue movements, whereas 'r' is a consonant that, in contrast, requires, marked tongue muscle involvement to be pronounced. The results showed that listening to words containing the double 'r' produced a significant increase of MEPs amplitude recorded from tongue muscles compared with listening to bitonal sounds and words containing the double 'f' (**Figure 5**).

Similar results were obtained using TMS technique and recording MEPs from a lip muscle and a hand muscle in four conditions: listening to continuous prose, viewing speech-related lip movements, listening to nonverbal sounds, and viewing eye and brow movements. Compared to viewing eye and brow movements, listening to and viewing speech enhanced the amplitude of MEPs recorded from the lip muscles. All of these effects were seen only in response to stimulation of the left hemisphere.

These and other similar data indicate that in humans, in addition to the mirror mechanism transforming observed movements into potential movements, there is a further system transforming heard phonemes in the corresponding motor representation of the same sound. There is little doubt that this system could play an important role in language learning. It is matter of debate, however, whether, and at what extent, it intervenes in the comprehension of word meaning.

Autism

The distinction between a direct mirror-mediated comprehension of others and a comprehension mediated by inferential reasoning appears a fundamental distinction for understanding not only the behavior of people in general but also that of individuals with atypical behaviors as children with autism.

Autistic spectrum disorder is a heterogeneous syndrome characterized by a marked impairment in social interaction and communication. Restricted repertoire of activity and interests, repetitive motion, and hypersensitivity to certain sounds are other symptoms that often accompany the other deficits. It has been noted that some of the functions impaired in autism are those mediated by the mirror mechanism (intention and emotion understanding, language). The hypothesis has been therefore advanced that the inability of patient with autism to relate to others in an ordinary way depends on an impairment of the mirror system.

First evidence in favor of this hypothesis came from EEG studies. It is well known that EEG rhythms recorded from the motor cortical areas are blocked any time a person makes a voluntary movement. These rhythms are also suppressed when a person observes another person performing a movement. This phenomenon has been

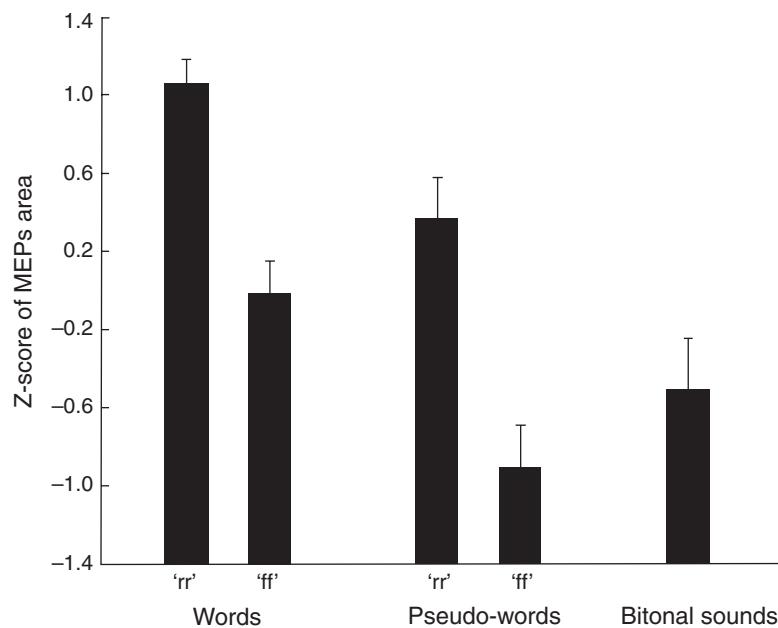


Figure 5 Speech listening modulates the activity in primary motor cortex. Average value of intrasubjects normalized MEPs total areas for words, pseudo-words and bitonal sounds. Data from all subjects; 'rr' and 'ff' refer to verbal stimuli containing a double lingua-palatal fricative consonant 'r,' and containing a double labio-dental fricative consonant 'f,' respectively. Reproduced with permission from Fadiga L, Craighero L, Buccino G, Rizzolatti G (2002) Speech listening specifically modulates the excitability of tongue muscles: a TMS study. European Journal of Neuroscience 15: 339–402.

used to test the functioning of the mirror mechanism in typically developed individuals and in autism. The results showed that individuals with autism present a suppression of motor cortical rhythms during voluntary movements,

but this suppression is lacking when they watch someone else performing the movement (**Figure 6**).

Strong evidence in favor of a deficit of the mirror mechanism came from an fMRI study. High-functioning

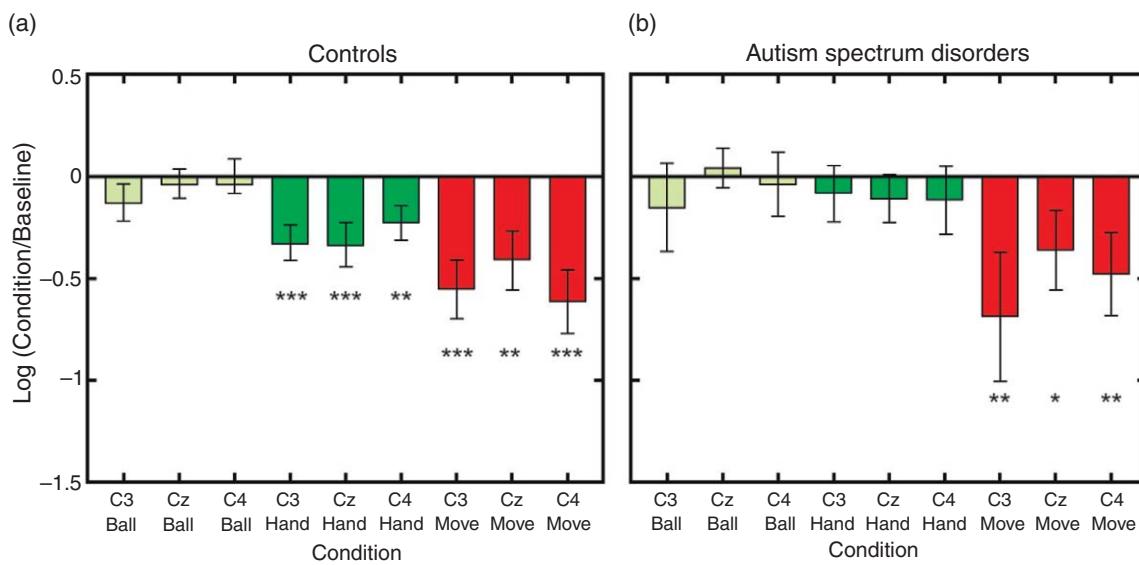


Figure 6 Absence of mirror EEG responses in autism. The charts show suppression of the mu rhythm in controls (a) and patients with autism spectrum disorder (b) during observation of movement of an inanimate object (ball, pale green) or movements made with a hand (hand, green), and during active hand movements made by the individual from whom recordings were being taken (move, red). The bars represent the amount of mu activity in central scalp locations; C3, Cz, and C4 refer to scalp coordinates of the 10/20 EEG system. Significant suppression of this activity, indicated by asterisks, is present for the hand observation condition only in controls, showing that patients with autism spectrum disorder fail to respond in a standard way to the observation of other people's actions. Reproduced with permission from Oberman LM, Hubbard EM, McCleery JP, Altschuler EL, Ramachandran VS, and Pineda JA (2005) EEG evidence for mirror neuron dysfunction in autism spectrum disorders. Brain Research. Cognitive Brain Research. 24: 190–198.

children with autism and matched controls were scanned while observing and imitating emotional expressions. The results showed a significantly weaker activation in IFG in children with autism than in typically developing (TD) children. Most interestingly, the activation was inversely related to symptom severity.

Recently, the deficit of the mirror mechanism in autism has been addressed from another perspective. TD children and children with autism were tested while they observed either an experimenter grasping a piece of food for eating or grasping a piece of paper for placing it into a container (**Figure 7**, right side). The EMG activity of the mylohyoid muscle (MH), a muscle involved in opening of the mouth, was recorded. The results showed that in TD children, the observation of food grasping determined the activation of MH muscle, while this activation was lacking in children with autism. In other words, while the observation of an

action done by another individual intruded into the motor system of a TD observer, this intrusion was lacking in children with autism. This finding indicates that, in autism, the mirror system is silent during action observation and the immediate, experiential understanding of others' intention is absent.

Both autistic and TD children were also asked to perform the two actions described above (grasp to eat and grasp to place) (**Figure 7**, left side). The EMG of MH muscle was recorded. In TD children, the muscle became active as soon they moved the arm to reach the food. In contrast, no MH muscle activation was observed during food reaching and grasping in autistic children. MH muscle activation appeared only when the children brought the food to their mouth.

These findings indicate that autistic children have a deficit in transforming their original intentions into motor

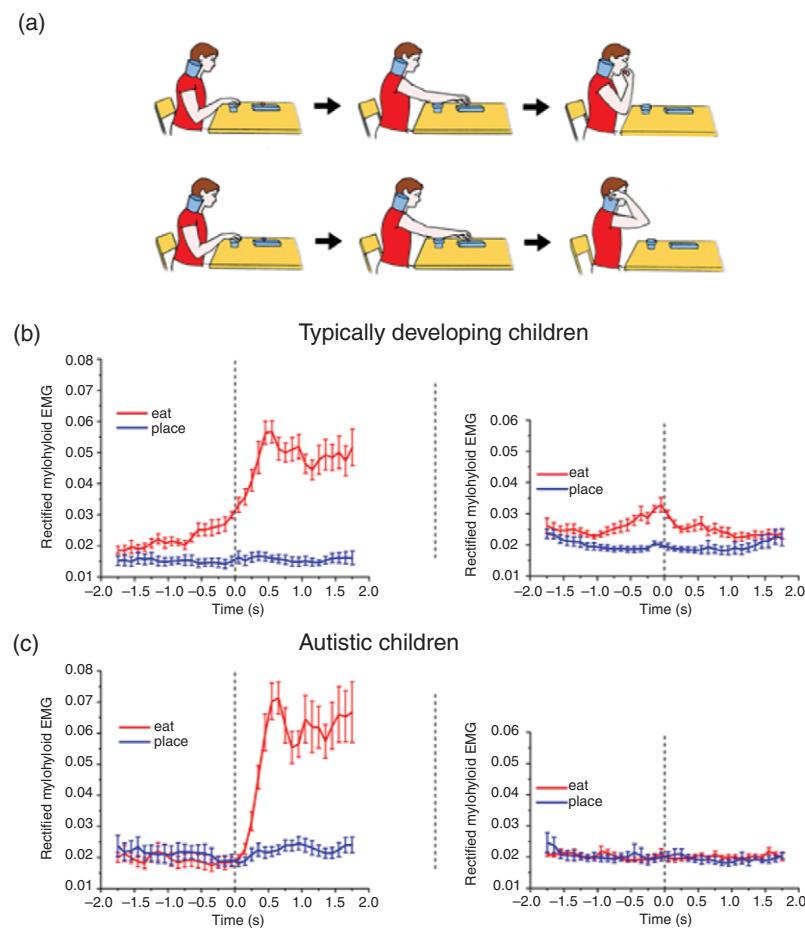


Figure 7 Motor behavior in typically developing children and children with ASD. (a) Schematic representation of the tasks. The individuals reach for an item on a plate, either to bring it to their mouth or to put it into a container placed on their shoulder. Time course of the rectified electromyographic activity of mouth-opening muscles during the execution (left side) and observation (right side) of the bringing-to-the-mouth action (red line) and of the placing action (blue line) for typically developing children (b) and for children with ASD (c). All curves are aligned with the moment of object lifting from the touch-sensitive plate (time = 0). The results demonstrate a lack of anticipatory motor activity during execution and a lack of mirror motor activation during observation of a given action in children with ASD. ASD, autism spectrum disorder; EMG, electromyography. Reproduced with permission from Cattaneo L, Fabbri-Destro M, Boria S, Pieraccini C, Monti A, Cossu G, and Rizzolatti G (2007) Impairment of actions chains in autism and its possible role in intention understanding. *Proceedings of the National Academy of Sciences of the United States of America* 104: 17825–17830.

intentions. It is plausible that the deficit in understanding experientially the intentions of others is linked to this primary motor deficit. When the capacity of endogenously recruiting the appropriate motor chains is impaired, the possibility to activate them exogenously is lacking as well and so also the capacity to understand other's intention.

This new view on autism requires further experiments, yet it appears to be very promising for establishing new rehabilitation procedures based on the acquisition of motor cognition.

See also: Communication of Emotions in Animals; Emotion–Cognition Interactions; Genetics of Language; Navigation in Virtual Space: Psychological and Neural Aspects; Peripersonal Space and Body Schema; Subjective Experience and the Expression of Emotion in Man.

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Navigation in Virtual Space: Psychological and Neural Aspects

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Glossary

Allocentric – A representation of a spatial environment referenced to an external coordinate system that is not dependent on the original view or direction navigated when first experiencing an environment.

Cognitive map – A representation of a spatial environment that contains information about the spatial layout of objects in that environment. By definition, cognitive maps are allocentric representations of space because they are not dependent on the original direction that a subject navigates and are instead general to any direction or viewpoint the observer experiences it from.

Egocentric – A representation of a spatial environment tied to a self or body-centered coordinate system.

Functional magnetic resonance imaging (fMRI) – A noninvasive method for looking at changes in neural activity in the human brain. It depends on changes in magnetic properties of deoxygenated hemoglobin, providing information on metabolic changes in various brain regions. fMRI is therefore an indirect measure for neural activity.

Hippocampal area – Comprises the hippocampus and surrounding cortical structures (i.e., parahippocampal region).

Hippocampus – A three-layered structure in the medial temporal lobes of the brain critically involved in memory and spatial navigation.

Parahippocampal cortex – Also known as ‘posterior parahippocampal cortex.’ Six-layered cortical tissue receiving strong input from visual areas; central to memory and spatial navigation.

Parahippocampal region – Cortical tissue surrounding the hippocampus, which is in turn composed of parahippocampal cortex, perirhinal cortex, and entorhinal cortex.

Lesion – Surgically or pathologically produced brain damage.

Place cell – A neuron that increases firing at specific spatial locations and not others. This increased firing is independent of the trajectory of the animal.

Place field – An area in space of increased firing of a place cell.

Path integration – Computation of the optimal, or shortest, path to a location based on previous paths.

Theories of Spatial Navigation and Navigation in Lower Mammals

Origin of the Cognitive Map Theory

How is it that we can return to a town we have not visited in many years and still have a feeling of where things are and the right way to go? When we learn the layout of one city, why is it we rarely confuse where things are in that city with a different city we know better? To explain these and other spatial memory phenomenon, in 1948, Edward Tolman proposed the idea that the brain forms comprehensive maps of a spatial environment, such that “the wider and more comprehensive...the more adequately it will serve . . . ” He termed these representations ‘cognitive maps,’ basing his ideas on observations of rat maze learning. If a rat first learned to find a reward by entering a tunnel and turning right, and then found the entrance blocked and replaced by several tunnels branching toward and away from the reward, the rat quickly learned to take the arm that went most directly to the reward. Furthermore, when the entire maze was rotated, the rat still navigated around the obstruction to find where the food had been. These results could not be accounted for by classical learning theory, the dominant theoretical paradigm at the time, which explained rodent navigational behavior based on learning associations between stimuli and responses such as “white wall, turn right.” Tolman’s data suggested that the

rodent representation for space was not dependent solely on egocentric coordinates, which would be based on the original set of stimulus-responses pairings learned, but instead on allocentric coordinates, a novel type of representation that was direction independent and referenced to landmarks in the room.

Neural Basis of Cognitive Maps in Rodents

Since Tolman's proposal an extensive literature in the rat points to a critical role for the hippocampus, a three-layered structure in the medial temporal lobes, in allocentric spatial learning. Electrophysiological recordings from the hippocampus showed that as a rat foraged in an open area, a significant percent of cells robustly increased in firing rate for certain locations of the room. These place cells also showed direction independence, thus fulfilling the criteria for an allocentric representation of space. Furthermore, recordings from large numbers (>30) of hippocampal neurons simultaneously demonstrated that the firing field (or place field) of each place cell had a fairly sharp tuning for a certain region of the environment. These place fields had firing rates of up to 40–50 Hz which fell off rapidly with distance, thus coding for a certain region of the room quite sharply. The collection of place fields recorded during a single session was shown to comprise a spatial code of the entire room.

The presence of hippocampal place cells in the rodent led O'Keefe and Nadel in 1978 to propose the idea that the hippocampus computes cognitive maps of place, representations of location in an environment referenced to the position of external cues or landmarks. In the rodent, the predictions of the cognitive map model have proven (largely) correct. Cognitive maps, which can be thought as collections of place fields representing a spatial environment in its entirety, rotate with the environment and are environment specific, thus suggesting a map-like quality to these representations. Hippocampal computational models that implement cognitive maps quantitatively account for a large amount of spatial neurophysiological data on the rat.

While the evidence for the involvement of place cells and the hippocampus in cognitive map formation is well demonstrated in the rodent, it is important to note that the rodent hippocampus also plays roles in other behaviors. Examples of stimuli encoded by rodent hippocampal neurons include odors and conjunctions of odors and places. A situation in which these types of representations typically manifest involves training of a rat to associate smells in certain locations with food reward. In these situations, hippocampal neurons come to represent specific odors, while other neurons code combinations of odors and spatial locations. These findings suggest that the neural mechanisms involved in representation of place in the rodent may not be unique to spatial representation and suggest a role for the hippocampus in more

than just spatial representation. Together, the place- and odor-representation findings suggest that the rodent hippocampus is important for representing stimuli in a manner that is task-driven and context dependent.

Neural Basis of Cognitive Maps in Primates

The conditions under which monkeys are tested in navigational paradigms have often differed from those of rodents due to the greater mobility of monkeys overall, compared to rats. In one particular study, monkeys navigated on a track from a head-restrained, moveable chair guided and controlled by a joystick. Monkeys were tested both on a real movement task (reward destinations were indicated by pointers on a monitor and monkeys navigated on the track to this location) and a virtual movement task (reward destinations were indicated by pointers on a monitor and the monkey moved the cursor on the monitor to this location). The study found that nearly half of the hippocampal and parahippocampal neurons recorded fired selectively at specific spatial locations (using criteria similar to that used previously in the rodent) in the virtual and real movement tasks, with a significantly greater number of neurons showing selectivity in the real navigation task compared to the virtual navigation task. These data argued for the presence of place cells in the primate hippocampus as well as the presence of place cells during both real and virtual navigation.

Some laboratories have developed technology for recording from freely moving and behaving monkeys and report different findings than Matsumara and colleagues. In one study, monkeys were tested in an unrestrained testing environment. Recording head direction, eye position, location in space, and neural firing rate, the location that the monkey viewed in space drove the firing rate of neurons in the hippocampus and parahippocampal region rather than spatial location. A subsequent study, however, which also examined freely moving monkeys, reported robust firing at specific spatial locations, regardless of the view direction from which the monkey approached the location. Additional studies are needed to resolve some of the discrepancies in findings with navigating monkeys and spatial coding. Both the place- and view-coding findings, however, show that the primate hippocampus forms allocentric representations of space because both the place- and view-based representations were direction independent in the studies mentioned.

Spatial Navigation in Humans

Behavioral Studies: Do Humans Employ Cognitive Maps?

Somewhat surprisingly, one of the first studies to carefully look for the presence of cognitive maps in humans reported little evidence for allocentric coding in the

human brain. Wang and Spelke set out to test behaviorally whether subjects use primarily egocentric or allocentric representations of space by having them view items arranged on a table and point to their locations in a darkened version of the room following blindfolded rotation. Consistent with an egocentric model of spatial representation, Wang and Spelke found errors accumulated during rotations rather than remaining constant across positions, inconsistent with an allocentric, or equal viewpoint representation of space. Subsequent studies also showed that subjects performed most accurately on viewpoints they had already experienced when pointing to a specific object from an imagined orientation, and that experiencing more viewpoints did not improve pointing accuracy to novel viewpoints. These findings were initially heralded as support for the idea that humans preferentially form egocentric rather than allocentric representations of space.

Part of the reason why these studies may have found support for the presence of egocentric rather than allocentric coding in the human (in contrast to what had been shown in the rodent) likely had to do with the testing situations employed by these studies. For example, the two studies mentioned relied, to some degree, on a bias toward a spatial representation from a specific viewpoint and not on a holistic representation of the room. Indeed, subsequent studies that naturally resulted in subjects referencing viewpoints outside of those they initially learned tended to demonstrate the presence of more allocentrically based representations. For example, when a salient landmark is experienced during active navigation, this information could override what viewpoints were best remembered egocentrically.

Why did Wang and Spelke find little evidence for allocentric representations during their task? Waller and Hodgson suggested that this could have arisen because Wang and Spelke did not interrogate subjects on their relative knowledge of items in the room compared to their absolute positions. Similar to Wang and Spelke, Waller and Hodgson showed that pointing accuracy increased following blindfolded rotation. Waller and Hodgson then went on to show that variations in errors in judging the relative distances of objects actually decrease following disorientation. Thus, pointing to objects appears to preferentially involve egocentric coding systems because the objects were originally centered on the first view-point experienced. Judging positions of objects relative to other objects, however, preferentially invokes allocentric coding as this system is instead initially referenced to landmarks in the room. The findings of Waller and Hodgon thus support the idea that both systems are active during spatial learning and therefore that our brain, similar to the monkey and rodent, codes both allocentric and egocentric references frames.

Brain Structures Underlying Human Spatial Navigation

The human hippocampus consists of several important and highly interconnected nuclei. These include CA1-4, dentate gyrus, and subiculum, which receive input from both lower brain modulating centers, higher neocortical locations, and local interconnections. Based on the hippocampus' apparent position at the top of a pyramid of inputs, it is in a unique position to integrate inputs from a variety of different sensory modalities and output this information for immediate action to motor regions. The parahippocampal region, found between six-layered neocortex and three-layered hippocampus, consists of entorhinal cortex, perirhinal cortex, and parahippocampal cortex, appearing histologically to represent a transition from hippocampus to neocortex. It receives reciprocal connections from the hippocampus and provides the majority of neocortical input to the hippocampus, thus containing many of the same inputs as the hippocampus (with the relative exception of lower brain modulatory centers the hippocampus receives via the fornix). A major source of input to the parahippocampal region, the retrosplenial cortex (an area of posterior cingulate cortex) provides ~20% of the cortical inputs into the entorhinal cortex. Since retrosplenial cortex also receives input from prefrontal cortex, it represents a way station for processing of multimodal input.

Patient lesion work

Lesions to the hippocampus, parahippocampal region, and retrosplenial cortex in humans produce a variety of deficits, including impairments in spatial processing. In one particular study, patients with hippocampal area lesions attempted to locate a sensor hidden under a rug in a room and then find the sensor again after a 30-min delay. Bohbot and colleagues reported that patients with parahippocampal lesions performed significantly worse than controls on this task, while patients with right and left hippocampal lesions did not. In subsequent studies, patients with parahippocampal lesions also showed deficits when navigating virtual environments, performing worse on exploration of a virtual environment than patients with lesions limited to the hippocampus or anterior parahippocampal cortex (i.e., perirhinal and entorhinal cortices). These studies suggest that the human parahippocampal cortex, and not the hippocampus, plays an important role in both real and virtual spatial navigation.

What role then does the lesion literature suggest that the hippocampus plays in spatial memory? Studies of the classic patient HM, who had damage to his hippocampus (and some surrounding structures), but whose parahippocampal cortex was largely intact, suggested a potentially different story than the rat. In one study, HM was tested

in the same navigation task used by Bohbot and colleagues. HM showed intact navigation to the location of the hidden sensor, comparable to controls, although was impaired when required to remember more than one location. These data suggested that HM had some intact allocentric memory, despite a lesion to the hippocampus. His hippocampal lesion appeared to produce the most pronounced impairments when he was required to remember more than one spatial location. In line with these findings, other studies reported that patients with damage largely prescribed to their hippocampus were similarly impaired at recalling the locations of several objects that had been placed in an open arena but not at drawing these objects correctly on a map. These findings further suggested that hippocampal lesions resulted in deficit in integrating multiple representations of objects in space and not simply locating a single object on a map. Thus, together these findings suggest that the human hippocampus may be most critical for integrating multiple representations of space, especially within different contexts. The lesion data suggest that the human parahippocampal cortex may be more involved in actual spatial representation of visually encoded environments than the hippocampus.

Some limitations with patient lesion work warrant consideration when evaluating what brain regions underlie navigation. Lesions impair both the computations a structure performs as well as the input it provides to other structures. Thus, damage to a structure, such as parahippocampal cortex, removes strong inputs to the hippocampus, thus altering its normal functioning as well. In both developmental amnesiacs and patients with even relatively recent lesions, significant remapping in brain structures may occur, which may still involve individual variability. Thus, other measures of brain function, particularly in nonpathological brain structures, are necessary to accurately identify the brain systems involved in navigation.

Functional imaging and virtual reality navigation

Since its inception about two decades ago, functional magnetic resonance imaging (fMRI) remains the dominant method in healthy volunteers for investigating the brain systems involved in human behavior. This is primarily because fMRI is noninvasive yet provides excellent spatial resolution (up to 1 mm) and acceptable temporal resolution (on the order of seconds) for most memory paradigms. fMRI relies on changes in the magnetic properties of hemoglobin as oxygen is delivered to tissue in the brain, and thus is not a direct measure of neural activity. This potential limitation with fMRI, however, appeared initially of less concern because, at least in visual neocortex because there is significant coupling between the fMRI blood-oxygenated-level-dependent responses (BOLD), synaptic activity, and single neural

firing. Unfortunately, the story in the hippocampal area appears more complicated. Current evidence suggests that activations in the hippocampal area largely reflect local field potential input from other brain regions rather than neural firing rate specifically, suggesting that the BOLD signal may largely represent input from nearby brain regions rather than the actual computations that structure performs. Despite this limitation, fMRI and positron emission tomography (PET) (which similarly measures changes in metabolism), remain a powerful technique for looking at changes in neural activity in brain regions and can provide valuable information about the types of information input for processing in a brain region.

Several fMRI and PET studies have looked specifically at the role of the hippocampal area in spatial processing. One of the first studies to look directly at virtual navigation using PET was a study by Maguire and colleagues, who looked at hippocampal activity as subjects navigated to spatial locations. Maguire and colleagues found that as subjects chose more accurate trajectories to locations, hippocampal blood flow increased in a linear fashion to the hippocampus. They also found significant hippocampal activation as subjects successfully navigated to locations compared with following arrows to locations.

In a subsequent virtual reality (VR) study, however, it was reported that the parahippocampal region, but not the hippocampus, was active during spatial navigation involving way finding (navigation requiring use of novel paths) compared with a control condition in which subjects traversed a virtual corridor back and forth. Subsequent work also demonstrated parahippocampal activation simply when subjects viewed spatial scenes compared to viewing faces or objects. What then explains the discrepancy between studies showing parahippocampal activations and those of Maguire and colleagues?

One possible explanation lies with some of the ambiguity in interpreting the BOLD signal in the hippocampal area as structures that provide input to each other (such as hippocampus and parahippocampal cortex) may make it difficult to disentangle where the BOLD signal originates. Another possible explanation, if we assume the BOLD signal at least conveys unambiguous information in both the hippocampus and parahippocampal region, is that different testing conditions tap into different navigational memory systems and thus, different testing conditions result in different brain activations. As discussed earlier, Wang and Spelke found that when subjects make absolute judgments about the positions of objects in a room, egocentric representations predominate. In contrast, in situations in which subjects are required to reference to landmarks, allocentric representations predominate. Thus, when subjects view static environmental scenes, there may be no particular reason for subjects to form allocentric representations of space, thus providing an

explanation for why hippocampal activation may not have been observed when subjects view static spatial scenes. The presence of parahippocampal and not hippocampal activation during way findings, however, suggests a more involved role in allocentric-based spatial navigation.

How do we reconcile fMRI findings with those of the lesion literature? One possibility is that the parahippocampal region not only processes view information experienced during navigation but also does some (preliminary) allocentric processing. This theory would help explain why parahippocampal lesions would have such a profound effect on navigation, in general, as its absence would greatly impair visual-based input to the hippocampus. This proposal also explains the presence of parahippocampal activations simply when subjects view scenes and spatial layouts but are not required to form allocentric representations. Since the parahippocampal cortex also does some allocentric-based, view point extraction, this proposal can also explain why there is greater parahippocampal activation when subjects use objects as landmarks to remember spatial routes compared to when these same objects do not serve as landmarks.

This proposal, however, does not directly address what types of cellular responses might be present in the parahippocampal region and what types of visual stimuli might preferentially drive its neurons during navigation. Rodent studies emphasize the critical role for the hippocampus in forming place cells although the human lesion and fMRI literature suggest a more complex picture at least in the human. fMRI and lesion work, however, are limited to the resolution of whole brain structures and cannot report on single neuron activity. Thus, direct recordings of single neuron from the human hippocampal area are necessary in attempting to resolve this issue.

Neurophysiology of Human Spatial Navigation

As discussed, the rodent and nonhuman primate both support the presence of place cells in spatial coding. Human behavioral literature suggests both egocentric and allocentric coding systems are involved in human spatial learning. Lesion and fMRI work suggest roles for both the hippocampus and parahippocampal region in navigation. At University of California, Los Angeles (UCLA) Medical Center, in conjunction with neurosurgeon Itzhak Fried, we have the opportunity to record from single neurons in the human brain in patients undergoing monitoring for seizures. In a study that we have reported previously and elaborated here with more patients and data, we report on single neuron recordings

from human patients undergoing seizure monitoring as they navigate a virtual environment.

Single Cell Recordings From Human Hippocampal Area during Navigation

The principle questions we wished to address were whether hippocampal area neurons responded at specific spatial locations, how they code landmarks, and whether these responses show allocentric coding properties. We additionally wanted to look at the view dependence and place dependence of neurons in these brain regions, given the previous literature. Would place representations show view dependence? Would representations for landmarks show place dependence? We recorded from hippocampal area neurons in 10 different patients as they navigated a virtual environment, binning firing rate according to behavioral epoch. We defined ‘place responsive cells’ to be cells that showed main effects of place with no main effects or interaction effects with view. We determined the number of cells responding to spatial positions (place) versus what was viewed during navigation (view) in an analysis of variance (ANOVA). We found cells in the hippocampus that responded robustly to place (see [Figures 1\(a\)–1\(c\)](#)); these neurons did not show changes in firing rate for viewing landmarks. We also found that cells showed effects of what landmarks a subject viewed ([Figures 2\(a\) and 2\(c\)](#)). These neurons were active from a variety of spatial positions (see [Figures 2\(b\) and 2\(d\)](#)) and thus, were place independent. We then tallied the total number of neurons responding to place and view across all the neurons we recorded. Place-responsive neurons were significantly clustered in the hippocampus compared to other brain regions, while view-responsive neurons were significantly clustered in the parahippocampal region compared to other regions ([Figures 3\(a\) and 3\(b\)](#)). Only place-independent view responses were clustered in the parahippocampal region; we did not observe any anatomical clustering of cells showing place–view interactions.

Our data both support previous ideas on human spatial navigation developed from the imaging and lesion literature as well as expand on it in important ways. Hippocampal neurons showed increased firing at specific spatial locations and not others – regardless of the patient’s trajectory – supporting the idea that these representations are both spatially specific and allocentric. These same neurons, however, did not respond to viewing landmarks. Interestingly, in many cases, these place-specific responses remapped depending on a subject’s navigational goals, demonstrating the context dependence of these responses. Thus, our data have several implications for theories of the human hippocampus. First, our data confirm the importance of the

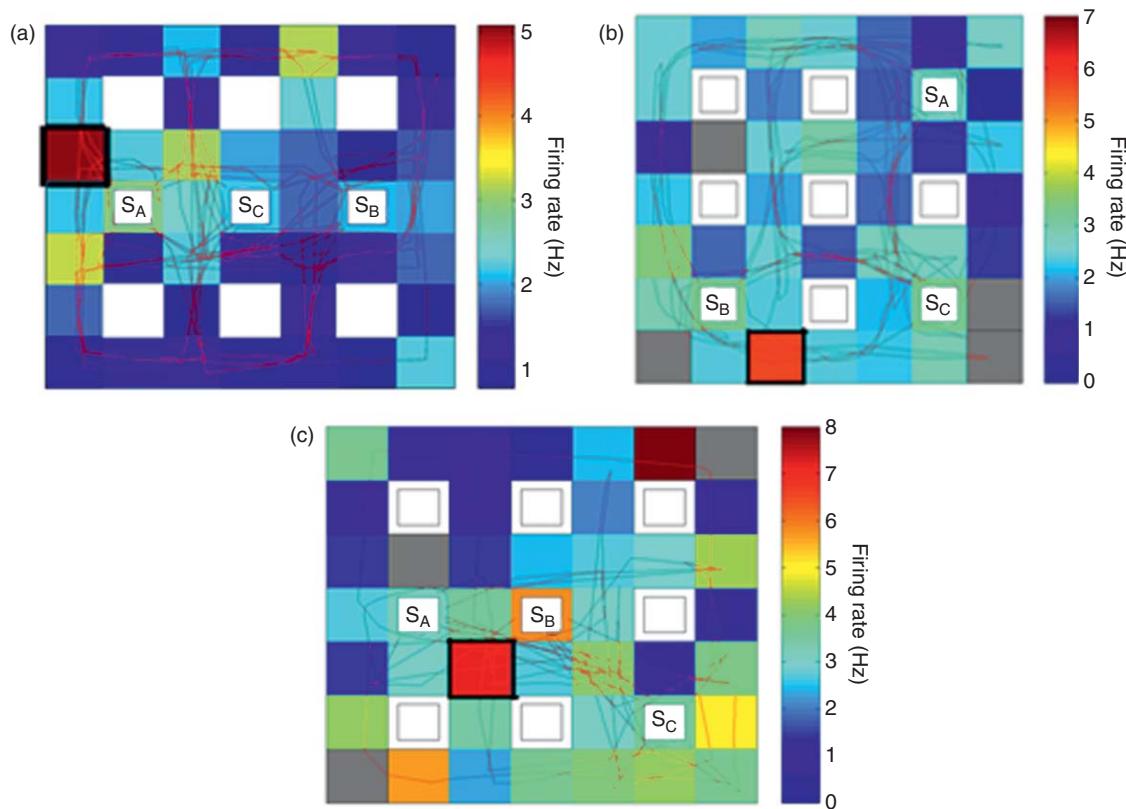


Figure 1 Place responsive cells. (a)–(c) Firing rate maps of hippocampal cells show significant place selectivity. Letters (S_A, S_B, S_C) indicate store locations, white boxes indicate nontarget buildings, gray boxes indicate unoccupied areas, red lines indicate the subject's trajectory, and black squares indicate regions of significantly high firing rate (determined using a resampling procedure at $p < 0.01$). From Ekstrom AD, Kahana MJ, Caplan JB, et al. (2003) Cellular networks underlying human spatial navigation. *Nature* 425: 184–188.

hippocampus in allocentric coding and extend previous work to demonstrate the conservation of place-selective firing from rodents to humans. Second, our data indicate that these place responses are context dependent, confirming a role for the hippocampus in representing information updated by the current experimental context.

Our data also confirm a role for the parahippocampal region in allocentric-based view coding. In our study, parahippocampal neurons showed increased firing when subjects viewed specific landmarks and did not show significant changes in firing rate for place (over the population of neurons recorded). While we found neurons that responded to viewing landmarks both independent and dependent on spatial position, only place-independent view responses were significantly clustered in the parahippocampal region. These data expand on previous ideas developed in the imaging literature and support a role for the parahippocampal region in both egocentric- and allocentric-view coding. These data support the parahippocampal region as an important source of input of visual information and also highlight its role in both egocentric and allocentric processing of spatial information.

Brain Activity in a Virtual Environment: Is it Comparable to Real Navigation?

One of the limitations with fMRI and clinical single cell recording is that subjects cannot navigate in an unrestrained manner, so all testing must be done with a computer laptop. The vast majority of research on the neural basis of spatial coding in the rodent, in contrast, has been conducted during real, locomotion-based, spatial navigation. In the literature, we reviewed previously regarding spatial navigation in patients with hippocampal area lesions, testing was performed during both real and virtual spatial navigation. As the reader will recall, both types of navigation depended on parahippocampal cortex. Similarly, monkeys tested both during real and virtual navigation showed formation of place representations in both conditions. One could still argue, however, that conclusions about spatial coding mechanisms derived in VR studies may not be the same as those observed during real navigation. Indeed, additional information is available during real navigation that is not available during virtual navigation. For example, real movement involves the additional input of head-direction and proprioceptive information into brain systems. Movement of the head

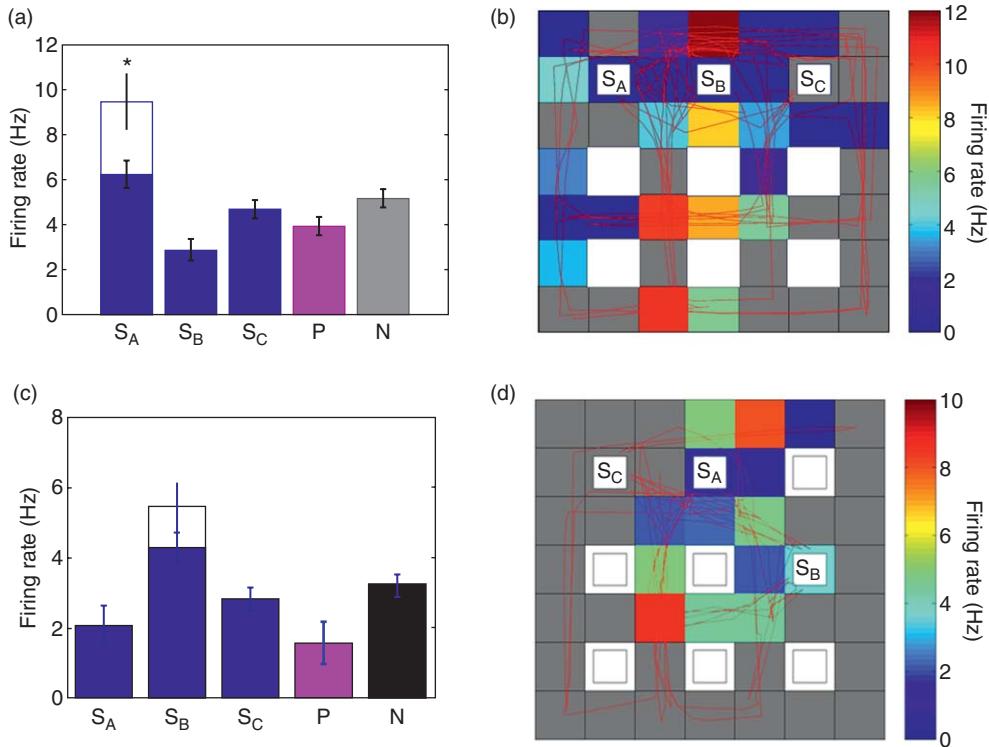


Figure 2 View responsive cells. (a) Mean firing rate for a parahippocampal cell that responded to viewing S_A (as compared with other stores, passengers (P) and control views (N)). The firing rate to viewing S_A (but not other targets) increased significantly when S_A was the goal (white bar). (b) Firing rate map shows that this cell responded to viewing S_A from disparate regions; gray regions indicate that S_A was not viewed from these locations. (c, d) Another parahippocampal neuron that responded significantly to viewing a store (in this case, S_B).

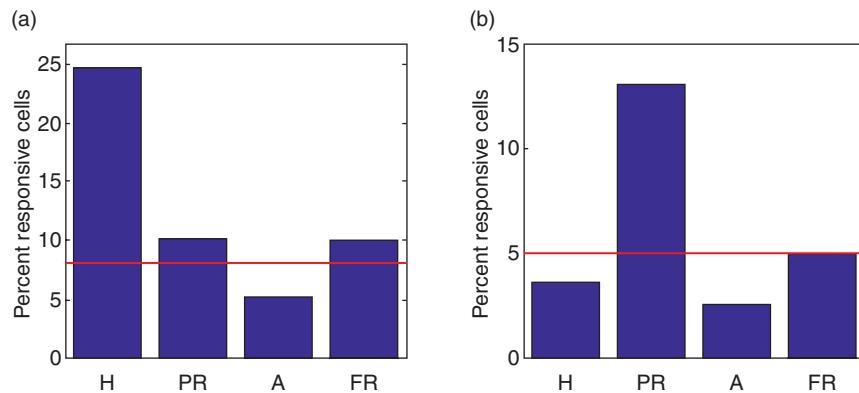


Figure 3 Anatomical clustering of responses. (a) Place-responsive cells were clustered in the H, hippocampus compared to A, amygdala; PR, parahippocampal region; and FR, frontal lobes. (b) View-responsive (location-independent) cells were clustered in the PR compared to other brain regions. Red line indicates bootstrapped type-I error rate.

invokes the head-direction system via the vestibular system, which provides important information about the subject's bearing not available in VR. Proprioceptive information, obtained through changes in muscle position, can provide information about speed. How does this additional information available during real navigation affect one's internal representation for space?

In several studies, the combination of proprioceptive information with head-direction information was shown to

provide the needed information for a rat to compute the optimal path to find a location, referred to as 'path integration.' A classic illustration of path integration comes from mother rats separated from their pups. When their pup is placed at a new location, rats can readily find the shortest path back to their nest. In support of the critical role of path integration in place coding, lesions of the vestibular system greatly impair path integration as well as the specificity and stability of place fields. Thus, vestibular and proprioceptive

input clearly provide important additional information to the navigator that are not present during virtual navigation.

Despite the lack of vestibular and proprioceptive input, human subjects can still path integrate in a virtual environment. Previous research suggests that humans can use optic flow and egocentric bearing in VR to guide themselves to visual targets. Human subjects can also readily find optimal paths to store locations in virtual space, similar to real-world path integration. But given that the inputs differ between real and virtual navigation, despite some of the similarities in how people navigate in both situations, how comparable is the utilization of information obtained during VR to that obtained during actual navigation? In one study, subjects trained in a virtual environment were more accurate at navigating a real-world version of the environment than subjects who received verbal instructions on how to navigate the environment. These data suggest that at least some aspects of what is learned during virtual navigation also apply to real-world navigation. Further studies that directly compare neural responses during virtual and real-world navigation, however, are needed to fully address how comparable VR and real-world navigation are.

Summary

How do we know where we are going when we navigate? What brain systems and neural representations are involved in this task? Are these systems conserved from lower mammals to humans and how can we test these questions in humans? In this article, we have attempted to address some of these questions. We first discussed the distinction between egocentric and allocentric representations as a way of distinguishing between navigation that is dependent on the original viewpoint learned (egocentric learning) and navigation that is independent of static view point (allocentric learning). Rodent electrophysiological studies demonstrated the presence of place cells, neurons in the hippocampus that increase firing for specific spatial locations in an allocentric manner. Nonhuman primate studies also support the presence of place cells as the basis for allocentric spatial coding. In humans, allocentric coding mechanisms have been more difficult to demonstrate, in part because certain coding systems may predominate under certain testing conditions. Under the correct testing conditions, such as making relative judgments about objects positioned in a room, subjects do indeed use allocentric coding schemes to solve a task. Human lesion work suggests the importance of the hippocampus and surrounding parahippocampal cortex in spatial representation, with a greater emphasis on the parahippocampal cortex in visual representation of an environment and the hippocampus in representing multiple spatial environments. fMRI research not only supports the role of the hippocampus in allocentric-based navigation but also

indicates the involvement of the parahippocampal cortex, particularly in extracting view information from spatial scenes. Both fMRI and lesion work are limited to broader statements about brain regions and brain circuits and cannot directly demonstrate whether place cells exist. Direct recordings from the human are rare and limited to clinical situations, yet offer promise for revealing the neural representations underlying spatial navigation. Recording from the human brain while subjects navigate a virtual environment, we report on both place responsive cells (clustered in the hippocampus) and view-responsive neurons (clustered in the parahippocampal region). These data support the idea that place cells are conserved from rats to humans. These data also show that our spatial coding mechanisms involve additional mechanisms, including a greater reliance on view than rodents.

See also: Animal Models of Learning and Memory; Brain Mapping of Language and Memory in Epilepsy; Cognitive Decline in Laboratory Animals: Models, Measures, and Validity; Cognition: Learning and Memory: Spatial; Knock-Outs: Learning and Memory; Role of Gene Transcription in Long-Term Memory Storage.

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Neural and Pharmacological Substrates of Aggression

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Glossary

Agonistic behavior – Behavior during aggressive confrontations, comprising threat displays and attack elements and defensive reactions, submissive displays, and escapes.

Dominance – Transmitting genes into the next generation via reproductive success, expressed by preferred access to fertile mating partners. Different types of dominance may comprise despotic dominance or relative dominance in a linear or more complex hierarchy.

Violence – Aggressive behavior of high intensity and injurious, often impulsive, oblivious to signals of appeasement.

has been challenged by the rare, but repeatedly documented episodes of intensely injurious aggression in chimpanzees that pursue neighboring groups in order to kill them. The significance of lethal raiding parties in hominids constitutes a problem for the typology of aggressive behavior, since it focuses on rare events that are carefully planned and coordinated and, at the same time, involve intense autonomic and behavioral excitement. The adaptive purpose of these lethal raids eludes an adequate theoretical framework. Conventionally, once the frequency, duration, and intensity of aggressive behavior escalate beyond the species-typical levels, it is considered maladaptive and may constitute a behavioral pathology in need of intervention.

Much pharmacological, neurochemical, and molecular biology research on aggressive behavior uses laboratory mice, often recombinant inbred strains and transgenic lines. These animals are housed in groups, preventing the species-typical formation of demes (breeding units), and only a small proportion of these mice actually engage in territorial aggressive behavior that is characteristic of this pugnacious species. Under captive laboratory conditions, group-housed animals may develop a despotic social organization, with one male dominating all other group members.

Types of Aggression

Social experiences shape future aggressive acts via action on molecular events in discrete ascending aminergic pathways from the midbrain and pons to the basal forebrain, and the cascade of these events is increasingly understood. The adaptive significance of aggressive behavior is apparent in all phyla of living creatures subserving chiefly reproductive purposes. Maladaptive types of aggressive behavior are the focus of human and veterinary medicine and also of the criminal justice system. Types of aggression differ in terms of their origins, motivations, expression or functions, and all indications are by their neurobiological mechanisms. Clinicians distinguish between those types of aggression that include hostile, affective, impulsive, and reactive features from those that are characterized by proactive, premeditated, instrumental, controlled, and also predatory elements. Behavioral researchers, by contrast, focus on adaptive types of aggressive behavior, mostly as part of reproductive strategies such as aggression during the formation and maintenance of dominance hierarchies or territories. Animal species that disperse during the reproductively active lifespan as well as those that live cohesively, engage in aggressive behavior in order to secure the resources for reproduction or to protect the offspring.

The classic ethological thesis that ritualized displays are the major means to maintain a dominance hierarchy

Neurotransmitters

More than any other neurochemical system, brain serotonin remains the focus for neurobiological studies of mechanisms, particularly those mediating impulsive, hostile and intensely violent outbursts, as well as predatory-like aggression. Relative to plants, only present in trace amounts in mammalian brain, this phylogenetically old transmitter, arising from cells along the center of the neuroaxis and acting on at least 14 different receptor subtypes, has a significant role in aggression ranging from invertebrates to humans. Several neurotransmitters comprising amines, acids, neuropeptides, and neurosteroids interact with serotonin (5-hydroxytryptamine, 5-HT).

Glutamate

The ubiquitous excitatory and inhibitory transmitters, glutamate and gamma aminobutyric acid (GABA),

modulate the cellular and behavioral effects of serotonin at several levels of the ascending pathways from the midbrain and pons to the basal forebrain pathways. Previous research has established an excitatory role of glutamate in violent outbursts during seizures, although the precise role of glutamate activity during ictal and inter-ictal events remains to be defined. The few studies that have investigated how pharmacological manipulations of glutamate receptor subtypes affect aggressive behavior suggest a potential role of *N*-methyl-d-aspartic acid (NMDA) receptors in escalated aggressive behavior. Low-affinity channel blockers such as memantine or partial agonists to the glycine binding site on the NMDA receptor may offer potential options in the pharmacotherapeutic management of escalated aggressive behavior due to their favorable side-effect profile. Regulatory changes in NMDA receptor systems occur also in individuals who are repeatedly victimized by aggressors as revealed by prevention of their sensitized response to psychomotor stimulants with protective administration of NMDA receptor antagonists. It will be of considerable interest to learn how glutamate modulates the ascending monoaminergic, especially dopaminergic and serotonergic projections to limbic and cortical target areas.

Gamma Aminobutyric Acid

In contrast to glutamate, GABA and particularly the GABA_A receptor complex have been consistently implicated in the neural control of several types of aggressive behavior. Especially positive allosteric modulators of the GABA_A receptors such as benzodiazepines, barbiturates, ethanol, and allopregnanolone can increase aggressive behavior after low acute doses or after tolerance to the sedative doses has developed. By contrast, at moderate and higher doses, the anti-aggressive effects of these substances are accompanied by sedation and motor impairment. The bidirectional effects of allosteric positive modulators of the GABA_A receptors depend not only on the dose, but also on the context and the prior experience with aggressive behavior. When social consequences lower the rate of aggressive behavior, benzodiazepines and ethanol are more likely to increase its occurrence. The current challenge is to understand how an individual's prior experiences with aggressive behavior modify the GABA_A receptor complex so that pro-aggressive effects of GABA_A positive modulators emerge. The prevalent current hypothesis attributes the divergent effects of GABA_A positive modulators on aggressive behavior to differential expression of genes encoding the subunits that form the pentameric GABA_A receptor complexes. Emerging data from gene deletion and pharmacological antagonism studies suggest a structural dissociation between the anxiety-attenuating, sedative

and aggression-heightening effects of GABA_A receptor positive modulation, primarily due to the differential role of alpha subunits. In addition to the GABA_A receptor, the GABA_B receptors are widely distributed throughout the neuroaxis. The population of GABA_B receptors in the dorsal raphe nucleus modulates serotonin cells, and this may be the mechanism via which GABA_B receptor agonists increase aggressive behavior in mice.

Norepinephrine

Reciprocal anatomical links between catecholamine and serotonergic pathways provide the basis for extensive functional interactions. Particularly intense arousal that is associated with salient life events, including certain types of aggressive behavior or just observing a fight, is based on elevated activity in noradrenergic cell bodies in the locus ceruleus and in cortical noradrenergic terminals. Pharmacological blockade of beta receptors may achieve its calming effects in patients with intensely aggressive, hostile outbursts by reducing noradrenergic hyperactivity, although alternatively, beta blockers also act as antagonists at 5-HT_{1A} receptors. Molecular manipulations of the genes encoding for the noradrenergic transporters or metabolic enzymes have so far resulted in inconsistent results.

Dopamine

Specific dopamine (DA) pathways and DA receptor subtypes critically contribute to the neurobiological mechanisms of species-typical and escalated, pathological types of aggressive behavior. Anatomical and pharmacological data provide evidence for serotonergic receptors on soma of DA neurons in the VTA and substantia nigra suggesting modulation of ascending DA pathways by 5-HT.

The most widespread option for pharmacotherapeutic management of aggressive individuals relies on antipsychotic medication that acts via blockade of DA D2 receptors, although the anti-aggressive effects of such agents as haloperidol or chlorpromazine are embedded in sedative and motor incapacitating side effects. At present, so-called atypical antipsychotic drugs with less selective mechanisms appear to be preferred as anti-aggressive medication on account of a more favorable side-effect profile. Clearly, there continues to be a need for more satisfactory medication development. Case studies point to amphetamine intoxication as a potentially triggering event for lethal violence. At intermediate doses, amphetamine disrupts many types of social behavior and at lower doses, it may increase aggressive behavior due to its antifatigue and arousing effects. Increased

corticolimbic DA can be detected via *in vivo* measurement and imaging techniques in individuals who react defensively to an aggressive confrontation and who prepare for such an event. Anatomical and temporal analysis with higher resolution may enable a more precise delineation of DA activity in different phases and types of aggressive behavior.

Disruption of the genes that are critically involved in the inactivation of catecholamines, Catechol-O-methyl transferase (COMT) and monoamine oxidase (MAO)-A can promote aggressive behavior in male mice. It is tempting to relate these preclinical data to the specific polymorphism in the gene for COMT which is associated with increased aggressive behavior in schizophrenic men.

Monoamine Oxidase

Considerable evidence links MAO-A to escalated types of violent behavior. Which of the monoamines is primarily responsible for the effects of mutations or deletions of the gene for this enzyme or for the effects of its pharmacological inhibition requires further analysis. An early study illustrated how acts of violence by the male members of a Dutch family suffering of mental retardation appear to be linked to a missense mutation in the gene for this enzyme on the X chromosome. Mice lacking this gene also initiate injurious aggressive behavior faster. Probably the most significant findings link the allelic variant with low activity of MAO-A to the antisocial and violent behavior only in those adult males who were severely maltreated in childhood, whereas the allelic variant with high MAO-A activity or the absence of maltreatment had no such influence in adults. A variable number tandem repeat polymorphisms of MAO-A may also contribute to the increased probability of a life history of aggressive behavior, particularly aggression involving dysregulated affect. However, there is also evidence that links lower MAO-A activity to aggressive tendencies independent from polymorphisms in MAO-A. While one of the leading candidates, a number of inconsistent findings obscure the causal relationship between the expression of MAO-A gene, its interaction with early life experiences and traits of hostile, antisocial aggressive outbursts.

The differential expression of specific genes for MAO-A in aggressive and nonaggressive individuals is often associated with alterations in the brain serotonin system, based on additional pharmacological studies. A rich array of methodological approaches has implicated the serotonin system in aggressive traits, impulsivity, and also in the initiation and termination of certain types of aggressive behavior. Beginning with the assay data from the hindbrain of isolated aggressive mice and from cerebrospinal fluid (CSF) samples of patients, an inverse correlation between 5-HIAA and trait measures of impulsive aggression was frequently, but not always, found. The

significance of CSF 5-HIAA measures is compromised by the uncertainty as to their precise anatomical origin. Direct challenges of brain 5-HT functions with either an agonist or a tryptophan-depleted diet demonstrated a blunted prolactin response in violent patients with various diagnoses, possibly due to actions on 5-HT_{1A} and 5-HT_{2A} receptors. Instead of relying on a single sample of CSF, a peripheral marker, or an endocrine response to a single pharmaco-challenge, *in vivo* microdialysis reveals no changes in cortical 5-HT during the phase of initiating an attack, but it begins to decline once the fight is progressing and terminating. The termination of an anticipated aggressive confrontation is accompanied by a decrease in accumbal 5-HT suggesting a potentially significant role for 5-HT in the inhibition of aggressive behavior.

Serotonin Transporter

Several receptor families and the serotonin transporter (SERT) have been characterized in terms of their genetic basis and molecular features. Pharmacological and molecular genetics studies have begun to implicate the 5-HT₁ and 5-HT₂ receptor families and SERT in different types of aggressive behavior. Agonists of the 5-HT_{1A} and 5-HT_{1B} receptors reduce aggressive behavior, and the anti-aggressive effects of the 1B receptor subtype are behaviorally specific and especially effective in situations that engender escalated levels of aggressive behavior, although this remains to be clinically verified. Microinjection studies provide evidence that 5-HT_{1A} and 5-HT_{1B} receptor agonists can achieve their anti-aggressive effects via action at either somatodendritic autoreceptors in the dorsal raphe nuclei or the presynaptic terminal autoreceptors or postsynaptic heteroreceptors (Figure 1). If in fact the decrease in extracellular levels of corticolimbic 5-HT after 5-HT_{1A} and 5-HT_{1B} receptor stimulation constitutes a critical mechanism of action for the anti-aggressive effects, a significant revision of the serotonin deficiency hypothesis is required. Genetic deletion studies of 5-HT_{1A} and 5-HT_{1B} receptors generate a more complex pattern of results that appears to be influenced by the genetic background of the mouse or by developmental compensations. Similarly, associations between polymorphisms of 5-HT_{1A} and 5-HT_{1B} receptors and aggressive traits in humans remain inconsistent.

5-HT Receptors

Antagonism of 5-HT_{2A} receptors represents the mechanism through which some atypical antipsychotic compounds achieve their calming effects in patients with diagnoses that range from schizophrenia, dementia, depression, and posttraumatic stress disorders. Yet, the

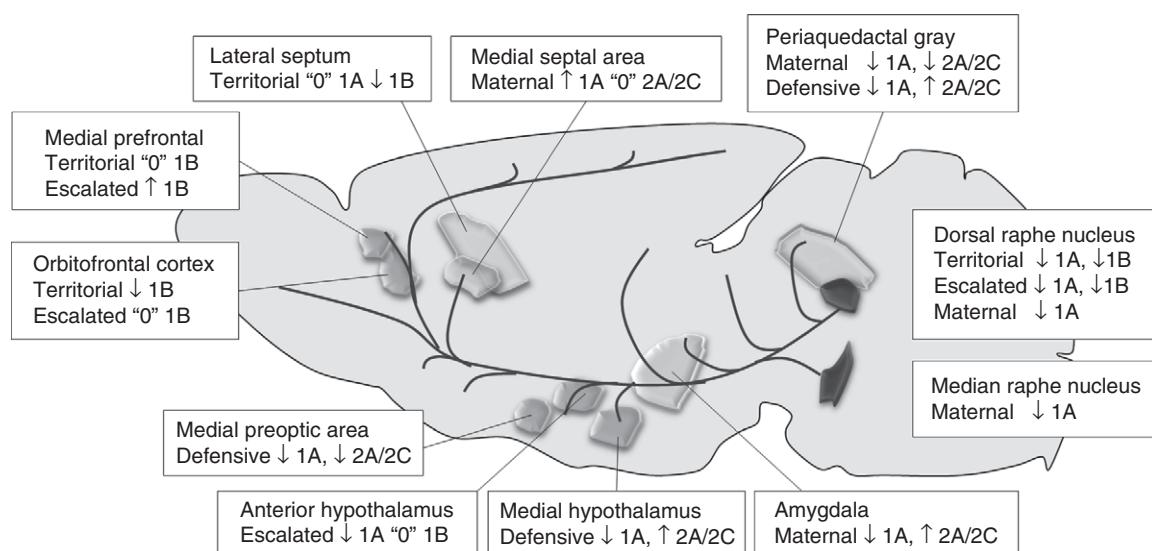


Figure 1 Modulation of aggressive behaviors in rodents by microinjections of 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A/2C} receptor agonists. Text boxes show that local injections of 5-HT_{1A} receptor (1A), 5-HT_{1B} receptor (1B), or 5-HT_{2A/2C} receptor (2A/2C) agonist increases (↑), decreases (↓), or has no effect ('0') on territorial, escalated, maternal, and defensive aggressive behaviors. Serotonergic neurons originate from the raphe nuclei and project to several brain areas. Reproduced with permission from Quadros IM, Takahashi A, and Miczek KA (2009) Serotonin and aggression. In: Muller C and Jacobs B (eds.) *Handbook of Behavioral Neurobiology of Serotonin*. Amsterdam: Elsevier.

side-effect profile of these agents highlights the problematic nature of this new class of antipsychotic agents. Preclinical studies of the 5-HT_{2A} and 5-HT_{2C} receptors have to await the development of more selectively acting molecular tools, since at present it is not possible to differentiate between the anti-aggressive effects of agonists and antagonists at these receptors. Similarly, linkage studies between polymorphisms in the 5-HT_{2A} receptor and impulsive-aggressive or antisocial traits require replication.

Blockade of the reuptake mechanism for 5-HT, the SERT, reduces aggressive episodes in most patients, especially when given over extended periods. Large meta-analyses have identified the exceptional nature of the occasional reports of increased aggressivity and suicidal tendencies among those treated with selective serotonin reuptake inhibitors (SSRIs). Preclinical studies have shown that acute and chronic treatment with SSRIs reduces aggressive behavior in species ranging from invertebrates to primates. Chronic SSRI administration can also restore competent agonistic interactions in laboratory species that do not show intact species-typical aggressive behavior.

The short length allele in the serotonin-transporter-gene-linked polymorphic region (5-HTTLPR) leads to lower SERT expression and lower serotonergic activity relative to those with the long length allele. Some evidence supports the association of the short allele with increased hostility, impulsivity, and aggressiveness, primarily in males. The contribution of the 5-HTTLPR to

the variation in aggressive personality traits is relatively small and appears to depend on epistatic influences and on environmental triggers. Early stressful life experiences in monkeys and humans may increase the probability of escalated aggression toward others and themselves in those individuals who carry the short length allele. A more adequate understanding of SERT expression in corticolimbic regions promises to be relevant for the display of aggressive personality traits.

Brain serotonin modulates and is modulated by other amines, amino acids, as well as neuropeptides and neurosteroids. For example, serotonergic projections in specific hypothalamic nuclei may regulate the release and action of vasopressin (VP), a neuropeptide that is associated with high rates of aggressive behavior in several animal species via action at 5-HT_{1A} and 5-HT_{1B} receptors. Similarly, the modulation of serotonergic neurons by corticotrophic releasing factor (CRF) and opioid peptides provides the anatomical basis for functional interactions that are relevant to aggressive behavior. The promising information on CRF, GABA, and glutamate in amygdaloid connections with hypothalamic and brainstem structures during displays of intense emotion should prompt a detailed examination of these mechanisms in escalated types of aggressive behavior.

See also: Genes and Behavior: Animal Models; Neural Bases of Defensive Aggression; Offensive and Defensive Aggression; Social Communication; Stress and Social Behavior.

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Neural Basis of Attention-Deficit/Hyperactivity Disorder

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Glossary

Amphetamines – Sympathomimetic drugs that are stimulators of the central nervous system.

Antidepressants – Drugs used to treat depression; the tricyclic and serotoninergic antidepressants are the most important pharmacological classes.

Basal ganglia – A group of deep nuclei located in the telencephalon and diencephalon; it includes the lenticular nucleus (subdivided into putamen and globus pallidum), the caudate nucleus (which forms the neostriatum with the putamen), the thalamus, and several small nuclei.

Continuous performance task – Task assessing the ability to sustain attention required to detect rare targets appearing among distractors; it usually requires answering to the target by the depressing of a response key.

Controlled processes – These processes involve executive functions and are opposed to automatic processes; they are operating under the direct conscious control of the subject and are especially involved in novel, conflicting, or complex situations.

Executive functions – Control functions mainly subserved by the prefrontal cortex and especially involved in nonroutine situations (novel, conflicting, or complex tasks); deficit of executive functions (labeled ‘dysexecutive disorders’) induces behavioral disorders and impairment of several tests such as Stroop, Go/No-go tests, set shifting, and planning tests.

Frontal lobe – This cerebral lobe, the largest in the human, is located in the anterior part of the brain. It is subdivided into three major parts: mesial regions (belonging to the paralimbic system and including the Brodmann’s areas 32 and 24), basal region, and the lateral region which contains most of the prefrontal cortex (corresponding to supramodal associative cortex involved in higher-order cognitive processes).

Go/no-go – Tasks requiring to answer to one class of stimuli (Go) and to suppress answers to another class (No-go); as ‘No-go’ stimuli are chosen to naturally trigger answers, the erroneous responses to ‘No-go’ stimuli reflect deficit of inhibition of prepotent responses.

Noradrenergic system – It originates in the locus ceruleus (a small nucleus located in the brainstem) and projects to the cortex.

Planning – It reflects the ability to preprogram complex actions prior to execution; it is usually assessed by various tower tasks requiring to move balls to achieve a predetermined spatial configuration. The complexity is assessed by the number of moves required.

Set shifting – The ability to shift from one set of action (e.g., sorting cards using the rule ‘color’) to another one (e.g., sorting cards using the rule ‘number’).

Stroop task – Task assessing the ability to focus attention on the relevant dimension of a stimulus; it is usually assessed using words of color names printed with ink of a different color; subject has to name the ink color (e.g., the word ‘RED’ printed in blue ink requires the answer ‘blue’).

Introduction

The domain of attention has evolved in the last decades. Thus, we first consider the major varieties of attention and its assessment for the nonexpert reader. The second section details criteria and major characteristics of attention-deficit/hyperactivity disorder (AD/HD), including underlying deficits of attention and cognition, which are critical to examine neural basis. Finally, brain regions associated with attentional processes are briefly mentioned before developing the neural basis of AD/HD.

Varieties of Attention

Attention represents a large variety of phenomena (**Table 1**) which are critical for enhancement of efficiency of cognitive processes. Attention is frequently subdivided into two main domains: (1) intensity which includes sustained attention and alertness and (2) selectivity which refers mainly to focused attention and selective preparation. ‘Sustained attention’ (also denoted vigilance) is the ability to maintain an optimal level of performance during a prolonged task (frequently longer than 10 min) which is usually monotonous (such as the detection of rare targets occurring among distractors). Sustained attention is usually examined by performance decline (indexed by reaction time (RT), hits, and false alarm) over time. Such paradigms have been extensively studied in normal

Table 1 Main attentional processes

Attention varieties	Definition	Performance Index	Illustration of key situation
Sustained attention	required to maintain an optimal level of performance during a prolonged task	performance decline over time	Radar monitoring
Alertness	ability to increase response readiness following a warning signal	Increased response speed following a warning signal	'attention!'
Selective attention	efficiency increase related to a cue providing information about the characteristic of target stimulus (e.g., stimulus location)	performance gain conferred by a cue	'Attention on your right!'
Divided attention	involved in situations requiring the performance of two tasks in combination (usually simultaneously)	performance decrement in dual-task condition	to drive and speak to someone

subjects. 'Alertness' (frequently denoted phasic alertness) is the ability to increase response readiness (usually indexed by RT) for a short time-period following a warning signal which indicates the stimulus occurrence. 'Selective attention' is defined by performance improvement following a cue which reduces uncertainties about target stimulus (e.g., stimulus location), task, or response processes. Selective attention is also involved in tasks requiring the inhibition of a prepotent response or process such as in the Stroop task. Selective attention is usually indexed by RT gain conferred by a cue delivering information about signal to be processed. Finally, 'divided attention' is involved in situations requiring the performance of two tasks in combination (usually simultaneously). Divided attention is indexed by performance decrement in the dual-task condition (i.e., when both tasks are performed simultaneously) as compared with the single-task condition (i.e., when performance is measured in each task performed in isolation). These varieties of attention have different neural bases and the present article focuses on attentional processes involved in AD/HD, that is, sustained and selective attention.

Attention-Deficit/Hyperactivity Disorder

General Characteristics of AD/HD

AD/HD is one of the most common childhood disorders. The core symptoms include a severe deficit of attention often associated with impulsivity and motor hyperactivity which interfere with many areas of normal development, learning, and functioning in childhood. In 1902, Still reported the first description of children with hyperkinetic syndrome. The symptoms of AD/HD, as defined by the 4th edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) include inattention and hyperactivity/impulsivity that lead to difficulties in educational, social, and family contexts. Diagnostic criteria require at least six of nine symptoms of each domain in the DSM-IV (AD and hyperactivity-impulsivity) lasting

more than 6 months. These symptoms need to be permanent, that is, present anywhere and independently from environment (at school and at home). The early age of onset is also important: symptoms have to be present before the age of 7 and are usually reported in the first 5 years of life. Motoric hyperactivity most often begins with walking while AD is generally observed when children start school.

DSM-IV provides diagnostic criteria for three subtypes of AD/HD: primarily hyperactive/impulsive (AD/HD-HI), primarily inattentive (AD/HD-IA), and combined type (AD/HD-C). The existence of AD/HD subtypes remains controversial. The hyperactive/impulsive form is relatively rare and is often conceptualized as a precursor of the combined subtype because attentional problems emerge during childhood in many children initially meeting criteria for AD/HD-HI.

Epidemiology

Epidemiological studies in the general population of children and adolescents provide different prevalence rates according to study population and methods, especially diagnostic tools and criteria. Studies using DSM-IV criteria have provided prevalence rates between 3% and 5%. Some studies have shown that the prevalence is higher for the urban than the rural population and increases as a function of lower socioeconomic class. AD/HD is much more common in males both in clinical (9:1) and epidemiological (4:1) samples. A referral bias may contribute to this sex-ratio since girls have primarily inattentive AD/HD and less frequently hyperactive and impulsive symptoms.

Diagnosis and Neuropsychological Assessment

The diagnostic evaluation is based on medical history, parents and teachers questionnaires, the AD/HD Rating Scale, and semi-structured interviews.

Neuropsychological assessment is useful to determine whether difficulties to sustain attention and distractibility are due to true AD. Children with AD/HD have usually a low processing speed index as assessed on the Wechsler Intelligence Scale for Children (WISC-IV). Computerized RT tests provide a more objective index of attention and inhibition, the Conner's Continuous Performance Task being the most widely used.

Attention and Cognitive Deficits Underlying AD/HD

Recent advances in cognitive neuroscience lead to a better understanding of mechanisms underlying AD/HD symptoms and related cerebral networks. Sustained AD is usually thought to be the key deficit in AD/HD. However, studies using computerized Continuous Performance Task showed inconsistent or negative findings supporting that symptoms in AD/HD are not attributable to a true sustained AD *per se*.

Recent studies indicate that AD/HD children suffer from disorders of executive functions and suggest the core deficit concerns inhibition. Studies have documented deficits of various executive processes, including planning, set shifting, and inhibition of prepotent responses. The inhibitory hypothesis of AD/HD provides a good account of clinical symptoms observed in daily life and clinical examination, especially excessive distractibility, as well as deficits on Go/No-go or Continuous Performance Tasks. Although less studied, children suffering from AD/HD show robust slowness and variability of RT in various experimental tasks. This finding could reflect a more diffuse AD than a specific inhibitory deficit. However, convergent data suggest that slowness in AD/HD especially concerns tasks involving controlled processes (i.e., involving executive functions) than those involving automatic processes.

Etiology and Comorbidities

The etiology of AD/HD is unknown and is probably multifactorial. AD/HD is probably the result of the interplay of both psychosocial and biological factors.

Genetic factors have been suggested. Family aggregation studies have shown that AD/HD do run in close family members and adoption studies favor the role of a genetic rather than environmental factor. Consistently, the concordance rate has been estimated between 50% and 66% in monozygotic twins and around 30% in dizygotic twins. Twin studies indicate heritability of 80–90%. A number of chromosomal regions containing potential AD/HD predisposing loci have been identified from family-based linkage studies, including 5p, 6q, 7p, 11q, 12q, and 17p.

Several medical conditions may be associated with AD/HD although AD/HD does not necessarily occur as a result of them. Some prenatal or perinatal factors have been found to be involved, such as fetal alcohol syndrome, severe prematurity, and consumption of caffeine during pregnancy. Genetic condition such as fragile X syndrome, Williams's syndrome, or peripheral resistance to thyroid hormones may also be associated with AD/HD. At least 20% of children with epilepsy have features of AD/HD that begin generally before seizure onset. Finally, postnatal brain injury, lead poisoning, or pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection are often complicated by AD/HD. Comorbid disorders are frequent in AD/HD and include learning disabilities, oppositional defiant disorder, conduct disorder, anxiety, depression, and Tourette's syndrome.

Treatment

The management of AD/HD requires a combination of pharmacological treatment and psychosocial interventions. The psychopharmacological agents are stimulants: amphetamines (mainly dextroamphetamine), pemoline, and methylphenidate, the latter – a stimulant that enhances catecholaminergic systems innervating the fronto-striatal regions – being the most widely used. A minimum of 70% of children with AD/HD have a positive response to psychostimulants. The stimulants bind to dopamine and norepinephrine transporters, blocking reuptake and facilitate release (d-amphetamine only) of dopamine. Efficacy has been clearly demonstrated in reducing AD, improving processing-speed index and inhibitive ability, as well as internalizing symptoms and positively impacting social behavior and academic performance. It is now clear that stimulants are not only 'school time drugs' but should be used throughout the waking day and on weekends. All stimulants share side effects (mainly decreased appetite, sleeping difficulties, headache, stomachache, irritability, and growth reduction related to decreased appetite). There is strong evidence that methylphenidate use does not lead to substance abuse. Second-generation, extended-release, and long-acting stimulants are now available. Other pharmacological nonstimulant agents are sometimes used 'off-label' such as tricyclic antidepressant, α_2 -agonists, or buproprion. More recently, atomoxetine – a specific norepinephrine reuptake inhibitor – has been approved for the treatment of AD/HD.

A recent review of evidence-based psychosocial treatments for children and adolescents with AD/HD indicates that there is adequate evidence to support their efficacy, especially behavioral parent training and behavioral school interventions. The largest and most influential study on the treatment of AD/HD is likely

the Multimodal Treatment Study of children with AD/HD study. This study demonstrates that combined pharmacological and behavioral therapy improves core areas of AD/HD and that families also benefit from this approach. Additionally, the combined approach (pharmacological and behavioral interventions) results in less challenging behaviors and allows prescription of lower doses of medication.

Neural Basis of AD/HD

Anatomy of Sustained and Selective Attention in Adults

Few studies have examined the anatomy of sustained and selective attention, and we only focus on those assessing (1) location of focal brain lesions associated with deficits of these attentional processes and (2) brain regions activated during functional imaging study involving these attentional processes.

Considering 'sustained attention,' the study of patients with focal lesion (especially stroke) has led to controversial results. Excessive fatigability and inability to maintain the ongoing activity are frequently observed clinically in frontal-lobe damaged patients. Although this behavioral disorder suggests a deficit of sustained attention, studies formally assessing performance-decline over time have showed either normal performance or a mild decline in both frontal and posterior lesions. This issue remains largely unresolved because of low test sensitivity or assessment of mixed pathologies, including traumatic brain injury (which induces diffuse lesions and thus prevents determination of the lesion location accounting for the cognitive deficit). The ability to 'sustain alertness' during a task has been found to be impaired in frontal damage, especially when the lesion concerns the superomesial region with a prominence in the right 24 and 32 Brodmann's areas. These regions are usually activated in functional activation studies using the sustained-alertness paradigm as well as other regions including the dorsolateral prefrontal, inferior parietal, thalamic, and pontomesencephalic regions. The role of the noradrenergic system originating in the locus ceruleus and projecting most strongly to frontal areas (at least in rats) has been suggested and is consistent with the finding of pontomesencephalic activation regions. Despite some negative results, most adult studies based on lesion and activational paradigms indicate that the superomesial region is critical for sustained alertness with a hotspot probably centered on the right 24–32 Brodmann's areas, and that a large attentional network is usually involved in activation studies including the lateral frontoparietal cortices and the thalamic and pontomesencephalic regions.

The neural basis of 'selective attention' has been examined in a large number of studies. Patient studies have

mainly used the Stroop and Go/No-go tests and have showed deficit in the frontal focal lesion. Within the frontal lobe, the impairment of selective attention is prominent when the lesion concerns the superomesial region in the Stroop task, and for the Go/No-go task, when it includes the superior part of the premotor–frontal regions with a prominence of the mesial side in some studies, or the lateral side in others. Most functional imaging studies have underlined the activation of the anterior cingulate (frequently with a right hemispheric prominence) usually associated with widespread activation of both right and left dorsolateral prefrontal cortices in the Stroop and Go/No-go tests where the activation of the inferior and middle gyri and medial regions were prominent.

Dysfunction of Brain Circuits Associated with AD/HD

Neuroimaging data suggest that the dysfunction of the cerebello-thalamo-prefrontal circuit may subserve the overactivity and deficits of motor control, inhibition, and executive function encountered in AD/HD.

Structural imaging studies

Structural imaging studies involve four cerebral regions in AD/HD: the prefrontal cortex with a right prominence, basal ganglia (especially the caudate nucleus), the splenium of the corpus callosum, and the cerebellum (vermis lobules VIII–X). These results are consistent both in boys and girls. Children with AD/HD have a reduced volume in the rostrum and rostral body of the corpus callosum, a lack of normal left-right asymmetry in the anterior brain and caudate nuclei, and smaller globus pallidus. These findings are consistent with an alteration of functioning of the prefrontal and anterior cingulated cortices – two regions involved in executive functions. Dysfunctions in the cerebello-thalamo-prefrontal circuitry underlying connections between the cerebellum and prefrontal associative areas may explain subtle neurological signs that are detected in over 50% of children with AD/HD as well as impairment in the cerebellar–vestibular test. The developmental trajectories of these brain-volume abnormalities (except the caudate) remain roughly parallel for children with AD/HD and controls during childhood and adolescence. The lack of asymmetry and reduced volume are fixed, nonprogressive, and unrelated to stimulant treatment. This finding suggests genetic and/or early environmental influences on brain development in AD/HD.

Functional imaging studies

Functional imaging studies also support the role of striato-prefrontal circuitry. Studies using single photonic emission computerized tomography (SPECT) or positron emission tomography (PET) demonstrate decreased

cerebral blood flow and metabolism within anterior frontal regions and striatum in adolescents and adults with AD/HD. Functional magnetic resonance imaging (fMRI) studies have assessed regions activated during various response-inhibition tasks. During the Go/No-go task, AD/HD children demonstrate less activation within a network important for inhibition including the caudate, anterior cingulate, and dorsolateral prefrontal cortex. Methylphenidate restores the normal activation in these regions.

Abnormalities of catecholamine regulation

There is considerable evidence that norepinephrine and dopamine play a critical pathophysiological role although they are certainly not the only neurotransmitters involved in AD/HD. Both agents contribute to maintain alertness, to focus attention, and to control emotions and behavior. Low rates of these monoamines or their metabolites have been observed in children with AD/HD. Increased dopaminergic striatal activity on PET in children with AD/HD suggests that their symptoms result from excessive dopaminergic activity in the striatum and probably the nucleus accumbens. Several studies have reported high levels of dopamine transporters in the striatum of AD/HD subjects. Moreover, high cerebrospinal fluid concentration of metabolites of monoamines is correlated with the severity of AD/HD and with the response to stimulants.

The positive response to psychostimulants and antidepressants also suggests that catecholamines are involved in the pathophysiology of AD/HD. Psychostimulants, such as methylphenidate, inhibit recapture of dopamine and noradrenaline and enhance the postsynaptic effect of dopamine. Stimulants may also reduce hyperactivity by reducing dopaminergic activation of the striatum probably via inhibitory presynaptic autoreceptors. However, adults with AD/HD have lower amounts of dopamine released in the caudate in response to intravenous methylphenidate, and this blunted response to methylphenidate is related to inattention.

Finally, genetic studies also point out the prominent role of dopamine. Polymorphisms in genes have been found for dopamine D4- and D5-receptors, dopamine β -hydroxylase, and the dopamine-transporter gene which is involved in the action of methylphenidate.

Motor and cognitive symptoms in AD/HD are probably mediated differently. The dopamine transporter is expressed in considerably lower amounts in the cortex than the striatum suggesting that transporter inhibitors would have a weaker ability to increase dopamine efflux in the cortex than the striatum. The positive effects of methylphenidate on cognitive function seem to be due to the facilitation of dopaminergic activity while behavioral improvement (hyperactivity and impulsivity) may be mediated by reduction in dopaminergic stimulation.

Hyperactivity may result from striatal dysfunction whereas AD may be related to prefrontal dysfunction. Indeed, decrease of dopamine concentration has been observed in prefrontal synapses and this may account for the deficit of inhibition. The increase of dopaminergic postsynaptic activity obtained with methylphenidate may enhance the integration of relevant signals from other cortical regions and the efficiency of executive functions.

Implication for General Management and Directions for the Future

AD/HD is a descriptive syndrome whose developmental pathways and causal mechanisms are not fully understood. The most scientific investigations to date focused on mechanisms involved in behavioral and cognitive dysfunctions that lead to a better understanding of the neural basis of AD/HD. The question about the causes and particularly the part of endogenous versus exogenous contributions is not resolved. The role of genetic factors, developmental pathways, and experiential factors needs further research. A better understanding of the pathophysiology of AD/HD would help for delineation of diagnostic tools and may contribute to finding alternative pharmacological and multimodal approaches for the treatment of AD/HD. The wide variability of response to medications and the heterogeneity of this syndrome need the development of personalized treatment.

See also: Aging and Cognition; Attention and Speed of Information Processing; Behavioral Pathologies in Nonhuman Primates; Behavioral Planning: Neurophysiological Approach of the Frontal Lobe Function in Primates; Cognition: Attention and Impulsivity; Neural Basis of Working Memory; Neuropsychological Aspects of Anxiety Disorders; Voluntary Movement: Control, Learning and Memory.

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Neural Basis of Classical Conditioning

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Glossary

Amygdala – An almond-shaped mass of nuclei that are located deep within the temporal lobes of the brain, adjacent to the hippocampus and medial to the hypothalamus. The structure has been implicated in emotional learning and memory.

Discrimination/reversal conditioning – A conditioning procedure that involves the presentation of two conditioned stimuli (CSs), one that is followed by an unconditioned stimulus (US; the CS+) and one that is not (the CS−). The task is for the subject to learn to respond with a conditioned response (CR) to the CS+ while not responding to the CS−. During the reversal stage, the CS+ and CS− are switched.

Hippocampus – A horseshoe-shaped area of cells buried deep in the forebrain. The structure has been implicated in learning and memory as well as in contextual and spatial processing.

Inferior olive – A large nucleus located deep in the medulla oblongata that has connections with the thalamus, cerebellum, and spinal cord.

Instrumental conditioning – A type of associative learning in which there is a contingency established between an observed behavior and the presentation of a biologically significant event.

Interpositus nucleus – The middle of three pairs of nuclei located in the brainstem that receives input from other brainstem nuclei, including the pontine nuclei and inferior olive as well as input from Purkinje cells in cerebellar cortex.

Latent inhibition – A conditioning procedure that involves presenting the CS alone several times before starting paired CS-US presentations. The pre-exposure to the CS slows down the rate of conditioning.

Long-term depression (LTD) – The weakening of a neuronal response at a synapse that could last from hours to days. LTD in the cerebellum is produced at the synapse between parallel fibers and Purkinje cells by the coactivation of climbing fibers from the inferior olive and mossy fibers from structures such as the pontine nuclei.

Long-term potentiation (LTP) – The strengthening of a neuronal response at a synapse that could last from hours to days. LTP has been demonstrated in the hippocampus and is produced by relatively high-frequency stimulation.

Medial prefrontal cortex – An anterior region of the frontal cortex that lies in front of the motor cortex. It has often been implicated in cognitive function.

Pontine nuclei – A large grouping of nuclei located in the pons. The brain region receives much input from virtually all areas of neocortex and, in turn, projects to many areas of the brain including the cerebellum.

Introduction

A very fertile area of research for experimental psychologists and behavioral neuroscientists for well over a century has been how the brain changes as a result of experience with the environment – that is, the neural substrates of learning and memory. For much of the twentieth century, research in this area focused on the involvement of neocortex and higher brain areas in learning and memory as exemplified by the classic experiments of Karl Lashley. Lashley used what on the surface seemed to be a very simple behavior – the learning and memory of mazes – to explore regions of neocortex involved in learning and memory. After many years of experiments he concluded that relatively large lesions of neocortex did not abolish learning and memory of this simple behavior.

Later studies involving amnesics suggested that at least one region of the brain, the hippocampus and associated temporal cortex, was involved in learning and memory processing. Subjects with damage to this brain area were found to be incapable of forming new memories for facts and events (i.e., declarative memories) even though they seemed to be able to form memories for skills and associative conditioning and able to demonstrate priming effects (i.e., procedural memories). The intact procedural memories suggested that learning and memory for these nondeclarative tasks might involve subcortical brain areas, a possibility that has subsequently been investigated by a number of researchers.

One approach that has been enormously successful in exploring how subcortical and cortical brain areas are engaged and changed during simple procedural learning and memory has involved the use of eyeblink classical conditioning. Indeed, use of this paradigm has arguably generated more data about the neural substrates of

learning and memory than any other approach. Eyeblink classical conditioning involves the presentation of two stimuli, a conditioned stimulus (CS) and an unconditioned stimulus (US), which are presented closely together in time. The CS is typically a discrete tone, light, or tactile stimulus while the US is typically an air puff directed onto the cornea of the eye or a very mild shock delivered to the skin overlying the musculature that surrounds the eye. The intensity of the US is normally set to elicit a robust eyeblink when presented, which is called the unconditioned response (UR). The intensity of the CS is normally set so that no behavioral response is elicited although in some species a small orienting or startle response that normally habituates after several presentations can be seen. Training consists of presenting the CS before the US on several discrete trials with onsets separated by 150–1500 ms (i.e., with a 150–1500-ms inter-stimulus interval, ISI). After several paired presentations, eyeblinks appear with onsets that begin before presentation of the US. These learned, anticipatory responses are called conditioned responses (CRs). Presentations of the US before the CS or unpaired presentations of the CS and US do not produce CRs.

The earliest eyeblink-conditioning studies were conducted using humans as subjects. However, as interest shifted to more cognitive phenomenon, humans were less frequently used as subjects and animal models of learning became more popular. In the late 1950s and early 1960s, Isadore Gormezano and his colleagues began using rabbits in eyeblink-conditioning experiments and developed an extensive data base on parametric and behavioral features of this basic form of associative learning. Recently, eyeblink-conditioning procedures for rats and mice have been perfected and these species have been used more frequently as subjects. In addition, the increased interest in translational research has resulted in a renewed interest in human eyeblink conditioning. Indeed, behavioral features as well as the neural substrates of eyeblink classical conditioning seem to be highly conserved across species, making this paradigm ideal for translational behavioral and neural research.

Much is known about the basic behavioral properties of eyeblink classical conditioning. For example, rates of conditioning increase when the intensities of the CS and/or US are raised. There is a well-defined ISI function that has been delineated by training subjects at various CS-US intervals and noting rates of conditioning. In rabbits, for example, the rate of conditioning is highest when the ISI is around 250 ms. Intervals less than 100 ms and greater than 2000 ms produce little learning. In general, two conditioning procedures have been employed – delay conditioning, in which the CS and US overlap in time to some extent, and trace conditioning, in which the CS is presented, a period of time is allowed to elapse, and then the US is presented.

Finally, an interesting feature of eyeblink conditioning is that in addition to the establishment of the CR to the tone, the acquired response is exquisitely timed. After learning, the peak of the CR occurs around the time that the air-puff delivery is anticipated, even on CS-alone trials. If the ISI is shifted during the experiment, the CR will move in time so that its peak corresponds to the new time when the air puff is expected. Thus, in studying behavioral features of eyeblink conditioning as well as the neural substrates of its learning and memory, two features of the learning have been explored – the acquisition and retention of the basic CR and the timing of its execution. This behavioral procedure has proven to be a powerful tool for studying how the brain changes during learning and memory.

Early Studies of the Neural Basis of Eyeblink Conditioning

In the late 1960s and early 1970s, a great deal of attention was focused on the involvement of the hippocampus and other higher brain areas in learning and memory due to a large extent on data from amnesics demonstrating severe memory impairments associated with hippocampal and temporal cortex damage. Of particular note were a series of studies that explored the learning-related activity of neurons in the hippocampus and related structures during eyeblink conditioning. In general, these studies showed that paired training, but not unpaired training, recruited populations of hippocampal neurons that discharged in close relation to the acquisition and execution of the learned response. Interestingly, the summed unit activity formed a rather precise amplitude-time course model of the CR, thus indicating that the hippocampus (and some related cortical areas) encodes the conditioning process. This recording work has been extended in several directions using variations of the basic conditioning procedure including studies of unit activity during (1) unimodal and multimodal discrimination and reversal training, (2) latent inhibition training, and (3) trace conditioning. Taken together, these studies indicate an engagement of the hippocampus and related cortical structures that occurs early in training. It also appears that the hippocampus is activated when features of the conditioning procedure are altered, such as when new CSs are introduced during training, the training context is changed, and perhaps when training is switched from one eye to the other.

The recording data provided strong evidence that the hippocampus was robustly engaged during eyeblink conditioning. However, even prior to when these recording studies were complete it was known that delay conditioning could be obtained in animals given complete removal of the neocortex and the hippocampus. The lesion data

suggested that while the hippocampal neurons were engaged during delay eyeblink conditioning, the structure was not necessary for CR learning and memory to occur; however, the same is not true for trace conditioning. There is solid evidence that hippocampal lesions given before training prevent learning of trace eyeblink conditioning and abolish CRs in animals trained before removal of the hippocampus. More recent data, however, suggest that this effect might be dependent to some extent on the relative lengths of the CS and the trace interval. From these data, the idea has emerged over the years that the hippocampus and related cortical areas are critically involved in trace conditioning and other relatively complex eyeblink-conditioning procedures (such as discrimination/reversal training and latent inhibition training). However, what is also clear is that while higher structures, such as the hippocampus, are engaged during the basic delay procedure, these structures are not necessary for conditioning to occur. This observation suggests that critical plasticity associated with the acquisition and performance of classically conditioned eyeblink responses occurs somewhere in the subcortical areas of the brain.

Later Studies of the Neural Basis of Eyeblink Conditioning

Around 1980, Richard Thompson and his colleagues at Stanford University reported a remarkable finding concerning the neural substrates of eyeblink conditioning that has defined the direction of research for the last 30 years. The Thompson group reported that lesions of the cerebellum, which included the interpositus nucleus, permanently abolished eyeblink CRs that had been established before the lesion. Lesions given before training prevented acquisition from occurring. These initial lesion studies have been followed up by several other experiments which have refined the location and extent of damage necessary to affect eyeblink conditioning. It appears that for rabbits, the critical area of the interpositus nucleus for eyeblink conditioning is about 1 mm³ of tissue located in the dorsomedial region of the anterior division of the nucleus; and the lesion effect is permanent – CRs do not reemerge with daily training sessions delivered for at least a year after the lesion. Perhaps the most compelling evidence for the critical involvement of the interpositus nucleus in eyeblink conditioning came from an infusion study conducted by Thompson and his colleagues, who infused the γ -aminobutyric acid (GABA) agonist muscimol into the interpositus nucleus during several days of paired CS and US training (which, in essence, produces a reversible lesion). During the training sessions with muscimol no CRs were seen. More interesting was the behavior of rabbits on subsequent training sessions when saline was infused instead of muscimol: the

rabbits behaved as if they were naive and had received no previous training (i.e., their rate of CR acquisition was identical to naive rabbits). These data provide irrefutable evidence that eyeblink conditioning is dependent on critical neuronal plasticity involving interpositus neurons. If plasticity, capable of supporting conditioning, occurred in one or more other brain areas one would expect CRs to emerge either immediately or relatively quickly after the training with muscimol was stopped. These data provide strong evidence that critical plasticity associated with basic delay conditioning is relatively localized within the cerebellum.

Lesions studies have also been conducted to explore the involvement of the cerebellar cortex in eyeblink conditioning. The results of these experiments have not been as definitive as the interpositus nucleus lesion studies. Two cortical regions have been studied: lobule HVI and a region located in hemispheric portions of the anterior lobe. A variety of effects have been reported for lobule HVI lesions that range from complete abolition of CRs to partial effects on the rate of conditioning and the size of the CR to little or no effect. Differences in results might be accounted for by factors such as lesion size and amount of postlesion training. What is clear is that it has been difficult to establish a precise role for lobule HVI in eyeblink conditioning using the lesion data. With regard to the anterior lobe cortical site, Mauk and colleagues have shown that anterior lobe lesions affect the timing and sometimes the execution of CRs, thus suggesting a role for the anterior lobe in conditioning. In addition to these ablation or electrolytic cortical lesion experiments, other manipulations have been used to study cortical function. For example, greatly impaired learning is seen in Purkinje cell-degeneration (pcd) mutant mice given eyeblink conditioning. Infusions of 6-cyano-7-nitroquinoxaline-2, 3-dione (CNQX) into lobule HVI, which reversibly blocks α -amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA)-kainate receptors, disrupts the expression of eyeblink CRs. It appears that picrotoxin infusions into the deep cerebellar nuclei, which disrupt cortical input to the deep nuclei by antagonizing GABA receptors, impair the acquisition of CRs when relatively long ISIs are used but not when shorter optimal ISIs. All of these lesion and inactivation studies suggest a role for cerebellar cortex in conditioning although the precise nature of that role is not as of yet well understood.

Neural recording studies have been used to strengthen the argument that the cerebellum is critically involved in eyeblink classical conditioning. Recordings taken from the anterior interpositus nucleus have revealed populations of neurons that alter the firing rates as a result of training. The predominant pattern of neuronal firing recorded from this population of cells was a significant increase in activity that preceded the onset of the behavioral response by 30–50 ms. Similar to what was seen in the hippocampus, the summed unit activity formed a

precise amplitude–time course model of the CR. The fact that activity precedes the behavioral response is extremely important – this is precisely what is expected if activity in that structure is responsible for generation of the behavioral response. The 30–50-ms lag time between interpositus activation and onset of the CR can be accounted for by synaptic delays between the cerebellum and brainstem nuclei responsible for CR generation. These recording data, coupled with the lesion data described above, argue strongly that the interpositus nucleus is a critical site for neuronal plasticity that underlies acquisition and performance of the classically conditioned eyeblink response.

Recordings from Purkinje cells in cerebellar cortex have revealed neurons that discharge in close relationship to the execution of the behavioral response. Two patterns of activation have been seen in lobule HVI: Purkinje cell inhibition, where the neuron slows or stops its spiking in relation to the CR, and Purkinje cell excitation, where the neuron increases its spiking in relation to the CR. Because Purkinje cells inhibit deep nuclear cells when they are activated, it is expected that inhibition of the cortical cells is involved in CR acquisition and performance. In fact, a cellular plasticity process known as ‘long-term depression’ (LTD) has been demonstrated in cerebellar cortex when populations of cerebellar projection fibers are conjunctively activated. The recording studies from lobule HVI, however, have consistently demonstrated a larger number of excitatory Purkinje cells than inhibitory Purkinje cells, which at this point in time begs an explanation.

Recordings from lateral regions of the anterior lobe of the cerebellum, however, have revealed Purkinje cell spiking patterns that are predominantly inhibitory in nature. An ISI discrimination procedure has been used to train rabbits to respond with appropriately timed CRs to two different ISIs signaled by two different CSs (i.e., low- and high-pitched tones). Recordings for individual Purkinje cells showed interesting patterns of CR-related responding. Some Purkinje cells discharged exclusively to one of the ISIs with a CR-related spiking train. Some Purkinje cells, however, responded to both ISIs with appropriately timed learning-related discharge patterns. Together, results of the lobule HVI and anterior lobe recording experiments provide strong evidence that discrete regions of cerebellar cortex are involved in eyeblink conditioning.

At about the same time, the critical involvement of the cerebellum in eyeblink conditioning was being established, the major neural pathways involved in getting the CS and US from the periphery to the cerebellum were delineated along with the output pathways from the cerebellum to motor nuclei responsible for recruiting eyeblink musculature involved in CR and UR production.

The crucial pathway for an auditory CS appears to involve projections from the cochlear nuclei to discrete

regions of the dorsolateral pontine nuclei, which, in turn, send mossy fibers axons to the interpositus nucleus and to granule cells in cerebellar cortex. Granular cell axons, known as parallel fibers, make synapses on Purkinje cells that, in turn, send axons to the same interpositus nuclear cells that receive direct mossy fiber input. This pathway was established through a series of stimulation, lesion, recording, and anatomical tract-tracing experiments. Direct electrical microstimulation of neurons in the dorsolateral pontine nuclei can be used instead of a tone CS for conditioning, and recordings from the region have shown auditory CS-related activity during tone conditioning. Discrete lesions placed in various areas of the pontine nuclei can selectively abolish learning to CSs of specific modalities and cutting the middle cerebellar peduncle, which is the source of pontine input to the cerebellum, results in loss of CRs. It appears that other CSs may engage other regions of the nuclei and relatively recent studies in rats have shown that there may be an important portion of the auditory CS pathway that engages the medial geniculate nucleus of the thalamus.

The major pathway for projecting US information to the cerebellum involves the dorsal accessory inferior olive. It appears that an air-puff US activates neurons in the trigeminal nucleus. The trigeminal nucleus, in turn, sends US input in two directions. First, some axons project to a region of the reticular formation, which then projects input to brainstem nuclei involved in eye-blinking – the reflexive UR pathway. Second, trigeminal axons course to the dorsal accessory olive where they make contact. The activated inferior olfactory neurons then send input to Purkinje cells along with collateral input to interpositus neurons that receive output from those Purkinje cells. A variety of experiments established this US pathway: lesions of the dorsal accessory olive produce either extinction or abolition of the eyeblink CR. Microstimulation of the dorsal accessory olive produced a variety of discrete movements (including eyeblinking if the stimulating electrode is positioned appropriately) that can be conditioned when paired with a peripheral or pontine-stimulation CS. In addition, a number of anatomical tract-tracing studies have established direct connections between critical regions of cerebellar cortex and the interpositus nucleus.

The output pathway for the CR from the interpositus nucleus has been delineated. Excitation of neurons in the interpositus nucleus activates neurons in the magnocellular division of the red nucleus. The red nucleus, in turn, sends axons to several brainstem nuclei that, dependent on the species being conditioned, are involved in generating the eyeblink CR, including the abducens nucleus, accessory abducens nucleus, and the facial nucleus. It appears that the interpositus nucleus also projects to the precerebellar regions involved in projecting the CS and US to the cerebellum. The projection from the

interpositus nucleus to the pontine nucleus appears to be excitatory in nature while the projection from the interpositus to the dorsal accessory inferior olive seems to be inhibitory in nature. It has been proposed that both of these projection systems may be important for regulating the activation of the CS and US systems during conditioning.

A Cerebellar-Brainstem System Critical for Eyeblink Classical Conditioning

Based on the relatively large volume of data described above, a number of formal and informal models have been developed to describe the neural substrates of eyeblink classical conditioning. These models differ from each other in the specifics as to exact locations where critical plasticity occurs during CS-US pairing (i.e., solely in cerebellar cortex, solely in the interpositus nucleus, or in both), but all agree that the cerebellum and associated brainstem structures contain the essential circuitry for this simple type of associative learning and memory.

It is clear that at least three regions of the cerebellum, the interpositus nucleus, lobule HVI, and the anterior lobe, are involved in eyeblink conditioning. This author has proposed that neurons in all these regions alter their firing patterns because of convergent CS and US input. Specifically, CS information is transmitted to the cerebellum through the basilar pontine nuclei via mossy fibers that terminate in cerebellar cortex and the deep nuclei. Information about the US is transmitted to the cerebellum through the inferior olivary complex via climbing fibers that terminate in cerebellar cortex and the deep nuclei. The firing patterns of neurons in the cerebellar cortex and deep nuclei are thought to be altered due to the convergent mossy fiber and climbing fiber input, presumably through LTD and long-term potentiation (LTP) mechanisms involving cortical Purkinje cells and likely through LTP-like processes in the interpositus nucleus (although scant data are available to support this conjecture). Because Purkinje cells only inhibit deep nuclear cells, it is assumed that LTD induced in the Purkinje cells phasically release nuclear cells from inhibition, thus encouraging or increasing their excitability. A powerful release-from-inhibition phenomenon has been reported for Purkinje cell input onto deep nuclear neurons and this may also play a role in establishment of the CR. The net effect of plasticity in the cerebellar cortex and interpositus nucleus is to increase firing of interpositus nucleus neurons, which in turn activates cells in the red nucleus, which then drive behavioral responses by activating neurons in brainstem nuclei known to be involved in generating eyeblinks. In other words, the changes in firing patterns in the interpositus nucleus neurons are critically involved in generating the

behavioral response – the interpositus nucleus is a critical location of learning and memory for this simple associative learning task.

In addition, as excitability changes occur in the interpositus nucleus there is evidence that these changes affect the CS and US pathways. For example, as neurons in the interpositus nucleus increase their firing rates after paired training, there is excellent evidence that output from the nucleus inhibits neurons in the inferior olivary complex that are known to transmit critical information about the US to the cerebellum. Thus, after CRs have been acquired it appears that further US input on subsequent trials is inhibited. Some elegant computational models have been forwarded that propose that the regulation of inhibition in the inferior olive by the interpositus nucleus may play a central role in the definition and regulation of the very precise timing of the CR that is observed. Ongoing work continues to refine this basic cerebellar model of eyeblink classical conditioning, especially in areas such as better defining the role of cerebellar cortex in the acquisition and retention of CRs.

The Involvement of Noncerebellar Brain Areas in Eyeblink Conditioning

As described above, the study of the neural substrates of eyeblink conditioning actually began with studies that examined the role of neocortex and the limbic system in conditioning. A focus on the cerebellum and brainstem emerged only after it was clear that the basic delay conditioning task could be learned with all tissue above the level of the thalamus removed. Does this mean that eyeblink conditioning does not engage or involve these higher brain regions? The answer to this question is an unequivocal no: while it is true that the cerebellum is necessary for all variations of eyeblink classical conditioning, it is clear that many brain regions are engaged during the learning and that these areas contribute in many ways to the learning and memory that takes place.

The involvement of the hippocampus and limbic system remains a central area of study. From data that has been collected, it appears that the hippocampus becomes increasingly engaged and important for conditioning as the procedure becomes more complex. For example, under some circumstances, trace conditioning is highly dependent on an intact hippocampus, perhaps especially so when the CS is of short duration and the gap between CS offset and US onset is relatively long (i.e., the demand for maintaining the CS in memory before US presentation is relatively great). It appears that the hippocampus is important for discrimination/reversal learning and perhaps for processing contextual information during conditioning. These data are consistent with a large

body of literature suggesting a central role for the hippocampus in memory processing.

Eyeblink classical conditioning, similar to many forms of learning, has an emotional component because it is an aversive conditioning procedure. During eyeblink conditioning as well as other Pavlovian procedures, changes in autonomic responding can be seen after the first few CS-US pairings, including changes in heart rate and respiration. A number of studies have shown that the medial prefrontal cortex and associated thalamic areas are involved in eyeblink conditioning. It appears that prefrontal lesions have little or no effect on simple delay learning. While the lesion studies did not support a role for the medial prefrontal cortex in the acquisition process or as a storage site for memory, it has been suggested that the cortical area is involved in promoting the persistence of the memory trace for the CS during the trace period, hence its critical role in this kind of conditioning.

There has been a lot of recent interest in exploring the involvement of the amygdala in simple associative learning, especially its role in encoding fear and emotional aspects of learning. For example, stimulation of the central nucleus of the amygdala causes an increase in the UR amplitude during conditioning. Amygdala lesions disrupt maintenance of facilitation of the eyeblink UR and slow down the rate of eyeblink conditioning, thus further suggesting that the amygdala may be involved in processing aspects of the US and UR. Recording data seems to support this idea. For example, the activity of amygdala neurons has been monitored during a variety of learning tasks, including eyeblink classical conditioning, classical fear conditioning, and aversive instrumental conditioning. Robust learning-related activation of the central nucleus of the amygdala was seen during acquisition of all of these tasks. The basolateral nucleus of the amygdala, however, showed little activation during the eyeblink-conditioning task but significant activation during the fear-conditioning and instrumental tasks. In general, the amount of learning-related activity appeared to be related to the relative intensity of the US presented during the task as well as the somatic requirements of the task.

Neural Substrates Appetitive Classical Conditioning

A number of years ago, an appetitive classical conditioning task in rabbits, known as ‘classical jaw-movement conditioning,’ was developed to parallel the aversive eyeblink classical conditioning task. During jaw-movement conditioning, a tone or light CS is presented before a rewarding intraoral water or saccharin US. The UR in this procedure is a rhythmic jaw movement that precedes consumption of the liquid. With CS-US pairings, presentation of the CS elicits the jaw-movement response in the absence of the

US. While initial studies used this procedure to study motivational influences on learning, later studies explored the neural bases of the appetitive learning. An important observation was that jaw-movement conditioning was not critically dependent on the interpositus nucleus of the cerebellum. While lesions of the interpositus nucleus completely abolished conditioned eyeblink responses, they had no effect on the performance of jaw-movement CRs recorded from the same rabbit. A central role for the hippocampus in jaw-movement conditioning has been demonstrated. For example, jaw-movement conditioning produces robust CR-related activity in the hippocampus, but the within-trial pattern of activity recorded during jaw-movement conditioning differed from activity recorded during eyeblink conditioning when both types of training were given to the same rabbit. These data suggest that higher brain areas are critically involved in encoding jaw-movement conditioning, and importantly, the cerebellum does not seem to play a critical role in encoding this appetitive conditioning task.

Classical Eyeblink Conditioning and Translational Research Approaches

Around 50 years ago, a shift was made away from using humans in eyeblink-conditioning studies and toward the development of animal models. As detailed above, the use of animal models have produced a wealth of data concerning how the brain encodes simple associative learning and memory. Interestingly, given the advancements recently made in our understanding of the brain correlates of eyeblink conditioning, there has been a recent renewed interest in the use of this procedure to study brain-behavioral correlates of clinical disorders – that is, eyeblink conditioning is being used in translational research programs interested in advancing our understanding of brain pathologies that accompany clinical disorders. The list of these translational efforts is indeed long and includes Alzheimer’s disease, Parkinson’s disease, fetal alcohol syndrome, obsessive-compulsive and anxiety disorders, schizophrenia, autism, and posttraumatic stress disorder, to name some.

The study of fetal alcohol syndrome provides a good example of this translational approach. This disorder is caused by excessive consumption of alcohol by pregnant mothers, which produces a variety of neural and behavioral deficits that depend on the timing and amount of alcohol that is consumed during pregnancy. One brain area that appears to be particularly susceptible to the alcohol-induced developmental insult is the cerebellum. Given the critical involvement of the cerebellum, eyeblink conditioning seemed to be an ideal behavioral paradigm to use concomitantly with experimental brain techniques to study brain-behavioral relations associated

with the disorder. A rat neonatal (i.e., third-trimester) model of the disorder was developed and it was shown that binge level exposures produced severe learning deficit in both juvenile and adult rats. Further, the neonatal alcohol exposure produced significant neuronal losses in regions of the cerebellum and brainstem known to be involved in eyeblink conditioning – lobule HVI of cerebellar cortex, the anterior lobe of cortex, the interpositus nucleus, and the inferior olivary complex. Unit recordings taken from the interpositus nucleus showed decreased CR-related activity that precisely predicted the observed learning impairment. In a recent study, children with fetal alcohol syndrome and related impairments were tested using the eyeblink-conditioning task. They showed severe deficits in conditioning as predicted by the animal model data and the large literature showing the involvement of the cerebellum in eyeblink conditioning. These data suggest there may be great value in working back and forth between the animal model and human clinical populations in describing the brain pathology and the behavioral phenotype associated with clinical disorders such as fetal alcohol syndrome.

Conclusion

The simplicity of the eyeblink classical conditioning procedure and the great experimental control that it affords have undoubtedly been key reasons that its use has advanced our understanding of the neural basis of conditioning more than any other behavioral procedure that is available. Indeed, the amount of neural and behavioral data generated through the use of eyeblink conditioning is perhaps rivaled only by the impressive amount of data on brain function that has been generated through the use of fear conditioning, another task that holds great promise for studying the neural substrates of learning. Future eyeblink-conditioning studies will undoubtedly further refine the nuances of the critical brain circuitries that have been described to date as well as explore molecular and cellular processes that underlie the neuronal plasticities that have been observed. In addition, the use of eyeblink conditioning in translational research aimed at uncovering the correlates of clinical disorders holds great promise for providing the keys to the mysteries of brain function and dysfunction that continue to be of interest to researchers from many fields.

See also: Active Avoidance and Escape Learning; Analysis of Learning in Invertebrates; Animal Models of Learning and Memory; Behavioral Planning: Neurophysiological Approach of the Frontal Lobe Function in Primates; Cerebellum: Associative Learning; Cognition: Learning and Memory: Pavlovian; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Eyelid Classical Conditioning; Fear, Anxiety, and Defensive Behaviors in Animals; Fear Conditioning; Neural Basis of Classical Conditioning; Neural Substrates of Conditioned Fear and Anxiety; Neural Substrates of Unconditioned Fear, Defense, and Anxiety; Neuron Excitability and Learning; Synaptic Mechanisms for Encoding Memory; Voluntary Movement: Control, Learning and Memory.

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Neural Basis of Gender

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Glossary

Activational effects – Effects of sex hormones that cause transient changes in brain and behavior. Examples are the effects of fluctuations of estradiol and progesterone levels during the estrous cycle on female sexual behavior.

Amygdala – Telencephalic area embedded in the temporal lobe that controls cognitive, behavioral, and autonomic responses to emotionally charged stimuli. In rats, the posteromedial nucleus is larger in males than in females, a difference that depends largely on activational effects of gonadal steroids. In humans, the amygdala shows sex differences in activation related to emotional stimuli.

Bed nucleus of the stria terminalis (BNST) – Telencephalic area located near the rostral pole of the lateral ventricle that controls behavioral and autonomic responses to emotionally charged stimuli. It shares architectonic and neurochemical characteristics with the central and medial divisions of the amygdala but differs in connectivity. In humans, its central subdivision is larger and contains more cells in males than in females except for male-to-female transsexuals, who show a female-typical anatomy. This suggests a role for the BNST in gender identity.

Kisspeptin – A recently discovered peptide that plays a role in the initiation of puberty as well as in the release of gonadotrophic hormone releasing hormone (GnRH). Kisspeptin neurons have been found in the arcuate nucleus and the anteroventral periventricular nucleus (AVPV). In rodents, the latter area is much larger and contains more cells in females than in males. The sex difference in the number of its kisspeptin neurons goes in the same direction, but is much more extreme. For example, male rats show virtually no kisspeptin cells in the AVPV.

Organizational effects – Effects of sex hormones exerted early in development that cause permanent changes in brain and behavior. Higher levels of testosterone during development, for example, make male mammals more likely to show male sexual behavior (masculinization) and less likely to show female sexual behavior (defeminization). If levels are low, a female pattern of behavior develops.

Random X inactivation – An epigenetic process by which one of the two copies of the X chromosome in female mammalian cells is inactivated.

Sexually dimorphic nucleus of the preoptic area (SDN)

(SDN) – Nucleus in the medial preoptic area that was first described in rats, where it is about 5 times larger in males than in females. Similar sex differences have been found in the preoptic area of other species, including humans. Currently, the most plausible function of the SDN is partner preference in males. There is no known function for this nucleus in females.

Sry – Gene on the Y chromosome that codes for sex determining factor, the protein that causes the primordial gonad to develop into a testis. Sry stands for sex determining region Y (chromosome). Sry is also expressed in the adult male brain and may thus influence neuron function in a sex-dependent fashion.

Vasopressin – Neuropeptide that was initially identified as a hormone synthesized by hypothalamic neurosecretory cells and released into the blood in the posterior pituitary (antidiuretic hormone). Vasopressin is also made by nonneurosecretory neurons and released in areas throughout the brain but especially in the limbic system, where it modulates autonomic process, social behaviors, and cognitive functions. In most vertebrates studied, vasopressin, or its nonmammalian homolog vasotocin, is expressed at higher levels in males than in females.

Practically, all reproductively active animals show sex differences in behavior. Sex differences in sexual behaviors are literally a fact of life. Likewise, sex differences in aggression and parenting were known long before there was any scientific interest in finding the cause of these differences. Our understanding of sex differences in cognitive behaviors has evolved from negative stereotyping and innuendo, usually intended to establish mental superiority of men, to a much more sophisticated understanding of the different ways in which humans and animals approach various cognitive tasks. Because behavior is generated by brains, they form a logical starting place for studying the physiological basis of behavioral differences. Sex differences have, in fact, been found throughout the brain, in almost any parameter imaginable. How these differences develop and what is their function forms the topic of this article.

Why Care about Sex Differences in the Brain?

There are sound medical reasons to study sex differences in the brain. Being born male or female influences the chances of developing specific neurological and psychiatric illnesses, the age at which symptoms develop, the course of illness, and perhaps even responsiveness to treatment. For example, women are twice as likely as men to become depressed, suffer from Alzheimer's disease, or develop multiple sclerosis, and at least 10 times more likely to develop eating disorders such as bulimia and anorexia nervosa. Boys are more than twice as likely to be dyslexic, suffer from Gilles de la Tourette syndrome, and 4 times more likely to be autistic or have an attention deficit and hyperactivity disorder than are girls. Men develop schizophrenia on average at an earlier age and the disease typically follows a more serious course than it does in women. Although societal factors may contribute to some of these differences, biology probably explains most of the variance. In some cases, the cause of the sex difference is clear. For example, Rett syndrome is a serious form of autism that is found almost exclusively in girls. It is caused by a spontaneous mutation of the MeCP2 gene. As this gene is found on the X chromosome, girls with Rett syndrome typically have one healthy and one mutated form of the MeCP2 gene. Having only one X chromosome per cell, male fetuses with this mutation typically do not make it to term. It has also been argued that the mutation occurs preferentially on the X chromosome derived from the father, which is found in girls only. The higher incidence of multiple sclerosis in women is another example that conforms to the higher incidence of autoimmune diseases in general. In this case, the cause of the sex difference appears to reside in the immune system rather than the brain. Rett syndrome and multiple sclerosis are exceptions, however. In most cases, we do not understand the cause of sex differences. As differences in the physiology and anatomy of the brain may contribute to differences in behavioral and neurological disorders, understanding the development and function of sex differences in normal brains is crucial.

There are solid basic scientific reasons to study sex differences as well. Sex differences present a unique perspective in figuring out how brains work. They allow us to compare sex differences in brain structure with those in brain function. The same is true for studying how brains develop the circuitry for stereotypical behaviors, in this case masculine and feminine behaviors. As sexual differentiation of brain and behavior can be manipulated hormonally or genetically, sex differences provide an alternative way of studying brain development in general. Although we are making inroads in understanding the cellular and molecular processes underlying sexual

differentiation, nature is not readily giving up its secrets as to how structural sex differences contribute to function, as is clear later in this article.

Sexual Differentiation

Central Role for Hormones in Sex Differences in Brain and Behavior

Hormonal effects on brain physiology and behavior

Alfred Jost's proposal in the 1940s that testes are crucial for the development of the mammalian male phenotype, and that without them bodies develop in a female direction, has driven research in the field of sexual differentiation for more than half a century, especially that of brains and behavior. The more recent discoveries of genes that direct the differentiation of the primordial gonad into a testis require minor reformulating of the theory on the mechanisms of sexual differentiation: sex chromosomal genes determine the differentiation of the gonads into testes or ovaries; the resulting differences in gonadal secretions cause all other differences.

For the following decades, differences in brain and behavior seemed to fall in line with Jost's principle. Initial research focused on processes linked with reproduction. For example, in the early 1950s, Harris and Jacobsohn demonstrated that male pituitary transplants placed directly under the hypothalamus sustained ovarian cycles in female rats, thereby disproving earlier claims that the pituitary was responsible for sex differences in gonadotrophic hormone release. Barracough and his collaborators then showed that single injections of testosterone propionate given shortly after birth caused permanent infertility in female rats. After identifying the anterior preoptic area as the site responsible for cyclicity in gonadotrophic hormone release, they concluded that the anterior preoptic area is undifferentiated at birth with regard to its subsequent control of gonadotropin secretion. They also concluded that in the absence of androgen the anterior preoptic area differentiates to sustain cyclicity whereas in the presence of androgen this area becomes refractory to both intrinsic and extrinsic activation, resulting in the tonic type of gonadotropin secretion observed in males.

At about the same time, William Young and colleagues demonstrated that administering testosterone propionate to pregnant guinea pigs increased the chances that their female offspring displayed male sexual behavior as adults while having the opposite effect on female sexual behavior. This work led to the important realization that gonadal hormones influence sexually dimorphic behaviors in two fundamentally different ways. Early in life, they direct the development of neural circuitry that will generate male- or female-typical functions and behaviors

in a male or female direction. These developmental effects are permanent and called organizational. For example, testosterone exposure during development increases the likelihood that animals will show male sexual behavior as adults. However, to show male sexual behavior, animals have to be exposed to testosterone in adulthood as well. This latter effect is transient and therefore called activational.

Sex differences in brain structure

Young and colleagues suggested that early androgen exposure causes a more subtle change in the brain than in genital morphology, one that is reflected in function rather than in visible structure. The first reports of sex differences hinted that differences were indeed subtle, but at least they were detectable. For example, in 1960, Kato reported higher serotonin levels in female than in male rat brains. A little while later, Pfaff showed that neonatal castration of rats permanently changed the size of nucleoli in hypothalamic neurons. In 1970, McEwen and colleagues showed that neonatal steroid treatment changed testosterone and estradiol uptake in rat brains. A year later, Raisman and Field reported the first sex difference in neural connectivity: male rats showed more synapses from nonstriatal origin on dendritic shafts and fewer on dendritic spines than did females. Although the functional significance of this finding is still unclear, it was a milestone because it showed that neonatal manipulations of gonadal hormone levels could reverse morphological sex differences. It mapped well, therefore, onto the concept of organizational effects of steroids.

After these admittedly subtle findings, Nottebohm and Arnold reported much more conspicuous differences in the size of song control nuclei in the brain of zebra finches and canaries. Males, which sing, had much larger song control nuclei than did females, which do not sing. This striking correlation between structure and function reinforced the notion that studying sex differences would allow one to link brain structure to function. Soon thereafter, equally impressive differences were found in the gross anatomy of mammalian brains. Arguably, the most famous of these was found in 1978 by Gorski and his colleagues in the sexually dimorphic nucleus (SDN) of the medial preoptic area of rats. This nucleus is 5 times larger in males than in females, which is remarkable considering that for years researchers must have overlooked this difference, which can be seen in Nissl-stained sections by the naked eye. Currently, hundreds, if not thousands, of sex differences have been reported, in almost any parameter imaginable. Remarkably, however, in most cases, we do not know the functional consequences of these differences for behavior.

Molecular mechanisms underlying hormonal effects

Gonadal hormones often have similar organizational effects on brain structure as they do on behavior. For example, the SDN attains a male size in females treated around birth with testosterone, and a female size in males castrated neonatally. Activational effects play no role, or at most, a minor role, as hormonal treatments of adult rats cannot reverse this sex difference. Other sex differences are caused in different ways. For example, the medial nucleus of the amygdala contains more cells that express the neuropeptide cholecystokinin in males than in females. This sex difference can be eliminated by treating males and females with similar levels of hormones. The same is true for sex differences in the size of subnuclei of the medial amygdala. Yet, other sex differences can be attributed to a combination of organizational and activational effects. For example, male rats have denser vasopressin projections from the bed nucleus of the stria terminalis (BNST) and amygdala than do females (**Figure 1**). This innervation is exquisitely sensitive to circulating gonadal hormones. Without these hormones, BNST and amygdala cells do not express vasopressin. Treating males and females with similar levels of gonadal steroids, however, does not equalize vasopressin content, unless males and females were exposed to similar levels of gonadal hormones during development. Clearly, sex differences in the brain can be caused by organizational or activational effects of gonadal hormones, or both.

A current focus is the cellular and molecular processes underlying organizational effects on the brain. Research at the molecular level, for example, indicates that masculinization, the process by which testosterone promotes the development of neural circuitry underlying male-typical behaviors, and defeminization, the process by which testosterone suppresses the development of neural circuitry needed to generate female-typical behaviors, can occur independently. For example, testosterone's effect on the development of male sexual behavior is mediated by prostaglandin E₂. Treating male rats with inhibitors of prostaglandin synthesis around birth blocks masculinization but not defeminization. On the other hand, blocking N-methyl-D-aspartic acid (NMDA) receptors during development inhibits defeminization, but not masculinization. These findings will help delineate the processes involved in the control of sexual behavior and may provide insight in the mechanism underlying sexual differentiation of other behaviors as well.

The use of genetically engineered mice is also helpful in this regard. For example, sex differences in the volume and cell number of distinct brain areas can be caused by differential cell birth, cell migration, cell differentiation, or programmed cell death. Of these four, cell death has been best documented. Areas such as the SDN, which are larger in males than in females, show higher levels of

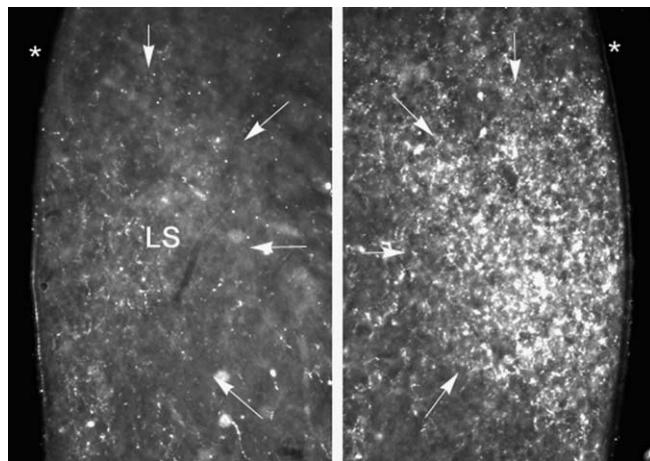


Figure 1 Sex difference in vasopressin innervation (arrows) of the lateral septum (LS). Females (left panel) show a much sparser innervation than males. Asterisks are placed in lateral ventricles. Modified from De Vries GJ and Panzica GC (2006) Sexual differentiation of central vasopressin and vasotocin systems in vertebrates: Different mechanisms, similar endpoints. *Neuroscience* 138: 947–955.

apoptosis, an orderly process of programmed cell death, in females around birth. The opposite is true for areas that are larger in females than in males. Mutant mice with abnormal apoptosis allowed a direct test of the hypothesis that this differential cell death contributes to sex differences in brain areas. For example, the principal nucleus of the BNST is larger and contains more cells in males than in females. This sex difference, however, is completely eliminated in mice unable to produce Bax, an obligate protein in the molecular cascade leading to neuronal cell death. The Bax mutation, however, leaves the sex difference in vasopressin expression intact, suggesting that in this case testosterone influences the capacity of individual cells to synthesize vasopressin. A change like that is likely caused by epigenetic changes, which is rapidly becoming a focus in research on sexual differentiation.

Direct Role for Sex Chromosomes

Model system to distinguish between chromosomal and hormonal contributions

The Jost doctrine that all sex differences in nongonadal tissues can be traced back to differences in gonadal secretions may not be as generally applicable as first thought. Several sex differences (e.g., those in songbird plumage, and in the development of pouch versus scrotum in wallabies (a marsupial)) likely depend directly on sex chromosomal composition in those tissues rather than on differences in gonadal hormone levels. Research on bird song had hinted that this might be true for brains as well. Sex differences in bird song or song control areas are not readily, or at all, reversed by hormonal manipulations. To test whether sex chromosomes may have direct effects in mammals as well, a mouse model system has been developed that differentiates between direct actions of sex

chromosomal composition (XX vs. XY) and gonadal hormones. In this model, female mice with the familiar XX genotype are crossed with males with an XY⁻ *Sry* genotype. The Y chromosome of these mice lacks the *Sry* gene on the Y chromosome, which normally directs the growth of the testis. XY⁻ *Sry* mice, nevertheless, develop a male phenotype because they have an *Sry* transgene inserted on an autosomal chromosome. The offspring of XX and XY⁻ *Sry* mice consist of XX and XY⁻ mice of either sex, depending on whether they inherit the *Sry* transgene.

Comparing XX and XY⁻ mice within sex (defined on basis of gonad) has revealed a number of traits that are influenced by sex chromosomal composition, for example, aggressive and maternal behavior. In addition, the vasopressin projections of the BNST show a modest but significant direct effect of sex chromosomes. XY animals of either sex have slightly denser projections than XX animals. In each of these cases, however, gonadal hormones seem to be the most important factors in sexual differentiation. Yet in some cases, sex chromosomes do seem to be the main cause of sex differences in neural structure or function. For example, gonadectomized XX mice are less sensitive to painful stimuli than XY⁻ mice, and express more of the neuropeptide precursors prodynorphin and preprotachikin in the striatum, irrespective of their gonadal sex. Similarly, fetal midbrain cell cultures from XX mice yielded more cells that express tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine production, than cultures from XY⁻ mice.

These findings suggest that sex chromosomes cause sex differences in brain and behavior indirectly as well as directly. The presence or absence of a Y chromosome determines whether an individual will develop testes or ovaries. Resulting differences in gonadal hormone levels then cause differentiation of the brain. In parallel,

differential expression of X and Y chromosomal genes in the brain causes additional differences. Currently, only a few traits are known to be directly influenced by sex chromosomes. However, as research on direct sex chromosomal effects on brain traits is relatively new, we may have seen only the tip of the iceberg.

Mechanisms underlying direct sex chromosomal effects

Seeking the molecular mechanisms underlying direct chromosomal effects may prove easier than identifying genes that mediate steroid action on the brain. Whereas gonadal hormones may target genes on any of the chromosomes, the genes primarily responsible for direct sex chromosomal effects are restricted to the sex chromosomes. The most obvious candidates are genes found exclusively on the Y chromosome, such as *Sry*. In fact, *Sry* is expressed in brain tissue of adult rats as well as mice, most notably in the substantia nigra. Treating animals with *Sry* mRNA antisense reduces TH production and causes motor defects in males but not in females, presumably by compromising dopamine neurotransmission. Therefore, the dopamine system, which on the surface is not strikingly dimorphic, harbors significant differences in biochemistry. Perhaps this difference contributes to the 50% higher incidence of Parkinson's in men than in women.

Other factors underlying direct chromosomal effects may be differences in the dosage of X chromosomal gene products. Although such dosage differences are in most cases prevented by random X inactivation, during the process by which the expression of most genes on one of the two X chromosomes is blocked, some genes escape inactivation, and they may therefore be expressed at different levels in male and female brains. Differential imprinting of X chromosomal genes may also cause sex differences. In male brains, all X chromosomes are inherited from the mother, and thus they carry maternal imprints that either block or alter the expression of select X chromosomal genes. In female brains, X chromosomes are derived from both parents and therefore they carry maternal as well as paternal imprints. As paternal and maternal imprints differ, this may lead to differential expression of X chromosomal genes. An interesting case is found in Turner's syndrome, where one of the two X chromosomes is lost early in development, leading to an XO genotype. Girls with Turner's syndrome who retained the paternal X chromosome have better social skills and a slightly higher verbal IQ than girls who retained the maternal X chromosome. It is tempting to correlate this difference with sex differences in social skills and verbal IQ, which tend to favor girls. Perhaps girls outperform boys in part because approximately half of their brain cells express paternal X chromosomal genes. Males will have to do with the maternally imprinted X chromosome.

Function of Sex Differences in the Brain

Our understanding of sexual differentiation stands in remarkable juxtaposition to our understanding of the function of sex differences, which is extremely limited. Research on the SDN illustrates how difficult it is to relate sex differences in brain structure to those in function. Because the medial preoptic area is essential for male sexual behavior, it has been attractive to link sex differences in this area to differences in male sexual behavior. However, lesions centered in the SDN produce little-to-no decrements in male sexual behavior. Discrepancies in the effects of perinatal endocrine manipulations on SDN morphology and male sexual behavior further weaken the link. For example, prenatal testosterone treatment increases SDN volume in female rats but not their propensity to show masculine behavior as adults.

Inconsistencies between sex differences in male sexual behavior and sexual dimorphism have also been reported for the male nucleus of the preoptic area (MN) in ferrets. This nucleus depends on the presence of testosterone before birth. If levels are low, as is true for females, no MN develops. If testosterone is given to females immediately after birth, females develop a masculine pattern of sexual behavior but not an MN. Interestingly, however, the MN may be important for partner preference as lesions centered in the MN cause male ferrets to prefer male over female conspecifics. Homologous sexually dimorphic structures may play a similar role in other mammals. Lesions of the SDN, for example, disrupt partner preference in rats as lesions of MN do in ferrets. Natural variation in male versus female preference also correlates with the size of sexually dimorphic nuclei in the MPOA. For example, about 8% of rams prefer mounting male rather than female sheep. These male-oriented rams have an ovine SDN only half the size of that of female-oriented rams. Interestingly, humans have an area in about same region, the third interstitial nucleus of the anterior hypothalamus (INAH3), which is larger in men than in women. In homosexual men, however, the size of INAH3 is intermediate to that of heterosexual men and women.

Even if a structural sex difference in the brain correlates well with a sex difference in behavior, such as appears to be the case with sexual preference, it remains still unclear what, for example, a difference in cell number buys one sex over the other in terms of function. To answer this, one has to identify the connections and functions of the components that make up a sexually dimorphic system. In the spinal cord, this is relatively easy. For example, the ventral horn in the lumbar region of the spinal cord contains a nucleus that is much larger in males than in females, the spinal nucleus of the bulbocavernosus in rodents such as rats and mice, and Onuf's

nucleus in dogs, hyenas, and humans. This nucleus contains motoneurons that innervate muscles at the base of the penis. Homologous muscles in females are much smaller or even vestigial. In this case, the function is obvious. Males need more motoneurons because the muscles that they target contain more muscle fibers. These muscle fibers are involved in genital responses that are clearly dimorphic. Similarly, sex differences in medullary motoneurons that innervate the muscles of the larynx in clawed frogs or muscles of the dewlap in green anoles have been linked to more frequent and intense use of these structures during courtship in males. In the brain, it is much harder to link structure to function, in large part because connections are far more numerous and complex.

Using Neurochemical Markers to Study the Function of Sex Differences

Studying neurochemical markers in sexually dimorphic systems has proven very helpful in this regard. Focusing on neurotransmitter systems, for example, helps trace the anatomical connections of subsets of cells within sexually dimorphic areas. It also allows more specific manipulations than lesioning entire cell groups or transecting projections. For example, injecting receptor agonists and antagonists or making conditional knock-outs or knock-ins have proven useful in delineating systems engaged in food intake and energy balance and may yield similar success in research on sex differences.

A recent success story concerns the anteroventral periventricular nucleus (AVPV) of mice and rats. In rodents, the AVPV is larger and contains more neurons in females than in males. This area is important for generating the surge in luteinizing hormone (LH) in response to rising levels of estradiol. This surge is found in females but not in males. A subset of AVPV neurons expresses Kiss1-mRNA and its gene product, kisspeptin. In mice, females have 10 times more AVPV kisspeptin neurons than males; in rats, males express almost no kisspeptin, making the sex difference nearly absolute. These kisspeptin neurons appear to contact neurons that synthesize gonadotropin-releasing hormone (GnRH). As kisspeptin triggers a surge of LH by stimulating these neurons, the higher number of kisspeptin neurons in females may explain the sex difference in the LH surge and thereby at least in part the sex difference in cyclicity of gonadal hormone release. The key to the success of the kisspeptin story may be similar to that of the sexually dimorphic nuclei in the spinal cord. Kisspeptin neurons form part of a final common pathway, in this case, of the sexually dimorphic control of the LH surge. The quest for the function of sexually dimorphic neural systems with no clear links to peripheral structures has turned out to be much more difficult.

Dual Function of Sex Differences in the Brain

One reason for this may be that the function of structural differences is too narrowly interpreted. We like to think that sex differences in brain structure beget sex differences in behavior. We typically overlook the possibility that sex differences in brain structure may allow males and females to reach similar behavioral endpoints even though their bodies and physiology differ. The need for such compensation is clear in behaviors that are shown by both sexes but depend on specific hormonal conditions in one sex that never occur in the other, for example, parental behavior in prairie voles.

Prairie voles form stable pairbonds, in which both parents take care of the pups. Once pups are born, males and females show similar parental behavior with the exception of nursing. Female voles have to experience the hormonal changes related to pregnancy as well as give birth naturally to become fully parental. Obviously, males need a different strategy. Part of this strategy appears to be engaging the sexually dimorphic vasopressin innervation, as blocking the vasopressin projections from the BNST inhibits parental behavior in males.

In rats, the same innervation can cause as well as prevent sex differences in brain function. For example, the higher levels in males probably make males more aggressive than females, as vasopressin stimulates aggressive behavior. On the other hand, the same higher levels appear to prevent sex differences in social recognition memory. Blocking vasopressin neurotransmission blocks vasopressin recognition memory in males, but not in females, thereby inducing a sex difference in performance that was not present before. In relation to this, in mice, a null mutation of vasopressin's V1a receptor reduces anxiety-related behaviors in males, but not in females. These differential effects are exactly what one would predict for a sex difference that may cause as well as prevent sex differences in behavioral output. Sex differences in vasopressin transmission may also influence human behavior. For example, a specific variation in DNA sequence upstream of the V1a gene has been linked to differences in pairbonding in men but not in women. Men with that variation are less likely to be married and, if they are, more likely to have had serious marital discord. Because the same variation has also been linked to disorders such as autism, it could potentially contribute to sex differences in these disorders.

Cause of Sex Differences in Behavioral and Neurological Disorders Revisited

Just as gonadal hormones and sex chromosomes can direct normal brain development in male or female direction, they may also increase the vulnerability for, and course of,

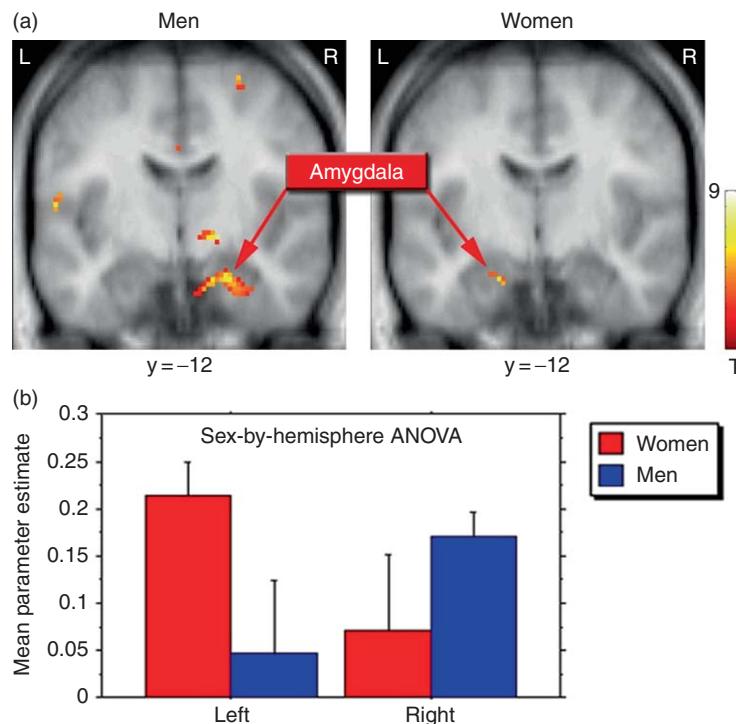


Figure 2 Functional magnetic resonance imaging study showing higher activation of the left and lower activation of the right amygdala in women than in men during a recall test for pictures with a negative emotional content ($F(1,13) = 7.99$, $P = 0.022$). From Cahill L, Uncapher M, Kilpatrick L, Alkire MT, and Turner J (2004) Sex-related hemispheric lateralization of amygdala function in emotionally influenced memory: An fMRI investigation. *Learning and Memory* 11: 261–266.

behavioral and neurological disorders. Although in most cases the nature of differences in these disorders is not well understood, there are many possible factors. For example, higher levels of circulating estrogen in women may offer greater levels of neuroprotection. Other factors may depend on sex differences in the brain. One possibility, for example, is directly related to males and females using different neurochemistry and neural circuitry to reach similar behavioral endpoints. This is probably not just vasopressin's prerogative. Strokes centered in similar areas in the brain can have different outcomes for men and women in, for example, language and visuospatial abilities. In addition, functional imaging studies show that male and female brains activate different areas during specific cognitive tasks even if there are no clear differences in performance. This is true, for example, for specific language tasks that show a more lateralized activation pattern in males than in females. It is also true for remembering images that evoke negative emotions, which activate the right amygdala more in males and the left amygdala more in females (Figure 2). All these studies suggest that men and women employ different neural circuitry for similar tasks, which almost by definition leads to different vulnerabilities to behavioral and psychiatric disorders.

See also: Hemispheric Specialization: Language, Space, and Sexual Differentiation; Male Sexual Behavior; Sex Hormones, Mood, and Cognition.

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Neural Basis of Recognition Memory in Nonhuman Primates

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Glossary

Familiarity – The mere judgment of knowing a stimulus that is currently present.

Recognition memory – When something previously experienced is identified as familiar when encountered again.

Recollection – The full recall of an event elicited by a stimulus that is currently present.

Setting the Stage: Recall versus Recognition, Familiarity versus Recollection

Recognition and recall are memory processes allowing conscious access to information previously encoded and stored in long-term memory. Recall is the self-organized retrieval of an event from long-term memory without any help (free recall) or based on partial information (cued recall). The tip-of-the-tongue phenomenon is a typical recall failure. Recognition, by contrast, is the mere decision that a currently present person, object, place, or event has been encountered or experienced before; hence, it is often, though not always, more efficient than recall.

Recall and recognition are the retrieval processes of a specific memory system – termed declarative, explicit, or cognitive – which include episodic memory for personal events in time and semantic memory for facts about the world. This system is distinct – functionally and anatomically – from the procedural, implicit, habit memory system, which simply expresses previously acquired skills through performance. Unlike procedural learning, which entails a lengthy acquisition phase, new information is rapidly committed to declarative memory. Accordingly, a characteristic common to all recognition-memory tasks is the use of a single exposure to the stimuli to be memorized.

Declarative memory is the system that is selectively disrupted in human amnesia. In most patients this syndrome leads to dense impairments in both recall and recognition, but relative sparing of recognition over recall has nevertheless been observed in a few amnesic patients. Recognition tasks are therefore markers of declarative

memory – crucial for understanding human amnesia – with the reserve that they cannot provide insights into all forms of declarative memory, but only into a subset of declarative memory processes.

Recognition itself is not a single process as it can yield two different outcomes: either a pure familiarity/novelty judgment or a full-event recollection akin to recall. The former is epitomized by what is known as the ‘butcher-on-the-bus phenomenon’: someone seen in an atypical context that we feel is familiar while failing to remember any information whatsoever about him or her. The latter consists, like recall, in remembering the information plus the spatiotemporal context of the latest episode in which it was encountered, for example, in remembering the butcher as well as his tiny shop down the block and the fantastic T-bone steaks he sold you last Saturday.

Determining whether familiarity and recollection form a unitary process depending on a single neural substrate or a dual process subserved by different brain structures is a challenge to current research. In the human, several protocols have been designed to distinguish recollective recognition from novelty/familiarity detection. They include the ‘remember/know’ procedure, which asks subjects whether the stimulus simply feels familiar (‘know’) or if they can recollect its context (‘remember’) and the ‘receiver operating characteristic’ (ROC) procedure, in which the relationship between the subjects’ confidence ratings of their recognition judgments and their accuracy is used to signal recollective recognition.

The above parsing of memory systems and recognition processes has a clear intuitive appeal and heuristic value when applied to the human with fully developed language skills. It is less helpful for nonverbal primates. Prelanguage human infants, as well as infant and adult monkeys, do possess recognition capabilities that can only be inferred from their changes in behavior. Which kind of ‘declarative’ memory do recognition tasks measure in these instances? Are familiarity/novelty judgments the only recognition tools available to nonverbal primates, or do they have access to some precursor form of recollection? In our current understanding of recognition, these questions remain open issues. Remarkable progress has, nevertheless, taken place with regard to the recognition memory of primates in the last five decades. The present article aims at emphasizing this progress.

A 50-Year-Old Plot: Amnesia, the Medial Temporal Lobe, and Recognition Memory

Brain regions other than the medial temporal lobe (MTL), in particular diencephalic structures, play a role in declarative memory and recognition processes – as long known from human Korsakoff patients (whose alcoholism-induced damage to this brain region yields global amnesia) and from numerous animal studies. However, the discovery of the crucial role of the MTL in human declarative memory, and the numerous subsequent monkey studies that this discovery prompted, has been the main driving force of research on recognition memory in primates. Hence, we chose to keep the present article focused on this brain region.

In 1950, Karl Lashley published the results of 30 years of lesions studies in rats spent “In Search of the Engram” only to conclude that, “It is not possible to demonstrate the isolated localization of a memory trace anywhere within the nervous system.” Thus, the theory of centralized memory storage in the brain had been laid to rest. At about this same time, however, quite different results emerged from Wilder Penfield’s investigations in epileptic patients about to undergo surgical removal of their epileptic foci. Presurgery stimulation of some cortical areas would repeatedly elicit detailed memories in the awake patient and subsequent unilateral temporal lobe resections could result in mild memory impairments. It is thus not surprising that we owe to one of Penfield’s collaborator, Brenda Milner, the description of the landmark case H. M. which, for more than 50 years now, has driven the study of memory and its neural underpinnings.

In 1953, Henry Molaison (26 February 1926–2 December 2008) – the now “Unforgettable Amnesiac” (*The New York Times* published on 4 December 2008 an obituary entitled “H. M. an Unforgettable Amnesiac, Dies at 82”) – was treated for intractable epilepsy by the bilateral removal of the MTLs. The result of this surgery was a devastating global (nonmodality specific) anterograde amnesia that persisted through his entire life, with a milder retrograde amnesia disrupting events close to the time of his operation without affecting his childhood memories. This profound deficit made the skill-learning capabilities that were later proved to be retained by H. M. all the more remarkable. H. M.’s case thus established three important points: (1) the MTL is required to encode some new memories, but some memory functions remain intact after MTL damage, (2) because some past memories survived, the MTL is not the permanent storage site of long-term memory, and (3) to the extent it is involved in retrieval, this role must be time limited as well. The now prevailing concept of multiple memory systems comes directly from H. M. and other patients suffering like him from global anterograde amnesia.

The MTL is a large region including two deep structures – the amygdala and hippocampus – which are wrapped rostrally by the rhinal (entorhinal and perirhinal) cortices, and caudally by the parahippocampal cortex (Figure 1). As estimated by William Scoville at the time of surgery, bilateral removal of the MTL in H. M. involved most of these structures. Why then was H. M.’s memory deficit attributed to the sole hippocampal damage? The original study by Scoville and Milner, in 1957, concerned 30 patients with bilateral temporal lobectomy. The three patients, who developed the amnesic syndrome, including H. M., had estimated excisions that extended sufficiently posterior to include a large portion of the hippocampus in addition to the amygdala. A subsequent study by Penfield and Milner, in 1958, involved 90 patients with temporal lobectomies that, although posterior enough to damage a large portion of the hippocampus, were only unilateral. Two patients, nevertheless, developed the amnesic syndrome.

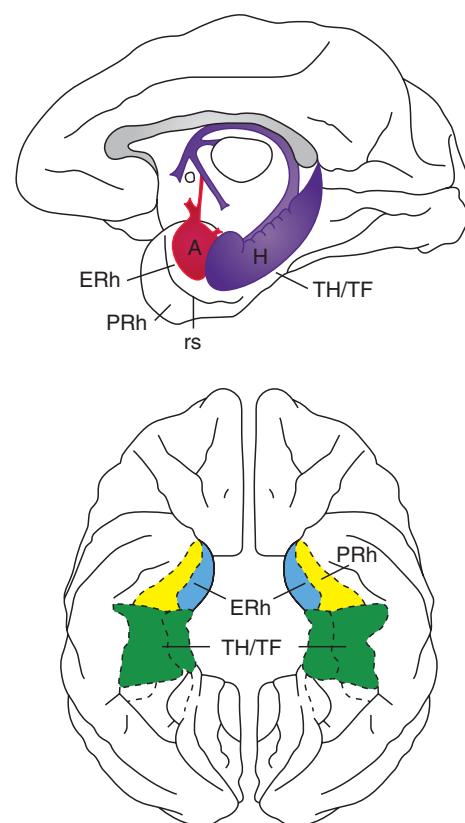


Figure 1 View of the medial surface (top) and of the ventral surface (below) of the brain of a rhesus monkey depicting the approximate location of the amygdala and the hippocampus – which lie deep in the temporal lobe and are shown in red and purple – and the extent of cortical areas, such as the entorhinal cortex (ERh, shown in blue), the perirhinal cortex (PRh, shown in yellow), and areas TH/TF on the parahippocampal gyrus (shown in green). These brain structures have a high degree of homology between rodents, monkeys, and the human.

Autopsy findings from one of this patient revealed a shrunken and necrotic hippocampus in the unoperated temporal lobe without any obvious damage to neighboring structures. A moderate amnesic syndrome was later described in a patient who suffered an ischemic event yielding a medial temporal damage seemingly restricted to a bilateral cell loss in the CA1 field of the hippocampus. These are the main findings that imposed the idea of the hippocampus as the structure whose bilateral damage was responsible for human amnesia.

What was not immediately forthcoming was similar evidence from the animal literature. Contrasting with the devastating effect of MTL damage on human memory, damage to the hippocampus in monkeys initially appeared to have very limited and somewhat specific effects on monkey memory. For some time it appeared that there was no comparison between the human and the monkey in spite of the high degree of anatomical homology between the two species with respect to the MTL structures.

In 1975, Mishkin and Delacour modified the matching-to-sample memory task developed by Gaffan in 1974 so that it could readily be learned by monkeys, while being closer to tests of human recognition memory, known as trial unique. They use a nonmatching rather than a matching rule to take advantage of monkeys' natural attraction to novelty. To better approximate the recognition tasks used in the human, they opted for a large pool of stimuli, creating a version of the task with 'trial-unique' stimuli; that is, rather than requiring the monkey to remember which stimulus had been seen most recently (recency memory), the new version simply required the monkey to indicate which stimulus was novel, while avoiding the previously seen other stimulus (recognition memory). An other crucial change was to combine this new task with extensive MTL ablations similar to that sustained by H. M., that is, encompassing both the amygdala and the hippocampal regions. Mishkin demonstrated for the first time in 1978 that, whereas amygdalectomy or hippocampectomy alone produce mild impairments at best, combined lesions, effectively replicating H. M.'s damage, produce a severe impairment.

Continued work in human amnesic patients further characterized the nature of the deficit as being limited to particular long-term memory processes, termed 'declarative,' 'episodic/semantic,' or 'explicit' memory, and those processes that are spared in human amnesics termed 'nondeclarative' or 'implicit' memory were also shown to be spared in the operated monkeys. This research has vastly increased our knowledge about the neural bases of memory and generated models in monkeys and rodents, and at the cellular level in invertebrates.

The Leading Actor: Delayed Nonmatching-To-Sample

As delayed nonmatching-to-sample with trial-unique stimuli (DNMS) was the first task to successfully demonstrate a recognition-memory deficit in monkeys accurately modeling that produced by human amnesia, it became the benchmark task in monkey research for about 15 years.

Delayed nonmatching-to-sample with trial-unique stimuli (**Figure 2(a)**) is simple in that monkeys learn to displace junk objects to obtain a hidden food reward. However, food is only located under objects that have not been seen recently; that is, animals are trained to associate novelty with reward. In the basic paradigm, the monkey is seated in a sound-attenuated chamber (Wisconsin General Testing Apparatus (WGTA)) behind an opaque screen. When the screen is raised, the monkey views a testing tray containing three equidistant food-wells, which can be covered with junk objects, hiding either a baited or empty well. Training takes place in two phases for each trial: sample and choice (**Figure 2**). During the sample phase, a single object covers the central food-well and when displaced, a food reward can be retrieved. The screen is then lowered and the now-familiar sample object is moved to cover a lateral well (empty) while a novel object covers the opposite lateral well (baited). After a brief period, typically ranging from 5–10 s, the screen is raised and the monkey must choose one of the objects. If the animal remembers the sample object, and correctly applies the nonmatching rule 'choose the unfamiliar item,' then the novel item is chosen and the food reward can be retrieved. When the animal reliably masters the rule governing the task, memory can be further manipulated (1) by introducing variable delays between the sample and choice phases (generally from 30 s to 40 min), occupied or not with interfering tasks, (2) by increasing the number of items to be remembered (list learning, generally from three to 10), and (3) by changing the nature of the information to be remembered (i.e., stimulus location, or nonvisual, e.g., tactile, features).

The early work by Mishkin and others on this task confirmed that large lesions to the MTL dramatically impaired performance while sparing performance on tasks such as visual discrimination learning in which monkeys learn a set of 20 concurrent discrimination problems that are presented only once per day, thus with 24-h delays. These results seemed to match the impaired and spared performance capabilities of amnesic patients, such as H. M., who could not retain new information for more than a few seconds without active rehearsal, but could improve over many trials to perform tasks such as mirror drawing, or the incomplete figures task. However, as a measure of combined amygdalo-hippocampal

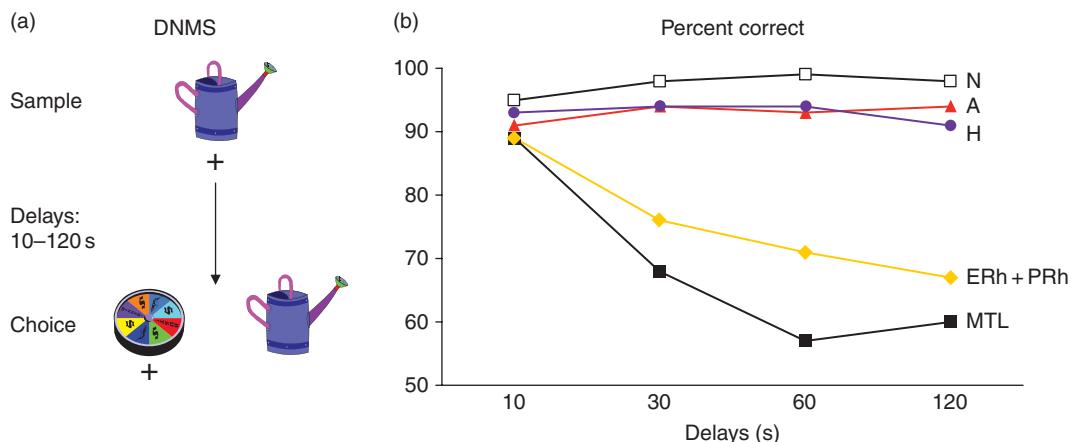


Figure 2 (a) The delayed nonmatching-to-sample (DNMS) task. During the sample presentation, central well of a 3-well tray is baited with a reward (+) and covered by an object that the subject displaces to retrieve the reward. After a delay varying from 10 to 120 s, the two lateral wells are now covered with two objects, one is the familiar positioned above an empty well and the other is a new object positioned above a baited well. Subject must displace the new object to retrieve the reward. The number of correct choice over 100 trials is used to assess visual recognition performance. (b) Performance scores obtained at each delay by normal monkeys (N) and monkeys with either amygdalectomy (A), hippocampectomy (H), combined amygdala and hippocampal lesions (MTL), or combined entorhinal and perirhinal lesions (ERh + PRh). Note that animals with the combined cortical lesions (ERh + PRh) are almost as severely impaired and those with the MTL lesions, whereas those with either amygdalar or hippocampal lesions alone are only mildly impaired. Adapted from Mishkin (1978) and Meunier *et al.* (1993).

memory function, the results from DNMS task were soon called into question.

More selective lesion techniques made it possible to parse out the individual contributions of MTL structures to recognition memory. In 1989, Zola-Morgan, Squire, and Amaral showed that the addition of cortical-sparing radiofrequency amygdala lesions to animals with hippocampal damage did not exacerbate their memory impairment. This raised for the first time the possibility that the hitherto overlooked cortical damage included in Mishkin's combined lesions contributed to the observed severe DNMS impairment. Subsequent studies, indeed, demonstrated that damage to the subjacent perirhinal and entorhinal cortices, removed along with the hippocampus and amygdala in the initial Mishkin studies (collectively referred to as rhinal cortex), devastates DNMS performance in monkeys (Figure 2(b)), producing as large an impairment as the combined hippocampo-amygda lesion.

The rhinal cortex is now unanimously viewed as a key substrate of recognition memory in primates. However, even the use of cell-selective neurotoxic lesions has not entirely solved the question of the hippocampal contribution. In 1998, Murray and Mishkin showed that monkeys with such neurotoxic, fiber-sparing damage to the hippocampus and amygdala showed normal DNMS performance out to 2-min delays. Nor were they impaired on lists of 40 items tested in reverse order and resulting in delays ranging from 30 s to 40 min. By contrast, Zola and colleagues, in 2000, found that irrespective of lesion method, hippocampal damage impaired DNMS performance at delays of 10 min and beyond. A meta-analysis of the results across several laboratories suggested that there

was a negative correlation, across and within studies, with the amount of damage on performance of the task. One possibility is that the lack of impairment in monkeys with selective hippocampal damage is due to the use of alternate strategies that allow the animal to bridge the longest delays. Indeed, when a distraction is inserted into the delay period (opening the screen at short delays, having the animal perform a motor task at longer delays, or removing it from the apparatus during 10-min delays), an impairment does emerge at the longest '10-min' delay that correlates 'positively' with the amount of hippocampal cell loss. In any case, in this study as in the earlier one by Meunier and colleagues in 1993, damage to the perirhinal cortex clearly produces a greater impairment on DNMS performance, with or without distraction and even at short delays.

Taken in context with what we now know of the anatomy of the region, it is clear that the perirhinal cortex is important for object memory, in particular for object identity, and that damage limited to this region produces a severe impairment in visual recognition memory. Indeed, it is currently being argued that the perirhinal cortex may be important for visual perception as well as memory. Hippocampal damage, by contrast, leaves short-term memory intact on this task, which is maintained both by the temporal cortical region, but also by active mechanisms when necessary, possibly controlled by the prefrontal cortex.

Likewise, the memory deficit produced by the selective hippocampal damage, resulting from forebrain ischemia, has been called into question. As mentioned above, ischemic events produce moderately severe memory

deficits in the human. Whereas ischemia was thought to damage only a single hippocampal cell field (CA1) in human patients, there is now evidence that the damage is more widespread. Furthermore, although ischemic damage in human amnesic patients impaired performance on a DNMS-like task, thus seemingly providing a direct link between the hippocampus and recognition memory, recent work by Mumby and colleagues, in 1996, showed that ischemia-related memory impairments were likely due to either extrahippocampal damage or dysfunction, as removing the entire hippocampus immediately after ischemia actually spared DNMS performance in rats. Thus, at best, the effect of hippocampal damage on DNMS for objects is mild and restricted to long delays and lists.

The Supporting Actor: Visual Paired Comparison

Similar to DNMS, the visual paired comparison task (VPC) utilizes a familiarization phase and a choice phase, but the task demands are quite different (Figure 3(a)). This task also takes advantage of the monkey's natural preference for looking at novel things in its environment but, contrary to DNMS, it requires neither rule learning, nor forced-choices between two objects to obtain a reward. For VPC, monkeys passively view a visual stimulus, typically a black/white image, and are allowed to look at it for a sufficient period to show habituation (i.e., they cease visual exploration). This familiarization period may range from 15 to 30 s of looking time. At this point, the image disappears and after a variable delay period (as brief as 1 s) the image reappears on the

screen, side-by-side with a novel image. Monkeys and the human naturally prefer to look at (explore) the stimulus they have not yet seen (novelty preference); thus, we infer that they remember the familiar stimulus. Given that the subject is not actively performing a task, it should not be surprising that performance levels on VPC are much lower than on DNMS, in which animals are trained to a 90%-correct criterion. With VPC, typical novelty preference is in the range of 65–70% preference for novelty, but this effect is reliable. Unlike the DNMS task, VPC has proven to be very sensitive to hippocampal damage at delays as short as 1 min. Similar to DNMS, however, damage to the medial temporal cortical areas produces impairments at shorter delays (~30 s for the parahippocampal gyrus and ~10 s for the perirhinal cortex). Thus, though the sensitivity may be greater in detecting recognition-memory deficits in the VPC task, the contributions of at least three regions of the MTL maintain a similar relationship to each other; that is, perirhinal contributes in the initial encoding and short-term retention of visual stimuli, whereas parahippocampal areas TH/TF and the hippocampus are required for longer retention (Figure 3(b)).

Similar to the DNMS task, the role of the hippocampus and parahippocampal areas in the VPC becomes more prominent when arrays or locations of objects are used as stimuli, making the task more spatial in nature, but the role of the perirhinal cortex is evident in all object conditions, irrespective of their spatial relations.

In sum, the two main measures of recognition memory show very different sensitivity to selective damage to hippocampus or temporal cortex. As noted above, however, in both tasks, each region contributes to recognition

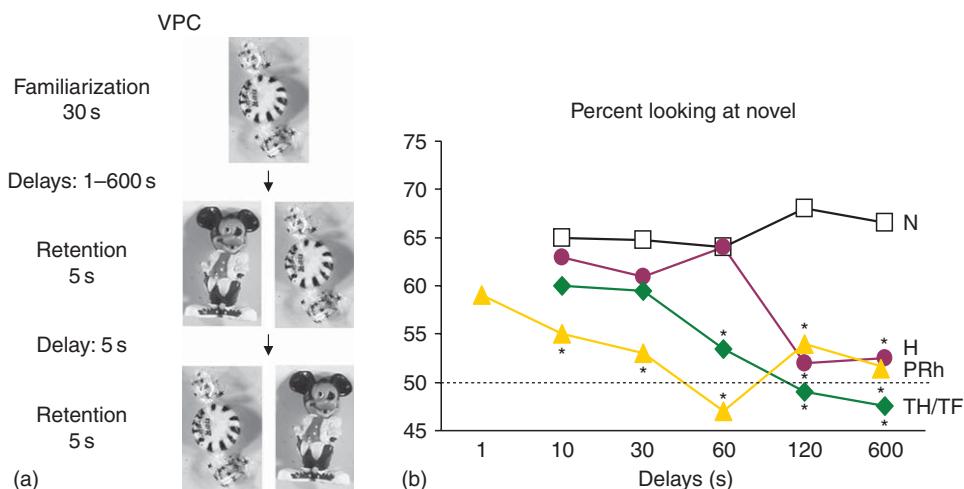


Figure 3 (a) The Visual Paired Comparison (VPC) task. The animal is familiarized with a picture of an object for a cumulative 30 s. After delays varying from 1 to 600 s, the familiar object reappears side by side with a new object for two short retention tests of 5 s each. Longer looking time at one of the two objects (generally the new one) during the two retention tests serves as a measure of recognition memory. (b) Percent looking at the novel images for normal monkeys (N) and those with neurotoxic lesions of the hippocampal formation (H) and aspiration lesions of the perirhinal cortex (PRh) or parahippocampal areas TH/TF. Dashed line indicates chance performance and asterisks defines scores that did not differ from chance. Adapted from Nemanic S, Alvarado M, Bachevalier J (2004) The hippocampal/parahippocampal regions and recognition memory: insights from visual paired-comparison versus object delayed nonmatching in monkeys. *Journal of Neuroscience* 24: 2013–2026.

memory in the same relative manner, but the absolute delay for which impairment occurred differs for each region damaged.

The Denouement: Performance of Human Amnesics on Animal Tests of Recognition

Human amnesia has, by and large, been tested with numerous tasks that are quite different from those used with animals. Thus, differences in the nature or extent of impairments between animal models and the human impairment may be due to task differences, to lesion differences, to evolutionary forces, in addition to the lack of language capabilities. This makes it important to test the human on animal memory tasks both to validate the behavioral assays, and better understand specific impairments in human patients. With regard to the lesion differences, the recent reports of patients with selective damage to individual components of the MTL provides an opportunity to test specific memory functions in the human with similarly selective damage, to compare the results with those from other species, and eventually, may inform the assessment of human patients.

Tests of human amnesic patients on animal tests of recognition, while relatively few, are largely in agreement with the animal findings when large temporal lobe lesions were used. For example, Squire and colleagues, in 1988, reported that a group of patients whose presumed damage was either diencephalic (Korsakoff's patients) or hippocampal (ischemic/anoxic patients) were severely impaired on the DNMS task, similar to the initial monkey studies. Similarly, Reed and Squire, in 1997, reported marked impairments on DNMS in patients with damage to the hippocampal formation. Unlike the monkey studies, however, they were also impaired on a concurrent discrimination task that is routinely spared in monkey studies and that presumably is a model of procedural memory. Based on the finding that performance levels on the task were directly related to declarative knowledge of the solution, Squire *et al.*, in 1988, suggested that, unlike monkeys, the human normally learns this task declaratively. By contrast, patients with damage restricted to the hippocampus have shown spared performance even on DNMS at delays up to 30 s, which follows more closely the results in monkeys and rodents and suggests that the impairment seen in the early studies of Squire might have resulted from damage to the adjacent MTL structures.

Similarly, recognition-memory impairments have been demonstrated on the VPC task, and, as in animals, these impairments are often larger than those obtained from matching or nonmatching tasks. For example, McKee and Squire, in 1993, reported novelty preference

in human amnesic patients when the delays were short (0.5 s) but not when delays were extended to 2 min or 2 h. Direct comparisons of the VPC and delayed matching-to-sample (DMS) performance have been investigated in the human amnesic patient Y. R. by Holdstock and colleagues in 2000. Patient Y. R. became amnesic after a possible ischemic infarct, resulting in reduced hippocampal volume and no other obvious pathology as measured by magnetic resonance imaging (MRI). This patient had previously shown impaired recall, but intact recognition on standard human memory tests. She also demonstrated severe impairments in memory on VPC demonstrating preference for novelty at the 0-s delay only. At the longer delays of 5 and 10 s, her performance fell to chance. By contrast, as described above, her performance was as good as that of controls on the DMS task with delays up to 30 s. Unfortunately, she was not tested at longer delays on DMS, but the reported results map onto those reported in monkeys in that hippocampal damage impairs VPC performance at much shorter delays than on DNMS. As for the animal studies, it is possible that the difference between VPC and DMS/DNMS performance is related to the forced choice required by the matching tasks that may encourage the subjects to rely on familiarity in the absence of recall to support good performance.

Importantly, the extensive knowledge accumulated in monkeys over the last decades combining the nonverbal tasks with selective lesions is now helping patients suffering from memory deficits. A human version of the monkey DNMS task developed by Barbeau and colleagues in 2005, and called the DMS48 has proved efficient to distinguish two subpopulations among patients suffering from mild cognitive impairment. Those who failed on the DMS48 were found to have grey matter loss predominantly in the MTL. As one hallmark of Alzheimer's disease is to initially develop in the perirhinal cortex, these patients seem at risk to develop the disease. By contrast, the patients who succeeded had damage elsewhere, making other etiologies such as Lewy body disease more likely. DMS48 could thus become a valuable clinical tool for early and accurate differential diagnosis of the different forms of dementia which plagues the world aging population.

Acknowledgments

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See also: Declarative Memory; Episodic and Autobiographical Memory; Psychological and Neural Aspects; Implicit Learning and Memory: Psychological and Neural Aspects; Learning and Memory: Computational Models; Neural Basis of Working Memory; Short-Term Memory: Psychological and Neural Aspects.

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Neural Basis of Working Memory

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Glossary

Delayed-response task – A task that involves the presentation of a set of stimuli with the instruction that it be held in memory across an interval of a few seconds (the ‘delay’) after which the subject must make a response to a query designed to assess the accuracy of the subject’s memory.

Domain-specific – Pertaining to a particular cognitive domain, such as verbal, visuospatial, visual-object, motoric, olfactory, etc. The domain may cut across sensory modalities, so that, for instance, auditory-spatial and visuospatial information might belong to the same processing domain.

Memory maintenance – A strategy for keeping memories ‘active’ that involves repeatedly and iteratively focusing internal attention on a set of items currently held in working memory.

Prefrontal cortex – A region of the frontal lobe that is located anterior to the motor cortices and constitutes, in the human, nearly one-third of the entire neocortex. The capacity for many higher-order functions, including reasoning, planning, and memory, is largely subserved by the prefrontal cortex.

Short-term memory – Memory for sensory/perceptual information that has been encountered only a few seconds ago. Unlike working memory, the concept of short-term memory is specifically related to time and, moreover, does not necessarily refer to cognitive processes associated with information maintenance and manipulation.

Working memory – A cognitive system for the maintenance and manipulation of information that is currently within the focus of attention.

for resurrecting a past experience at the precise moment when we need it. To some extent this idea of memory as a passive phenomenon – consisting of certain properties and governed by certain rules, but not terribly amenable to strategic control or manipulation – was assumed in much of the early research on the psychology of human memory.

In the last 30 years or so, however, the study of memory in the human and other species has focused a great deal more on how ‘executive control’ – the ability of an animal to organize and regulate its own internal cognitive milieu – allows for memory to take on a dynamic and interactive quality, whereby its contents are hardly accidental or desultory, but rather are under the direct control of the conscious animal. A typical example of this sort of ‘top-down’ memory is when one has to keep a telephone number in mind during the interval that may intervene between hearing the number and actually having to dial the digits. If memory were always passive, we would not be able to keep the telephone book more than an arm’s distance away from the telephone – memory decay and interference would always ensure that by the time we were ready to dial the numbers they would have vanished from our mind. As everyone knows, however, the best way to succeed at this basic task is to mentally ‘rehearse’ the numbers – to say them over and over again to ourselves (overtly or covertly) – until the time comes to dial them into the telephone. Of course, all this rehearsing is a lot of ‘work,’ and it takes discipline, effort, and cognitive agility to keep the numbers mentally aloft, but in the end this kind of memory – this ‘working memory’ – is essential to the efficient and effective performance of the task at hand.

Defining Working Memory

A basic definition of the term ‘working memory’ – as it has come to be used in the neuroscience and psychological literature – is the online maintenance and manipulation of information that is not directly available in the current environment. Most of the time, in the context of behavioral paradigms testing some aspect of working memory, the information retained in mind has only recently arrived to the senses. This is also the case in our phone-book example in which the telephone numbers are perceived and then immediately transferred to a working memory rehearsal loop. It is important to emphasize, however, that this need not be the case. Information ‘in’ working memory may have been recently received from

Introduction

In everyday life we often complain of memory ‘coming and going,’ or ‘not being there when you need it,’ as ‘fading away with time,’ or as occasionally ‘escaping’ us. The ways we talk about memory often suggests that it is a passive phenomenon, something that more or less has a ‘mind of its own’ and is not to be summoned at will. Indeed, this characterization of memory is often accurate: there is not always a tried and true method, a strategy, or secret recipe

the environment but it may also have been retrieved from long-term memory. For instance, if a person were asked to retrieve his or her own telephone number from memory and then to covertly rehearse that number for some period, then the contents of memory would consist of an informational sequence whose proximal source is not the immediate sensory environment, but rather, a stored trace in long-term memory. This is an important aspect of working memory that distinguishes it from ‘short-term’ or ‘immediate’ memory in that both of the latter terms refer to the retention of information that has only just arrived to the senses. Thus, although working memory is often thought of as a special case or more detailed conceptualization of short-term memory, in fact, it refers to a broader class of memory phenomena involving the maintenance or manipulation of ‘active’ representations, without respect to their proximal source (i.e., long-term memory or recent sensory experience). Nevertheless, because recently received information very often lacks a preexisting representation in long-term memory (for instance, when the information is novel), many tasks designed to tap working memory are generally also considered short-term memory paradigms. As a result, there is a great deal of overlap between short-term memory and working memory research and the terms are sometimes used interchangeably despite the above-mentioned differences between the concepts.

The next section introduces the working memory model of Alan Baddeley and colleagues, provides a description of its main principles and underlying logic, and then describes its functional architecture. The remaining sections will be devoted to the neural organization of working memory as it has been established in neurobiological research in the human and other animals in the last 30 years.

The Working Memory Model

The central tenets of the Working Memory model of Baddeley and colleagues are as follows: (1) it is a limited-capacity system; at any moment in time, there is only a finite amount of information directly available for processing in memory; (2) the specialized subsystems devoted to the representation of information of a particular type, for instance, verbal or visuospatial, are structurally independent of one another; the integrity of information represented in one domain is protected from the interfering effects of information that may be arriving to another domain; and (3) storage of information in memory is distinct from the processes that underlie stimulus perception; rather, there is a two-stage process whereby sensory information is first analyzed by perceptual modules and then transferred into

specialized storage buffers that have no other role but to temporarily ‘hold’ preprocessed units of information. Moreover, the pieces of information that reside in such specialized buffers are subject to passive, time-based decay as well as inter-item interference (e.g., similar sounding words like ‘man,’ ‘mad,’ ‘map,’ ‘cap,’ ‘mad’ can lead to interference within a specialized phonological storage structure); finally, such storage buffers have no built-in or internal mechanism for maintaining or otherwise refreshing their contents – rather, this must occur from without – through the process of rehearsal, which might be a motor or top-down control mechanism that can sequentially access and refresh the contents that remain active within the store.

The initial working memory model proposed by Baddeley and Hitch argued for the existence of three functional components of working memory (**Figure 1**). The ‘central executive’ was envisioned as a control system of limited attentional capacity responsible for coordinating and controlling two subsidiary slave systems, a ‘phonological loop’ and a ‘visuospatial sketchpad.’ The phonological loop was responsible for the storage and maintenance of information in a verbal form, and the visuospatial sketchpad was dedicated to the storage maintenance of visuospatial information. These two slave-systems are thought to be independent in the sense they do not rely on the same storage resources – phonological and visuospatial codes are, as it were, implemented atop different mental hardware – but, nevertheless, they must be coordinated by a unitary central executive, and thus there can be a competitive relation between the two systems.

In the last 20 years, the concept of working memory as a system for the active maintenance and manipulation of information has been embraced by researchers studying the brain basis of memory and cognition, and the following sections trace the progress that has been made in uncovering the neural basis of just such a system. As a result of this effort to resituate working memory in the brain, the concept has not merely been dressed up in neural garb, but rather has been enriched and expanded in the process, and what has emerged is a better and more nuanced understanding of working memory.

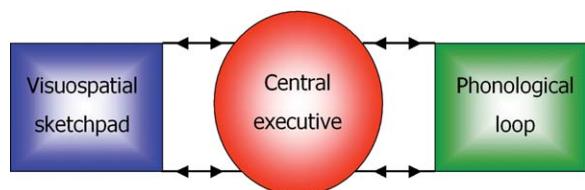


Figure 1 The Working Memory model comprises a control system, the central executive, and two storage systems – the visuospatial sketchpad and the phonological loop.

The Emergence of Working Memory as a Neuroscientific Concept

The first insights into the neurobiological underpinnings of a memory whose purpose is to bridge an interval of time comes from the work of Jacobsen, who studied nonhuman primate behavior after ablation to the prefrontal cortices (PFCs). Jacobsen showed that damage to the PFC of the monkey produces selective deficits in a task requiring a delayed response to the presentation of a sensory stimulus. The delayed-response tasks were initially devised by Hunter in 1913 as a way of differentiating between animals on the basis of their ability to use information not currently available in the sensory environment to guide an imminent response.

In the classic version of the delayed-response task (**Figure 2**), a monkey is shown the location of a food morsel that is then hidden from view and placed in one of two wells. After a delay period of a few seconds, the monkey chooses one of the two locations and is rewarded if his choice corresponds to the location of the food. Delayed response tasks measure a complex cognitive ability that requires at least three clearly identifiable subprocesses: to recognize and properly encode the to-be-remembered item, to hold an internal representation of the item ‘online’ across an interval of time, and, finally, to initiate the appropriate motor command when a response is prompted. Jacobsen showed that lesions to the PFC impair only the second of the above three functions, suggesting a fundamental role for the region in immediate or short-term memory. Thus, monkeys with lesions to PFC perform in the normal range on a variety of tests requiring sensorimotor behavior such as visual pattern discrimination and motor learning and control – that is, tasks without a short-term mnemonic component. Although the impairments in the performance of delayed-response tasks in Jacobsen’s studies were caused by large prefrontal lesions that often extended into the frontal pole and orbital surface, later studies showed that lesions

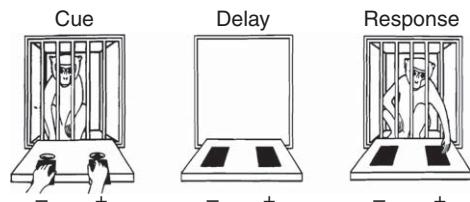


Figure 2 Three components of a spatial delayed-response trial. Left, the monkey observes the experiment place a morsel of food in a well (cue) before both wells are covered. Middle, an opaque screen is lowered for several seconds (delay). Right, the screen is raised and the monkey chooses one well by removing its cover. Adapted from Goldman-Rakic PS (1987) Circuitry of primate prefrontal cortex and regulation of behavior by representational memory. In: Plum F (ed.) *Handbook of Physiology – The Nervous System*, vol. 5, pp. 373–417. Bethesda, MD: American Physiological Society.

confined to the region of the principal sulcus produced deficits equally as severe.

In 1971, Fuster and Alexander reported the first direct physiological measures of PFC involvement in short-term memory. With microelectrodes placed in the PFC, they measured the firing patterns of neurons during a spatial delayed-response task and showed that many cells showed increased firing, relative to an inter-trial baseline period, during both cue presentation and the later retention period. Importantly, some cells fired exclusively during the delay period, and therefore could be considered pure ‘memory cells.’ The results were interpreted as providing evidence for PFC involvement in the focusing of attention on information that is being or that has been placed in temporary memory storage for prospective use.

Many subsequent electrophysiological studies have demonstrated memory-related activity in the PFC of the monkey during delayed-response tasks of various kinds, although it was Patricia Goldman-Rakic who first drew a parallel and then firmly linked the phenomenon of persistent activity in PFC to the cognitive psychological concept of ‘working memory.’ In a 1987 monograph, Goldman-Rakic – citing lesion and electrophysiological studies in the monkey, human neuropsychology, and the cytoarchitectonics and cortico-cortical connections of the PFC – argued that the dorsolateral PFC (the principal sulcus of the monkey) plays an essential role in holding visuospatial information in memory before the initiation of a response and in the absence of guiding sensory stimulation. In this and later work, Goldman-Rakic and colleagues developed a model of PFC in which visuospatial and (visual) object working memory were topographically segregated, with the former localized to the principal sulcus and the latter localized to a more ventral region along the inferior convexity of the lateral PFC (**Figure 3**). This domain-specific view of the prefrontal organization, which was supported by observed dissociations in the responsiveness of neurons in dorsal and ventral areas of the PFC during delayed-response tasks, could be viewed as an anterior expansion of the dorsal (where) and ventral (what) streams that had been discovered in the visual system in the posterior neocortex by Ungerleider and Mishkin. In addition, the parallel and modular nature of the proposed functional and neuroanatomical architecture of the PFC was in keeping with the tenet of domain independence in the Working Memory model of Baddeley and colleagues.

The Neural Organization of Working Memory

The early evidence from the animal literature emphasized the profound importance of the PFC in the performance of working memory tasks. Indeed, one

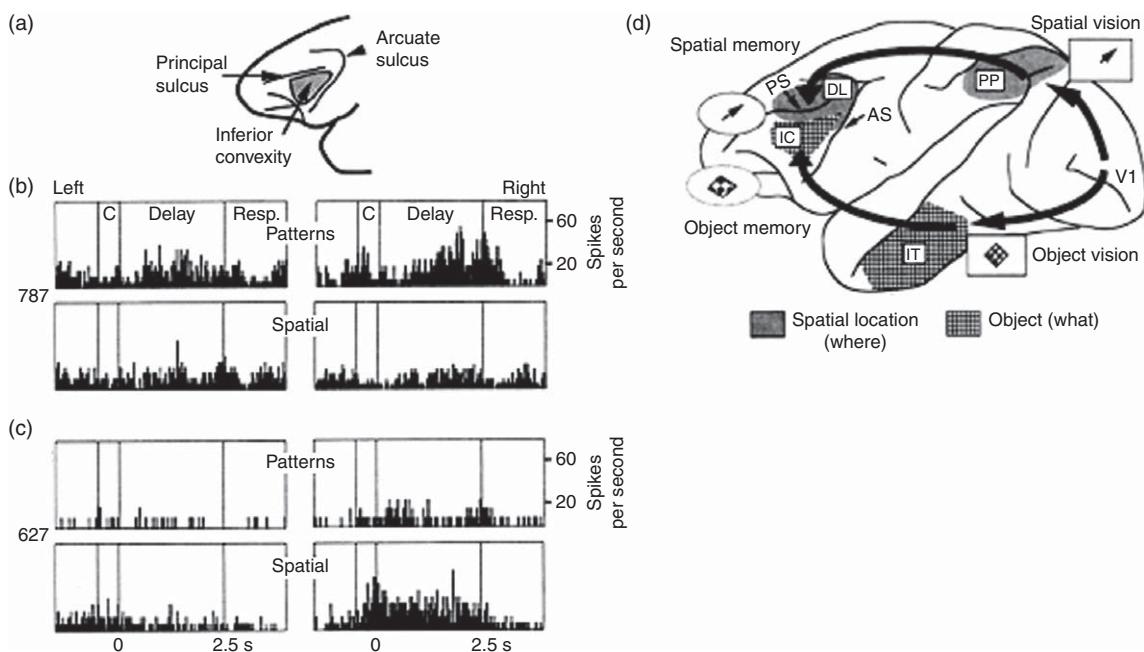


Figure 3 (a) Diagram of the frontal lobe and location of the principal sulcus and inferior convexity. (b) Responses of inferior convexity neuron with visual object-specific activity in the delayed-response task. The upper panels show delay-period activity for object-memory trials; the lower panels show lack of response on spatial memory trials. (c) Responses of the dorsolateral prefrontal neuron with spatial memory selectivity. The upper panels show lack of responsivity on object-memory trials; the lower panels show delay-period activity on spatial memory trials. (d) Schematic diagram illustrating the dorsal and ventral streams in the visual system and their connections with PFC; PS, principal sulcus; AS, arcuate sulcus; The posterior parietal (PP) is concerned with spatial perception, and the inferior temporal (IT) cortex with object recognition. These regions are connected with the dorsolateral (DL) and inferior convexity (IC) prefrontal cortices where, according to the Goldman-Rakic model, memory for spatial location and object identity are encoded in working memory. Adapted from Wilson FA, Scalaidhe SP, and Goldman-Rakic PS (1993) Dissociation of object and spatial processing domains in primate prefrontal cortex. *Science* 260: 1955–1958.

might conclude on the basis of these initial studies that an animal's ability to maintain information in an active state over a delay – a primary function of working memory – is the exclusive province of the operations of the PFC. One might have also concluded, based on the influential work of Goldman-Rakic and colleagues, that information stored within the PFC is segregated according to the processing domain, for instance, that certain subregions (i.e., dorsolateral portion) of the PFC store spatial information and other regions (i.e., ventrolateral portion) store information pertaining to object identity. Recent research on the neural basis of working memory has clarified and tempered these initial conclusions about the neural basis of working memory and the specific role of the PFC.

Working Memory and Domain Specificity in the PFC

As already mentioned, evidence for the neuroanatomical segregation of the what and where processing domains into dorsal and ventral pathways, suggested that a similar functional-anatomical organization might also hold for memory processes. Funahashi and colleagues – using a

memory-guided saccade task in the macaque and recording from neurons in the posterior part of the principal sulcus in the dorsolateral PFC (DLPFC) – showed that cells in this region code for the location of spatial cues (**Figure 4**). Moreover, lesions of the same region in the principal sulcus of the macaque impair performance on the spatial memory task, indicating the importance of the area for spatial working memory. In addition, Wilson and colleagues showed a double dissociation between object and spatial working memory in the macaque in which the DLPFC coded for spatial location memory in the delayed-response task and the ventrolateral PFC (VLPFC) coded for the identity of objects in memory (**Figure 3**).

Evidence for this neuroanatomical dissociation between spatial and object working memory was further bolstered by some human functional neuroimaging studies that revealed similar dissociations in the PFC between spatial and object domains. For instance, an early event-related functional magnetic resonance imaging (fMRI) study by Courtney and colleagues showed a neuroanatomical dissociation between delay-period activity during working memory maintenance for either the identity (object memory) or location (spatial memory)

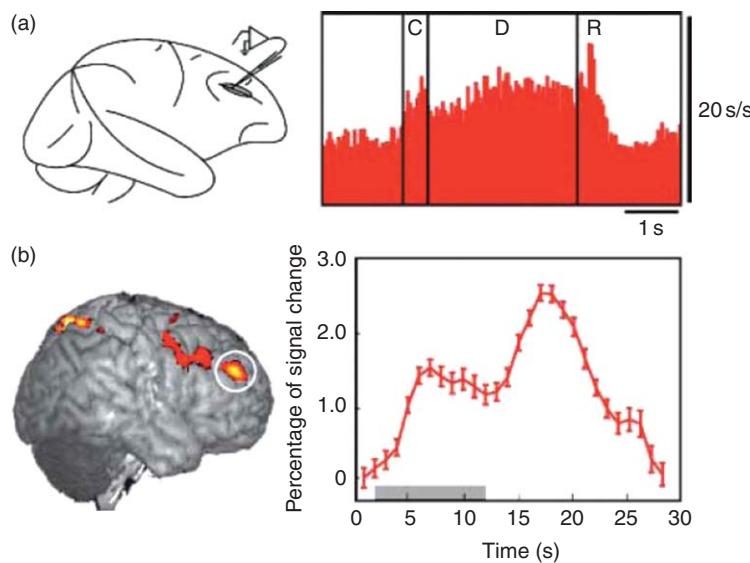


Figure 4 Neural activity in the monkey and the human lateral PFC during the retention interval of a spatial oculomotor delayed-response task. (a) average of single-unit recordings from 46 neurons with delay-period activity from the monkey principle sulcus; C = cue, D = delay period, and R = response. Adapted from Funahashi et al. (1989). (b) Human: significant delay-period activity (left) and average (right) fMRI signal from the right lateral PFC in human subject performing oculomotor delayed-response task (unpublished data from laboratory of M. D'Esposito). The gray bar represents the delay interval.

of a set of three face stimuli. Greater activity during the delay period on face-identity trials was observed in the left inferior frontal gyrus, while greater activity during the delay period of the location task was observed in the dorsal frontal cortex – a finding consistent with the spatial/object domain-segregation thesis of Goldman-Rakic. This dorsal–ventral dissociation has been observed in a number of human neuroimaging studies, using a variety of visual paradigms, and has been extended to the auditory modality – where Rama and colleagues observed a similar dorsal ventral dissociation for a task requiring working memory maintenance for either voice location or voice identity in separate trials.

The Integration of Information in the PFC

The domain-specificity hypothesis has not, however, received unequivocal support either from monkey electrophysiological investigations or from studies in the human. For instance, Miller and colleagues trained monkeys to perform a delayed object-matching task (what) and a delayed spatial-response task (where) within the same trial – a design that allowed a direct comparison of a neuron's response properties across the two domains. The results indicated that most of the neurons showing delay-period activity were equally responsive during the object and spatial memory tasks. This finding was essentially replicated in a human functional neuroimaging study by Postle and

D'Esposito who found that no region in the PFC was differentially active across the delay periods of the object and spatial working memory-trial phases. Other data from monkey physiological and human functional imaging studies also seem to be inconsistent with the domain-specific hypothesis because they provide evidence that certain dorsal and ventral PFC regions do not appear to be specific to one domain of information. For example, cooling of PFC and dorsal PFC lesions causes impairments on nonspatial working memory task and ventral PFC lesions cause spatial impairments. In addition, ventral PFC lesions in monkeys did not lead to delay-dependent defects on a visual pattern-association task and color-matching task. There are also numerous human functional imaging studies that have failed to find different patterns of PFC activation during spatial versus nonspatial working memory tasks. Postle has argued, moreover, that even if one accepts that some human neuroimaging studies have shown differences in delay-period activity across spatial and object domains, the magnitude of these effects is relatively small, and that the most salient aspect in the neural patterns observed in these studies is the extraordinary degree of overlap in the distribution of brain activity in the frontal cortex in an assortment of working memory tasks. Thus, there is good evidence that the role of the PFC in working memory, for the most part, cuts across stimulus domain, and this evidence argues for a role that is rather more integrative and flexible than is indicated by the strict domain-segregation view of Goldman-Rakic and colleagues.

Working Memory Processes and the PFC

Another possible axis along which the human lateral PFC may be organized is according to the type of operation that is performed on the contents of memory, rather than the type of information that is being maintained. For example, Petrides proposed that there are two processing systems – one dorsal and the other ventral – within lateral PFC. He proposed that the ventral PFC (Brodmann areas 45 and 47) is the site where information is initially received from posterior association areas and where active comparisons of maintained information are made. In contrast, the dorsal PFC (areas 9, 46, and 9/46) is recruited only when monitoring and manipulation of this information is required.

This model received initial support from an empirical positron emission tomography (PET) study performed by Owen and colleagues in which dorsal PFC activation was found during three spatial working memory tasks thought to require greater monitoring of remembered information than two other memory tasks that activated only the ventral PFC. For instance, in a study by D'Esposito, subjects were presented two types of trials in random order in which they were required to either (1) maintain a sequence of letters across a delay period or (2) manipulate (alphabetize) this sequence during the delay in order to respond correctly to a probe. In every subject, delay-period activity was found in both dorsal and ventral PFC in both types of trials. However, dorsal PFC activity was greater in trials during which actively maintained information was manipulated. These findings suggest that the dorsal PFC may exhibit greater recruitment during conditions that require additional processing of actively maintained information, supporting a process-specific PFC organization.

One resolution to the issue of domain specificity and working memory in the PFC comes from a close examination of the particular PFC regions that do or do not exhibit persistent activity that is specific to a particular type of information. For instance, domain specificity may exist within the superior frontal sulcus (Brodmann area 6/8) and portions of the inferior frontal gyrus (areas 44, 45, and 47), but other lateral PFC regions such as the middle frontal gyrus (areas 9, 46, and 9/46) may not show domain specificity. A coarse subdivision of the PFC into dorsal and ventral regions fails to account for the possibility that both domain-specific and process-specific organization may exist. A hybrid model of PFC organization could accommodate the empirical findings but may not be able to capture the specific types of processes that are carried out by the middle frontal gyrus (Brodmann areas 9, 46, and 9/46). Are the processes attributed to this region, for example, ‘monitoring’ and ‘manipulation’, distinct from active maintenance processes? For example, one possibility is that ‘monitoring’ and ‘manipulation’

tasks recruit the middle frontal gyrus because they require active maintenance of more abstract relations (e.g., semantic, temporal, etc.) between items. In this view, the PFC is not organized by different types of processing modules, but by the abstractness of the representations being actively maintained. This organization could be hierarchical, ranging from features of an object (e.g., red), to more abstract dimensions (e.g., color), to superordinate representations such as goals or task context (e.g., color-naming task). Evidence from functional neuroimaging studies has begun to provide support for this idea, although other explanations of the division of labor of the domain general portion of the PFC are also viable and this is an active area of research.

Working Memory Storage in the Posterior Neocortex

Although much of the focus of working memory research in the neurosciences has been on the PFC, it is now clear that regions in the parietal, temporal, and occipital cortices in the posterior part of the brain play at least a large role as the PFC in working memory. Indeed, there is good evidence to suggest that the informational content of working memory – that is, the constellation of sensory and perceptual features that comprise a memory representation – are primarily stored in the posterior neocortex. In contrast to the PFC, where evidence for functional neuroanatomical dissociation across stimulus domain has been equivocal, delay-period activity for a large number of stimulus and feature classes have been identified in sensory cortices. Single-unit recording studies have shown, for instance, that inferior temporal neurons show persistent delay-period activity as monkeys retain objects in memory across a short delay. Moreover, disruption to areas in the inferior temporal cortex of the monkey via cooling or lesions leads to severe impairments in working memory maintenance for visual object features. Similarly, application of repetitive transcranial magnetic stimulation (TMS) to the posterior temporoparietal cortex – a region known to be important for verbal memory storage in the human has been shown to disrupt verbal working memory maintenance.

As a general principle, it appears that regions that are preferentially involved during the perceptual processing of a given stimulus feature – that is, color, shape, spatial location, pitch, motion, and so on – also show reliable delay-period activation during tasks requiring working memory maintenance. For instance, the fusiform face area in the human inferotemporal region – a region that has repeatedly been shown to activate most strongly when subjects passively view pictures of human faces – also shows robust delay-period activation when subjects are required to retain face stimuli in memory across a

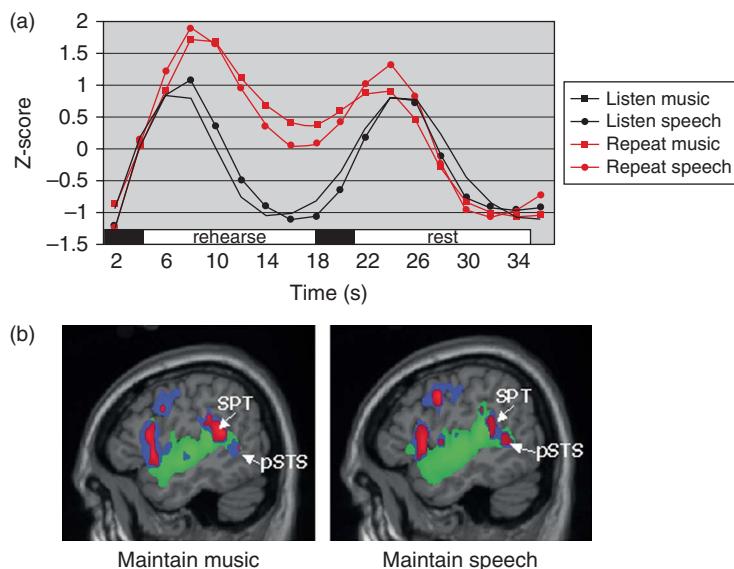


Figure 5 Main results from Hickok *et al.* (2003) study of verbal and musical working memory maintenance. (a) Averaged time course of activation over the course of a trial in the planum temporale for speech and music conditions. Timeline at bottom shows structure of each trial; black bars indicate auditory stimulus presentation. Red traces indicate activation during rehearsal trials and black traces indicate activity during listen-only trials in which subjects did not rehearse stimuli at all. (b) Activation maps of in the left hemisphere (sagittal slices) showing three response patterns for both music-rehearsal (left) and speech-rehearsal trials (right): auditory-only responses shown in green; delay-period responses shown in blue; and auditory + rehearsal responses shown in red.

short retention interval. Similarly, fMRI studies of the perception of human voices has been shown to preferentially recruit the anterior portion of the superior temporal sulcus and this same area shows delay-period activity during working memory for voice-specific information; in contrast, a more dorsal temporal region at the posterior end of the Sylvian fissure is active during the rehearsals of verbal and tonal sequences (see Figure 5).

Similar correspondences across perception and memory have been demonstrated for motion stimuli, for manipulable versus nonmanipulable objects, visual-verbal versus auditory-verbal word stimuli, olfactory stimuli, and even memory for line orientations in the primary visual cortex to name but a few examples. The evidence, therefore, is now overwhelming that domain-, stimulus-, and feature-specific memory codes are represented in the very posterior cortical centers that are known to be crucial for the sensory and perceptual encoding.

The important conclusion to be drawn from the numerous demonstrations of functional-anatomical congruence between perception and working memory storage – especially considering the relative lack of such fine-grained differentiation in the PFC – is that the maintenance of information in working memory is an interactive process in which general attentional control mechanisms in the PFC serve to modulate and bias sites in the posterior neocortex wherein specific patterns of activation that represent specific perceptual memories are stored. According to this guided activation view, the

delay-period activation observed in the PFC may actually correspond to the maintenance of task goals, abstract rules, or the maintenance of attention that is required to stave off irrelevant or distracting information. Thus, working memory requires the concerted collaboration of prefrontal and posterior areas – where the former operates as a kind of central executive whose role is to sustain attention on the currently relevant stimulus representations, even in the face of competing demands and possibly irrelevant and distracting sensory information.

Summary and Conclusions

The emergence of the concept of working memory, with its emphasis on the utilization of the objects stored of memory in the service of behavioral goals, has enlarged our understanding and broadened the scope of neuroscience research concerning working memory. It appears that the PFC has functional subdivisions that are organized according to the domain (verbal, spatial, object, etc.) of the topographical inputs arriving from posterior cortices. On the other hand, a level of representational abstractness is achieved through the integration of information converging in the more anterior and dorsal portions of the PFC. Finally, working memory function is not localized to a single brain region but is rather an emergent property of the functional interactions between the PFC and other posterior neocortical regions. Many questions remain about the neural basis of this complex

cognitive system, but studies such as those reviewed in this article should continue to provide converging evidence that may provide answers to the many residual questions.

See also: Declarative Memory.

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Neural Representations of Direction (Head Direction Cells)

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Glossary

Attractor states – In the context of neural networks, this term refers to a stable state of ongoing neural activity within a set of interconnected neurons. In any such state, each neuron has its own stable activity level (typically, for many of the cells, this level would be zero). It is assumed that these stable firing patterns are enabled by strong, mutually excitatory connections between the active cells, with strong inhibitory projections from these active cells onto those cells which are silent (or less active).

Beacon following – A navigational strategy in which the goal (or an intermediary goal) is immediately observable from the current position. In this case, it is necessary only to directly approach that goal.

Dead reckoning – A navigational strategy in which information about one's own direction and speed of movement through space is integrated over time to provide a constantly updated representation of current position. Note that this term is the less formal term for path integration.

Grid cell – A recently discovered type of navigation-related cell which resides in a portion of the entorhinal cortex. As an animal travels through a region of space, each of these grid cells will fire only in certain circumscribed hot spots. Remarkably, these spots form a hexagonal grid pattern across the two-dimensional floor of the environment.

Hippocampal formation – A set of cortical regions thought to be involved in learning and memory, perhaps especially spatial learning and memory. The hippocampal formation includes the dentate gyrus, the hippocampus proper, the subiculum complex, and the entorhinal cortex.

Path integration – A navigational strategy in which information about one's own direction and speed of movement through space is integrated over time to provide a constantly updated representation of current position. See also 'Dead reckoning.'

Place cell – A type of navigation-related cell originally discovered in the hippocampus. As an animal travels through a region of space, each of these cells will fire in only one (or, rarely, two or three) hot spot within the area. Unlike the entorhinal grid cells, these place cell firing fields do not form any regular pattern, even when they have more than one hot spot.

Postsubiculum – Sometimes also referred to as dorsal presubiculum. This is a cortical region which is one of the subregions of the subiculum complex. The subiculum complex is, in turn, part of the hippocampal formation. The postsubiculum is characterized by distinctive cell islands in layer 2. In addition, it has a somewhat different set of connections with other limbic and sensory regions, when compared to the other parts of the subiculum complex. Head direction cells were first discovered in the postsubiculum.

Subiculum complex – A set of interconnected cortical regions which form a border along much of the longitudinal extent of the hippocampus. This subiculum complex has been divided into a set of subregions based on anatomical and connectional characteristics. Depending on the species and the exact criteria used, these subregions can include the postsubiculum, prosubiculum, parasubiculum, presubiculum, and the subiculum proper. Each of these subregions contains cells which appear to signal navigation-related information.

Animals and Humans Depend on Sophisticated Navigational Abilities for Survival

The ability to navigate over extended terrain is an essential skill for many animals, including humans. Thus, it is often necessary to leave the safety of the home in order to search for food, water, mates, warmer climate, and so on. Typically, it is also then necessary to make the return trip back to the starting location. This extended travel requires that the individual, in some manner, keeps track of the relative distance and direction of travel during the outward journey, so that an accurate calculation can be made for the trajectory needed to return home.

Behavioral scientists have discovered that this ability depends on a variety of interwoven capabilities, including calculations based on the relative positions of environmental landmarks, beacon following, route following, and inertial guidance.

One component of this complex navigational capacity is a sense of direction. Thus, for example, when one is sitting in the office at work, it is typically possible to point

toward the approximate relative direction of various other familiar locations, such as home, the grocery store, the library, and so on. This ability can be demonstrated behaviorally in animals as well by testing their ability to head out in the correct direction toward a goal.

Head Direction Cells: The Basic Phenomenon

An insight into how this sense of direction is generated in the nervous system was provided by James Ranck in 1984, with the discovery of head direction (HD) cells. Ranck recorded the activity of the cells in a cortical region known as the 'hippocampal formation,' which had already been identified as being critical for navigation (see below). In particular, he had placed the electrodes in a subregion of the hippocampal formation known as the 'postsubiculum.' There, he observed cells which had the remarkable property that any one cell fired only when the animal faced one particular direction, relative to the surrounding environmental cues.

Figure 1 shows a diagram of the recording system used to document these cells in the seminal work of Taube and colleagues. Rats were trained to constantly locomote within a 76-cm-diameter recording chamber while they foraged for tiny food pellets which were continually dropped onto random locations on the cylinder floor. The rat carried with it two small lights which were rigidly attached to its head. These lights enabled an overhead tracking system to continually record the rat's momentary

directional heading. At the same time, action potentials from cells in the postsubiculum were recorded.

Figure 2 provides an illustration of three different, prototypical HD cells. **Figure 2(a)** shows an overhead view of the cylindrical recording chamber, along with the radially organized numeric scale used to indicate directional heading. The left side of each row in **Figure 2(b)** shows the average firing rate for one HD cell over the entire 30-min recording session, plotted as a function of directional heading. The right panel in each row shows a pictorial diagram of the cell's directional firing pattern. As can be seen, each cell was active only over an approximately 90° portion of the 360° range of possible headings. Within this 90° preferred directional range, each cell showed a pronounced peak rate located at the center of this range, and the average rate for each cell falls off symmetrically for directions on either side of this peak. Thus, each cell provides a signal that the rat is currently facing one particular direction within the cylinder. Since different cells have different preferred directions, this indicates that for any direction the rat can face, there will be a unique pattern of cell activity across the HD cell population.

Note that it does not matter where the animal is within the chamber or what behavior it is engaged in. As long as the animal obtains a directional heading within the range of a given HD cell, that cell will fire.

It should be noted that these HD cells have also been observed in the subiculum complex of the monkey, *Macaca mulatta*, by Edmond Rolls and colleagues. In addition, those brain regions in which the HD cells have been

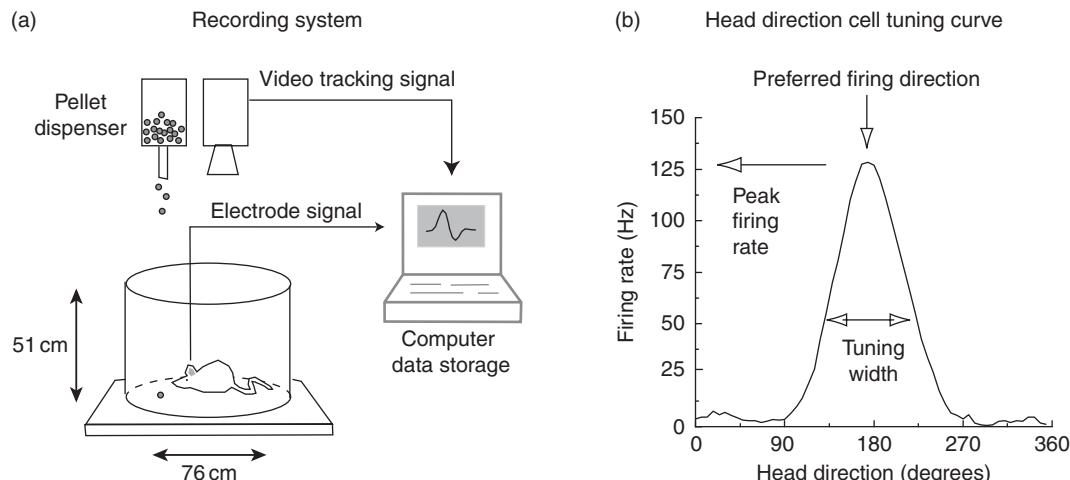


Figure 1 (a) A schematic diagram of the recording setup used for many studies of the head direction (HD) cell system. The rat is first trained to search for tiny food pellets which are continually dropped to random locations within the chamber floor. This training results in a behavioral pattern in which the rat constantly zigzags over the chamber floor, continually circling through all possible directional headings over the course of recording sessions which can last up to an hour. Throughout the recording sessions, the rat's location and directional heading are constantly monitored. In addition, microelectrodes implanted in the navigation-related brain regions constantly record the action potentials of single neurons. (b) An illustration of a typical directional tuning curve from a HD cell. Here, the average firing rate of the cell over the course of the recording session is shown as a function of the rat's momentary directional heading.

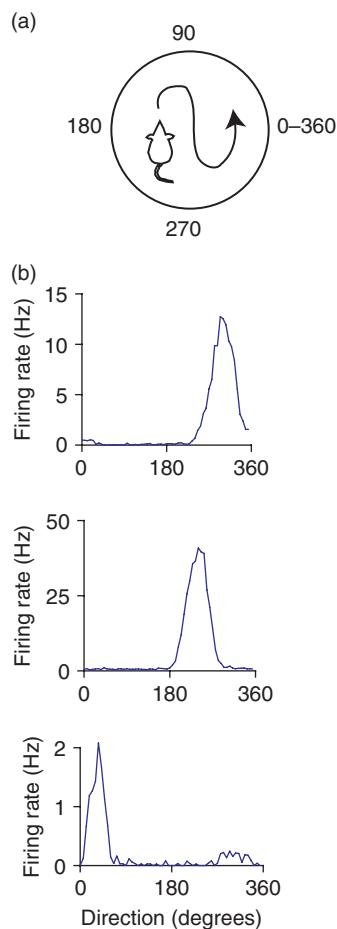


Figure 2 (a) Here, an overhead view of the recording chamber from **Figure 1** is shown. The numbers placed around the outside of the chamber indicate the angular scale used to calculate the directional tuning curves for the head direction (HD) cells. (b) Each row illustrates one typical HD cell. The left panel of each row shows the directional tuning curve for the cell, while the right panel shows an illustration of the directional heading at which that cell fired maximally.

documented in animals are also known to be important for navigation in humans. Thus, it appears that the HD cell system may be a highly conserved component of the vertebrate neural circuitry for navigation.

HD Cells Are Guided by Both Environmental Landmarks and Path Integration

Since the initial discovery of HD cells, follow-up studies have investigated how these cells work; that is, how can a neuron buried deep within the cortex manage to somehow track which direction the rat is facing at each moment?

HD Cells Are Partly Controlled by Familiar Landmarks

One possibility would be that the cells somehow receive information about the local geomagnetic field. Thus, they could possibly sense the directional relationship between the head and actual geocentric coordinates.

Work conducted in laboratory settings has indicated that if such an influence is present, its influence is minimal. **Figure 3** provides a diagrammatic illustration of one of the early findings by Taube and colleagues. For this work, the gray, cylindrical recording chamber was typically equipped with a salient white cue card which served as an orienting stimulus. The top row in **Figure 3** shows this card in the standard position, which was used on a daily basis for the routine screening and recording of these cells. The second row illustrates what happened during occasional probe sessions in which the card position was rotated prior to the rat's entry into the chamber. Here, it can be seen that this angular rotation of the position of the card caused an equal rotation of the angle of the preferred direction of the HD cell. Thus, it appeared that this familiar, salient landmark controlled the directional preference of the cells, rather than (or at least more strongly than) any geomagnetic cues which were available.

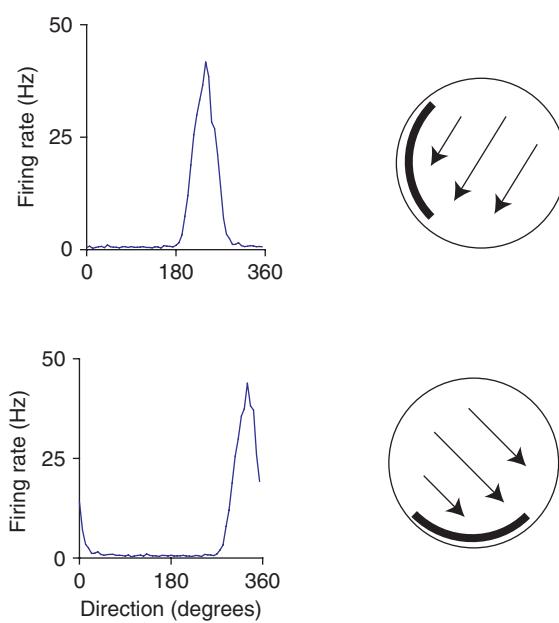


Figure 3 An illustration of the landmark control of head direction (HD) cells. The upper row shows the directional firing properties for a typical HD cell recorded in the cylindrical chamber equipped with a cue card in the standard position, on the west portion of the wall. The second row shows the data from that same cell during a probe session in which the cue card position was rotated by 90°. As can be seen, in this familiar chamber, the position of the cue card exerted control over the directional preference of the HD cell.

This finding is compatible with everyday experience, in that we often use available landmarks, such as mountains or tall buildings, to help us get our bearings. The above finding also suggests the possibility that, indeed, the only explanation necessary for the behavior of these cells is that they are tuned to respond to particular stimuli, when those stimuli are positioned within a certain limited region of the visual field. Thus, it is possible that the cell portrayed in **Figure 3** responds to any large, white stimulus whenever it is in the right portion of the visual field. Within the cylindrical chamber used here, this would only happen when the rat was facing the direction indicated by the arrows.

HD Cells Are Also Guided by Path Integration (Dead Reckoning)

Additional work, however, has clearly shown that the explanation for direction-specific firing in these cells is not as simple as this. Indeed, it seems that the HD cells have a remarkable ability to keep track of directional heading even when the animal travels into a novel environment, so that there are no familiar landmarks, and even if it is in a situation in which the salient landmarks are ambiguous.

Figure 4 illustrates findings from an experiment conducted by Taube and Burton. Here, HD cells were first recorded in the standard cylinder used for training and recording. Thereafter, during probe sessions, the rat was

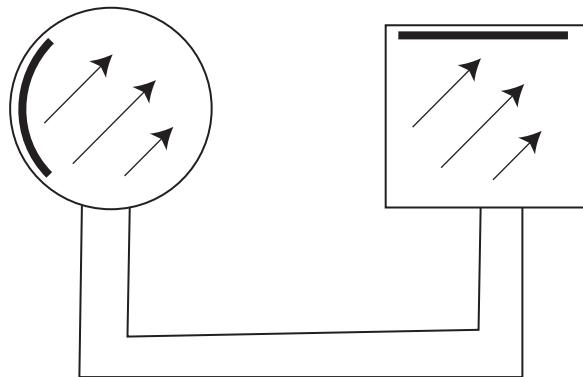


Figure 4 An illustration of path integration in a head direction (HD) cell. During training and standard recording sessions, the rats were confined to the cylindrical chamber which forms the left compartment of this apparatus. Note that this cylinder is equipped with a single, white cue card on the west portion of the wall. Then, during a probe session, the rat was allowed, for the first time, to travel down the corridor to locate the novel square chamber on the right. As illustrated, HD cells in this experiment retained the same directional preference in this new chamber. Note that a white cue card is also present in the square, but placed in a different angular position here. This suggests that the HD cell system tracked the animals as they traveled heading into the new terrain, and was not controlled by the cue card in this unfamiliar context.

allowed to travel along a novel route provided by the opening up of a hallway which led to a novel chamber. Note that this new chamber was also equipped with a single white cue card, but then it was placed in a different angular position. In this case, the cells retained the same directional preference (relative to earth) as for the original standard cylinder. Thus, even when the rat traveled into a chamber it had never previously visited, it retained a single, fixed, directional preference for each of its HD cells. In this novel setting, the familiar relationship to the cue card was broken.

These findings are also compatible with everyday experience. Thus, if one heads north, out of town, and drives into novel terrain, it is possible to retain some ongoing sense of which way one is headed, even if there are no available directional indicators, such as the sun (i.e., the sky being overcast) or distant mountains. For example, if the road is straight, and one has not turned onto any other road, then one must still be headed north. Similarly, if one then makes a 90° right turn onto another road, then one must now be heading east.

Note that in this new environment, one is likely to see examples of familiar stimuli, such as gas stations and fast-food restaurants. When we see these stimuli in our familiar home territory, they can often help us get our bearings. Thus, for example, if the Exxon station is on the north end of Main and we are able to view this station from the center of town, it must be the case that we are currently facing north. In novel terrain, however, the same sort of Exxon station may be in any arbitrary position relative to nearby towns. Thus, this new Exxon station is not initially a useful directional landmark. The experiment illustrated in **Figure 4** suggests that the HD cell system recognizes that familiar landmarks do not retain a constant relation to directional heading when in a novel terrain, since the HD cells do not snap into line with the cue card in the novel setting, even though they did so in the familiar environment (**Figure 3**).

This ability to keep track of directional heading even in the absence of learned environmental landmarks is thought to depend on a process known as ‘path integration,’ or, less formally, ‘dead reckoning.’ According to this idea, directional heading can be tracked by a process in which any angular motion of the head is constantly integrated to calculate the current directional heading. Thus, in the example above, a 90° right turn taken from a start position heading north would result in a position in which the head faces east. In relation to the HD cells, this implies that the right turn somehow turns off the north HD cells and turns on the east ones.

Another example of evidence for the path integration process for these cells is illustrated in **Figure 5**. This study used a chamber equipped with eight alternating, vertical, black and white stripes. These stripes were all identical in width and designed to provide an

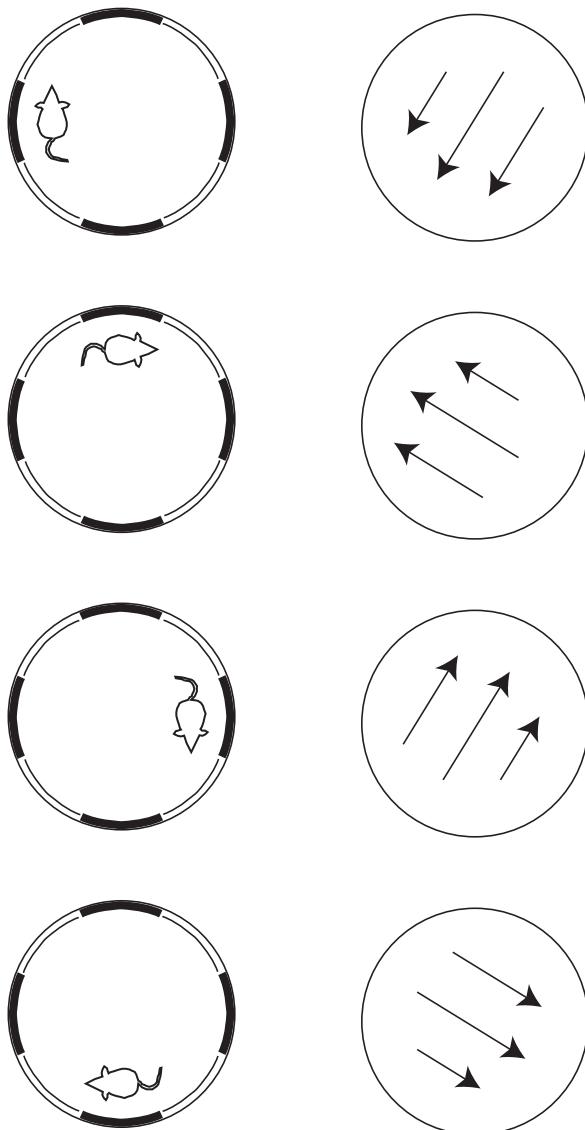


Figure 5 An additional example of path integration in the head direction (HD) cell system. Here, the cylindrical recording chamber has been equipped with a set of alternating black and white stripes, meant to provide an ambiguous stimulus situation. Each row illustrates a different session for the same HD cell. The only variable which was manipulated from one session to the next was the rat's start position. As indicated by the drawn rat figure, each session began by initially placing the rat down next to a different black stripe. As shown, this initial placement determined the preferred direction of that HD cell for the duration of that session.

environment in which all salient visual cues were ambiguous in terms of their relationship to directional heading. Thus, for example, if the rat stood in the middle of the chamber and faced due north, its view would include a black stripe surrounded by two white stripes. This same view would also be available when facing each of the directions east, south, and west.

Note that if the HD cells were controlled just by the immediate input from environmental landmarks, then this environment might be expected to cause directional firing patterns with four, equally spaced peaks along the directional range. However, in keeping with the path integration idea presented above, this is not what happened. Indeed, during any given recording session, each HD cell showed only one preferred direction. This preferred direction was robust and stable throughout the 15-min session.

Interestingly, however, this preferred direction could be changed from one session to another simply by changing the position at which the rat was initially placed down into the chamber. Specifically, each 90° rotation of the start position caused a corresponding 90° rotation of the cell's directional peak. This suggests that the initial placement of the rat into the chamber served to initialize the HD cell system to some constant value (such as north). From that initial setting, the rat's subsequent movements served to constantly update the firing pattern in the HD cell system, so that all cell activity was stable relative to the start position in that particular session.

HD Cells Appear to be Wired Together to Form a Single, Internally Consistent Set of Direction-Signaling Cells

One additional general finding is relevant here that serves to capture the flavor of these cells. This finding comes from cases in which two or more HD cells were recorded at once. Imagine, for example, that in the standard cylinder there is one cell which fires when the rat faces north, and another which fires when the rat faces south. Next, imagine that the rat is placed into a covered carrying cage and is spun around, one way and then the next, many times, so that there is no way the HD cell system could track so many different turns and twists. During this spinning process, the animal is also transported and placed down into a completely novel chamber in a new laboratory room. In this case, there is no way to predict which direction will be selected by either of the two cells; that is, in keeping with common experience, if they have no way of keeping track of their ongoing position, and if there are no familiar cues available, then the system simply has no way to tell which direction is which, relative to earth.

What can be predicted, however, is that each of the cells will show a single preferred direction in the new environment; that is, they will be well-formed HD cells. In addition, these two cells will, once again, have a 180° difference between their two preferred directions. Thus, if cell 1 chooses east (in earth-centered coordinates), then cell 2 will choose west.

There have been numerous additional studies conducted to examine the relative influences of

environmental landmarks and path integration on the firing rates of the HD cells. All of these data are compatible with the idea that the HD cells are somehow connected together to provide a single, internally consistent set of direction-specific cells. This cell population receives input about the animal's angular head motion from vestibular, motor, and other sources of movement information, and uses this input to update the population firing vector. In addition, however, the cells can become attached to familiar landmarks, so that these can sometimes reset the directional firing pattern. This attachment to landmarks is thought to result from Hebbian increases in synaptic strength, which result when signals from environmental inputs are paired with activity in certain HD cells.

Neural Network Models for the HD Cells

Investigators in several laboratories have worked to encapsulate the above ideas into a neural network model of the HD cell system. All the models incorporate the same general set of principles, and these are illustrated in **Figures 6 and 7**.

All the models begin with the idea that the HD cells are linked together in a ring-like architecture in which

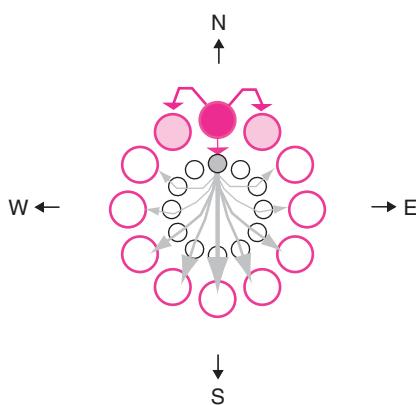


Figure 6 The basic ring architecture is shown here which forms the core of most neural network models of head direction (HD) cells. The to-be-modeled HD cells themselves are drawn in pink. For illustrative purposes, these cells are drawn in a circular formation, with each cell occupying a radial position corresponding to its preferred firing direction. Each HD cell has a companion inhibitory cell (drawn in black), to which it sends an excitatory projection. Each inhibitory neuron, in turn, sends inhibitory projections to the remaining HD cells. In addition, there are excitatory connections between neighboring HD cells. This architecture enforces a winner-takes-all behavior in which, at any one time, activity is allowed only for a small subset of adjacent cells located within a small range of the circle. Note that cell activity here is indicated by coloring in the cell bodies. In addition, the excitatory and inhibitory connections are shown here only for the north HD cell and its inhibitory companion. However, each of the cells is assumed to have a corresponding set of connections.

cells meant to represent similar (nearby) directions are linked through a combination of excitatory and inhibitory connections, while those meant to represent different directions (such as north vs. south) are linked through strong inhibitory connections. This basic ring structure is illustrated in **Figure 6**.

This architecture ensures that the HD cell population can never represent two different directions (such as east and north) at the same time, and thus matches the real-world fact that it is not possible to face two different directions at once. Indeed, these structures form a ring attractor in which a single, localized packet of activity can come to rest at any position along the ring.

The models also incorporate cells which provide information about angular head velocity, as shown in **Figure 7**. These angular velocity cells fire tonically, so that they are active even when the rat is at rest. This rate is modulated by the angular motion of the head, however, so that the head turns to one side (e.g., to the right or clockwise) cause increases in the rate which are linearly related to the speed of the turn. In contrast, turns to the opposite side (e.g., left or counterclockwise) result in firing-rate decreases which are linearly related to the turning speed.

Cells like this have, in fact, been discovered in many of the same brain regions in which HD cells are located (see discussion of the 'anatomical location of HD cells' below). These angular velocity cells are thought to receive information from a variety of sources. These include the vestibular system, which contains cells that code the angular acceleration of the head, as well as optic flow induced by head rotation, and also motor commands which produce head motion.

These angular velocity cells are incorporated into the models in such a way that when the animal turns its head, this angular motion will cause the firing pattern in the HD cell population to be updated. For example, if the animal starts out heading north and then makes a 90° clockwise turn, this motion will result in turning off the north HD cells and turning on the east ones.

An example of this idea is provided in **Figure 7**. Here, the angular velocity cells described above are attached to a set of intermediate cells (angular velocity by HD cells), which also receive input from the HD cells themselves. Specifically, each of these angular velocity by HD cells receives input from just one HD cell. In addition, they receive input from only one of the two possible kinds of angular velocity cells (clockwise vs. counterclockwise). This means that any one angular velocity by HD cell will fire only when the rat is facing one particular direction and turning one particular way (clockwise or counterclockwise). Thus, each of these cells detects one particular conjunction of current heading direction and current angular motion. The solid blue cell in the lower panel of **Figure 7**

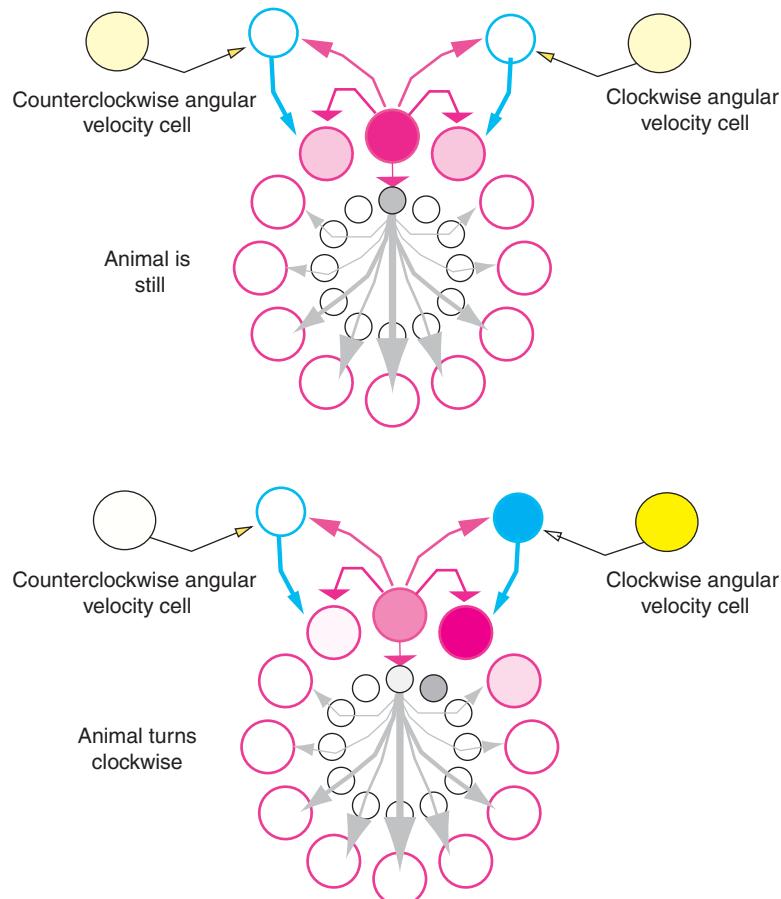


Figure 7 Here, angular velocity cells (yellow) and angular velocity by head direction cells (blue) have been added to the ring structure shown in **Figure 6**. When the rat is still (upper panel), the two angular velocity cells fire at the same low, tonic rate. This is not sufficient to activate the angular velocity by HD cells. When the rat turns to the right (lower panel), the angular velocity by HD cell on the right is activated. This cell, in turn, provides excitation to the next HD cell in the ring. Thus, as described in the text, the activity pattern in the HD cells is updated to reflect the rat's angular head turns.

represents a cell which requires joint activity in the north-preferring HD (pink) cell and the clockwise angular velocity (yellow) cell. Note that each of these angular velocity by HD cells, in turn, sends an excitatory projection onto the appropriate adjacent HD cell. In this case, activation of this blue cell causes activation of the HD cell one step clockwise from the north cell. As this next HD cell begins to increase its firing rate, it activates its own companion inhibitory cell, which then begins to inhibit the other HD cells. In this way, the overall pattern of cell activity is shifted clockwise by one step. Continued movement will cause continued shifting.

Anatomical Location of HD Cells

Since the initial discovery of HD cells in the postsubiculum region of the hippocampal formation, these cells have been discovered in numerous additional brain

regions. Indeed, much of the rat limbic system seems to contain HD cells. **Figure 8** shows a diagram of the portions of the rat limbic system in which HD cells have so far been documented. These areas include a set of interconnected regions which form a loop from the hippocampal formation down to the mammillary bodies, continue up to the anterior thalamus, and then back up to the hippocampal formation. Within each of these limbic regions, the HD cells usually constitute only a small subset of all the cells.

Interestingly, many of the cortical regions which contain HD cells also contain additional cell types thought to be critically involved in navigation-related cognition. These other types include the well-known hippocampal place cells and the recently discovered entorhinal grid cells.

It is thought that the place cells, grid cells, and HD cells all work together to form a spatial tracking system which constitutes the basis of navigational tracking abilities at the behavioral level.

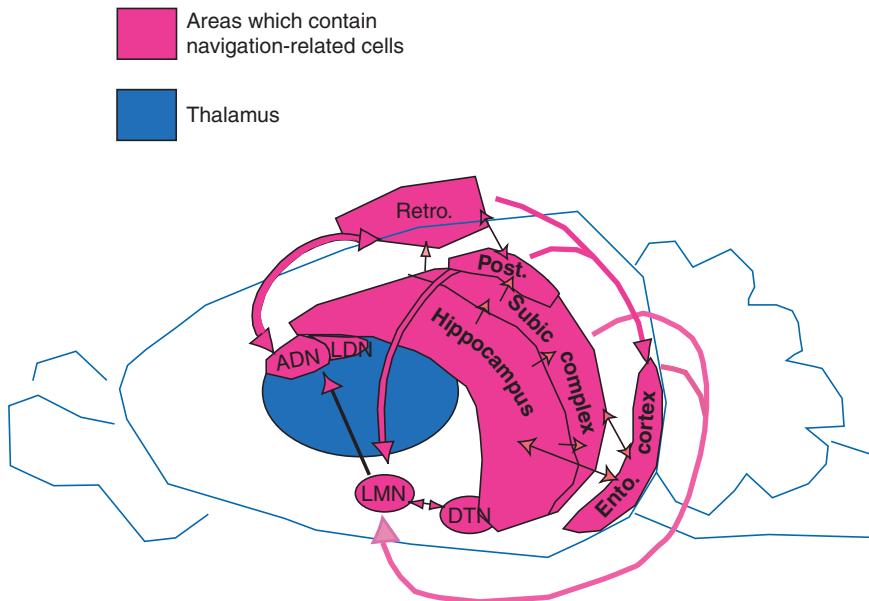


Figure 8 A schematic diagram of the rat limbic system areas in which head direction cells, as well as additional navigation-related cell types have been discovered (see text). ADN, anterodorsal nucleus of anterior thalamus; DTN, dorsal tegmental nucleus; Ento. cortex, entorhinal cortex; LDN, lateral dorsal nucleus of thalamus; LMN, lateral mammillary nucleus; Post., postsubiculum; and Retro., retrosplenial cortex.

See also: Cognition: Learning and Memory: Spatial; Neural Representations of Intended Movement.

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Relevant Websites

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Neural Representations of Intended Movement

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Glossary

- Coordinate transformation** – A computation that converts the position of an object in one reference frame into its corresponding position in another reference frame.
- Efference copy** – A copy of the brain's motor command that could, in principle, be shared among brain areas.
- Intention** – The goal or purpose behind a particular action or set of actions.
- Proprioception** – The sense of position and orientation of the parts of the body. This sense is derived from receptors in the skin, joints and muscles.
- Reference frame** – A coordinate system or set of axes used to describe the position or movement of an object.
- State estimation** – The process of computing the position and velocity of a time-varying system from online measurements.

Intentions represent early plans for movement. Rather than specifying the endpoint forces, joint torques, or muscle activations required for movement execution, intentions represent 'earlier' aspects of movement planning. Intentions specify the goal of a movement as well as the effectors (eyes, limbs, etc.) that will be used to accomplish that goal. Thus, the formation of intentions depends upon a host of cortical functions, including spatial perception, action planning, and decision making.

Where are intentions encoded in the brain? In humans, neurological deficits following injury to the frontal and parietal cortices are consistent with these regions playing an important role in early aspects of eye and arm movement planning and spatial perception and thus in the formation of intentions. For example, injury to the superior parietal lobule (SPL) of the posterior parietal cortex (PPC) can lead to disturbances of planning such as optic ataxia, which is characterized by an inability to accurately reach objects under visual guidance. In contrast, injury to the inferior parietal lobule (IPL) and premotor cortex leads to disorders that have traditionally been thought to be more perceptual or cognitive in nature: (1) neglect, characterized by a failure to perceive space (extrapersonal or intrapersonal) on the side contralateral to the injured half of the brain, and (2) apraxia, defined as an inability to perform complex gestures that is not caused by weakness, loss of sensation, or other obvious motor-related deficits. Importantly, these deficits do not reflect difficulties in

movement execution but rather in the planning and preparation of movement.

As in humans, the frontal and parietal cortices of non-human primates have also been implicated in the formation of intentions. For example, effector-specific areas responsible for the planning of saccades and limb movements have been identified in both cortices. In addition, activity related to movement goals can easily be elicited in these areas. In many instances however, this intention-related activity is combined at the single cell level with activity that encodes the state of the animal, that is, the position and configuration of the eyes, limbs, and/or other body parts. Thus, borrowing terminology from engineering, these areas can be thought of as being involved in 'state estimation' as well as in the formation of intentions. The discussion here focuses on how state- and intention-related activity are encoded and interact in limb movement-related areas of the brain, with special attention given to the dorsal premotor cortex (PMd) of the frontal lobe and parietal area 5 and the parietal reach region (PRR) of the SPL ([Figure 1](#)). It will be argued that the particular form of this interaction allows for maximum flexibility in planning and executing movements of the eyes and limbs under constantly changing environmental conditions.

State Estimation in the PPC

State estimation refers to the process of computing the current state (i.e., position and velocity) of a time-varying system from online measurements. In the context of limb motor control, optimal state estimators monitor incoming sensory information as well as outgoing motor commands in order to reduce uncertainty in the estimate of limb position and velocity. Estimating limb position on the basis of motor commands is facilitated by a forward model, a system that uses efference copy and an internal model of the dynamics of the limb to predict the limb's state in response to these commands. A recent study of nonhuman primates trained to control a computer cursor by moving a joystick has shown that many PPC neurons encode the future position and velocity of this cursor, suggesting that this area may also play an important role in estimating the state of the limb.

Psychophysical experiments in humans also implicate the PPC in this important function. For example, in humans damage to the SPL has been shown to result in

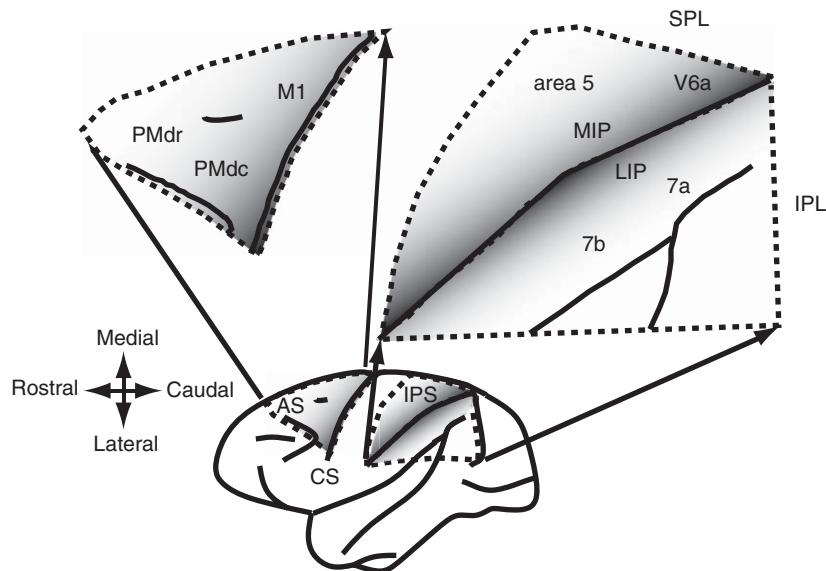


Figure 1 Lateral view of the rhesus macaque monkey brain with the PPC and parts of the frontal lobe highlighted and expanded. Shaded regions indicate the banks of sulci. In the PPC, PRR corresponds to what is labeled MIP (medial intraparietal area) and V6A. AS: arcuate sulcus. CS: central sulcus. IPS: intraparietal sulcus.

a profound difficulty in maintaining limb position and grip force in the absence of vision, supporting a role for this structure in integrating sensory and motor information to estimate limb state. Studies of patients with known optic ataxia exhibit marked difficulty in making corrections to their ongoing arm movements in response to a sudden change in target location, suggesting impairment in the system responsible for rapidly updating movements. Importantly, these patients can correct their ongoing movements; they just do so in a slower and more deliberate fashion than their neurologically intact counterparts. These observations have led to the proposition that there are two distinct brain systems subserving the online control of visually guided arm movements: a fast-acting automatic system, based largely in the SPL, and a slower more voluntary one, based in the IPL and inferotemporal cortex, that augments the automatic system. The ability to rapidly update ongoing movements requires accurate information about the current position and velocity of the moving limb; thus, it is possible that the automatic and voluntary systems, and their constituent brain areas, may be distinguished from each other by their relative contributions to online state estimation.

Sources of Limb-State Information

What signals contribute to the encoding of limb state in the PPC? In nonhuman primates, both the SPL and IPL have been shown to receive visual and somatosensory inputs. Within the IPL, sensory inputs appear to be largely segregated, with 7a receiving input primarily from visual areas and 7b receiving input from areas considered

to be involved in somatosensory processing. Although it has been known for some time that neurons in area 7a respond during arm movements, these responses have not been nearly as well characterized as those in the SPL. Recent studies indicate that 7a neurons are strongly modulated at the time of, or slightly after, movement onset. These observations, in addition to anatomical data showing a lack of direct projections from the motor cortex (M1) or PMd to 7a, suggest that area 7a responses during movement are related largely to sensory processes. However, it should be noted that 7b receives input from area 5 and projects in turn to 7a, providing a possible neural substrate for an efference copy of limb movement commands to 'leak into' the IPL reach areas (see below). In addition, probably the strongest neural evidence to date of the existence of an efference copy signal, though in the context of oculomotor control, comes from studies of a part of the IPL immediately adjacent to 7a, that is, the lateral intraparietal area (LIP). Subsets of neurons in this area begin to increase their firing rate in anticipation of a visual target falling within their receptive field at the completion of a planned saccade. This suggests that LIP neurons have access to an estimate of the future position of the eyes that is derived from efference copy and a forward model of the eye plant. In a similar way, it is conceivable that arm movement-related areas of the IPL could receive an estimate of the future position of the limb based on efference copy and an internal model of the arm.

In nonhuman primates much more is known about the anatomy and physiology of arm movement-related areas of the SPL, particularly area 5. Neurophysiological

studies have consistently reported that area 5 neurons encode information about static limb position as well as limb position and velocity information during movement, both strongly suggestive of a role for this area in state estimation. However, the relative roles of efference copy and sensory signals in this process remain unclear. Historically, activity in area 5 has been assumed to reflect high-level somatosensory processing though recent studies have provided evidence that static limb position signals in this area are based, in part, on visual input. Regarding efference copy, lesion studies suggest that the SPL receives a copy of ongoing arm movement commands. This finding is supported by anatomical studies, which report direct projections from PMd and M1 to area 5 of the SPL. Lastly, neurophysiological studies have shown that many cells in area 5 often respond before movement onset in reaction time tasks and continue to respond even following deafferentation of the arm by dorsal rhizotomy. It is unlikely that activity preceding movement in area 5 reflects a descending motor command, as direct projections from area 5 to the spinal cord synapse on cells in the dorsal, rather than ventral, horn. Instead, this activity may represent an efference copy of a command generated elsewhere.

Thus, evidence obtained from nonhuman primates suggests that limb movement-related areas of the SPL and IPL can be distinguished not only by the type of sensory signals (visual, proprioceptive) that they process during movement, but also by the extent to which they integrate sensory inflow with ongoing movement commands. Even within each subdivision of the PPC, the relative contribution of these signals varies anatomically. For example, in the most dorsal parts of area 5, somatosensation (i.e., proprioceptive and tactile sense) seems to dominate the representation of limb position; neurons here respond strongly to passive manipulation of the joints and have large tactile receptive fields that are sometimes bilateral. In addition, reach-related neurons in dorsal area 5 appear to encode information about the configuration of the limb and not simply its endpoint, further supporting a strong role for proprioception in this area. However, as one moves caudally in the PPC, that is, toward the crown and bank of the intraparietal sulcus, the relative contribution of vision gets stronger, as discussed below.

Interaction of State and Intention Related Activity in the PPC

Coding of Intention

Areas located with the banks of the intraparietal and parieto-occipital sulcus, such as the medial intraparietal area (MIP) and V6A, are generally more responsive to visual stimuli than adjacent areas on the cortical surface.

These anatomically defined areas appear to correspond to what has been termed, on functional grounds, as the PRR. Cells in PRR respond briskly to briefly flashed visual stimuli and continue to respond even after these stimuli have been extinguished, that is, during the delay periods of instructed delay tasks. Despite being strongly visually driven, it appears that this area is involved in the early planning of limb movements rather than simply being involved in sensory and/or attentional processes. For example, cells in this area have been shown to be much more active during the planning of reaches than during the planning of saccades. In addition, PRR neurons are preferentially activated with movements of the contralateral limb and their activity during delay periods is correlated with reach but not saccadic reaction times, findings that might be expected of areas fairly far along in the computations involved in specifying limb movements. Interestingly however, goal locations in this area are encoded with respect to the current point of visual fixation, that is, in eye-centered, rather than limb- or body-centered coordinates. Even more surprising is the observation that the responses of some PRR neurons are further modulated in amplitude (i.e., gain modulated) by the position of the limb, an effect which also appears to be best described in eye coordinates. This hand-in-eye coding appears to persist even in the absence of concurrent visual input about hand position, suggesting the representation of limb position in this area is constructed, at least in part, by transforming somatic signals (proprioception/efference copy) into a reference frame that is more aligned with the visual one.

Coordinate Transformations for Reaching

The benefits of such an encoding scheme are now clear. If both the goal location and current hand location are encoded in eye coordinates, a representation of the desired arm movement vector can be computed by vectorially subtracting the position of the limb from the position of the goal location in this reference frame (**Figure 2**). Evidence for such a scheme has been observed in area 5. Here the responses of many neurons are most consistent when goal locations are identical in both eye and hand coordinates. In other words, these neurons encode reach goals not with respect to the eyes or limbs alone but with respect to both effectors. This supports the idea that an eye-centered representation of the reach vector is generated within the PPC, with PRR and area 5 both playing a role in this computation. A critical component of this scheme is the gain modulation of PRR activity by the position of the limb in eye coordinates. This gain modulation is a distributed effect, that is, the precise position of the hand in eye coordinates in PRR cannot be determined from the activity of a single cell but must instead be read out from a population of

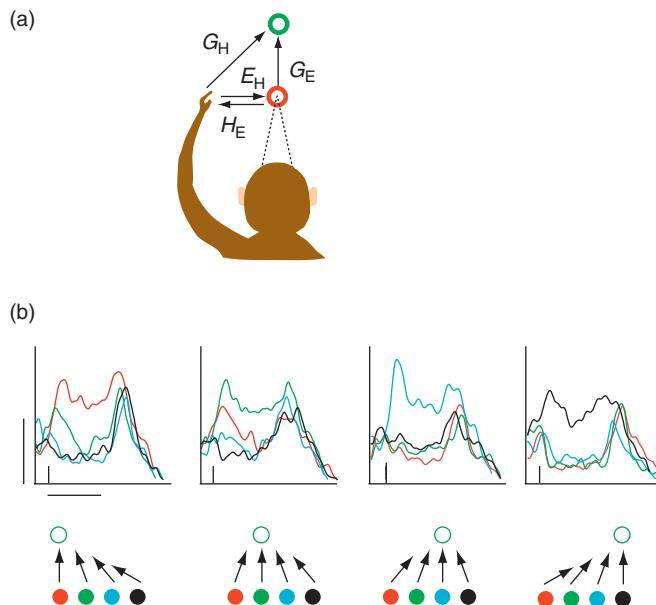


Figure 2 (a) The location of the goal in hand-centered coordinates (G_H) can be computed by vectorially subtracting the hand location in eye coordinates (H_E) from goal location in eye coordinates (G_E). (b) Example of a parietal neuron that encodes the vector G_H during the memory period of an instructed delay task. Each panel represents a different horizontal target (goal) location. The histograms in each panel show neural activity associated with movements from different starting positions (located just below the targets) and are aligned on target onset (small vertical tick mark). For this neuron, planning activity was greatest for purely vertical movements. Vertical scale bar: 40 spikes/s. Horizontal scale bar: 1 s.

gain-modulated neurons. A representation of the position of the limb in eye coordinates is also observed in PMd, with one very important difference: here rather than a distributed representation of this vector, the hand-in-eye effect is observable at the single cell level. This representation appears to be ideally suited for coordinating movements of the eyes and hand.

Parieto-Frontal Intention Networks

It is now well established that the reach areas of the PPC are richly interconnected with those of the frontal lobe. The pattern of connections has been termed a ‘neuroanatomical gradient,’ as areas located more medially or posteriorly in the PPC are connected more strongly to rostral parts of PMd, while more anteriorly located parietal areas are more strongly connected to more caudal areas of the frontal lobe, that is, caudal PMd and M1. It is also clear that many of the areas in this parieto-frontal network are not involved in specifying the forces and muscle activations necessary to move the arm but in monitoring the state of the body in relation to high-level movement goals. Other aspects of intention may also be encoded in this network. For example, it has recently been shown that correlations in spiking and local field potential activity between PMd and PRR are greater when monkeys freely choose a sequence of targets to which they are reaching than when

the sequence is instructed, suggesting that this intention network is also involved in decision making.

The pattern of connections described above coexists with another network connecting areas of the IPL with the ventral premotor cortex (PMv). As stated above, neurons in 7a and 7b of the IPL are active during limb movements. Neurons in these areas have also been shown to signal the positions of the eyes and limbs and, at least in 7a, the position of the body in space. Although these areas project to PMd and other areas of the dorsal frontal lobe, they appear to be more strongly connected with PMv. PMv neurons encode goal-related information. They are also active during ongoing reaching movements but appear to be more involved in signaling visual feedback of these movements rather than somatomotor feedback. In addition, PMv neurons do not appear to encode effector-related information (i.e., left or right arm) as strongly as neurons in PMd. This suggests that PMv, and the parietal areas to which it connects, may play a different role in intention than PMd, PRR, and area 5. In humans, the specific role of this ventral intention network is only beginning to be unraveled. However, a recent imaging study has demonstrated that recovery from neglect is associated with a rebalancing of activity in the ventral frontal and lateral parietal lobes, highlighting the importance of cooperation among the various nodes of this network for high-level cortical functions such as spatial attention and orienting behavior.

Interaction of State- and Intention-Related Activity in PMd

As indicated earlier, certain forms of neglect and apraxia are associated with damage to frontal lobe areas such as PMd, further evidence from humans for a role for this area in the formation of intentions. In humans damage to PMd can also result in asomatoagnosia, a condition where subjects describe parts of their body as missing or disappeared. This suggests that human PMd integrates visual and somatosensory information to construct the body image. A recent transcranial magnetic stimulation (TMS) study indicates that this integration may serve another purpose as well. Application of TMS over PMd impairs the ability to update movements online, a phenomenon previously described for the SPL. Thus, PMd may also integrate visual and somatic information in order to estimate the state of the limb during movement.

Intention-Related Activity

Like the areas of the parietal lobe to which it is connected, the PMd of nonhuman primates has been shown to reflect information about intended actions. For example, PMd neurons are active during the planning of arm movements and appear to specify information about both the direction and amplitude of these movements. Neurons in PMd have also been shown to encode reach goals as well as aspects of the limb trajectory and this goal-related information is combined with information about the effector that will be used to attain that goal.

State-Related Activity

PMd neurons encode not only intention-related activity but also information about the state of the body. For example, several studies have reported sensitivity to eye position in PMd though the magnitude of these effects appears to vary under different behavioral conditions. PMd neurons also encode information about both the static position and configuration of the limb as well as limb position and velocity during movement, strongly suggestive of a role for this area in state estimation. However, as in the PPC, the relative roles of motor and sensory signals in constructing an estimate of arm state are unclear in PMd. The existence of strong projections from M1 suggests that efference copy information should be readily available to PMd. PMd is also known to receive visual and somatosensory information via the parietal lobe. Thus, similar to the SPL, PMd appears to have access to all signals relevant for estimating limb state.

Coordinate Transformations Underlying Eye-Hand Coordination

In what manner are movement goals and state information integrated in PMd? Like neurons in PRR and area 5, neurons in more rostral parts of PMd appear to encode goal- or target-related information in eye-centered coordinates. Many of the same cells also encode goal-related information in limb-centered coordinates, similar to what has been observed in area 5. More recently, it has been shown that PMd neurons also encode the position of the limb in eye coordinates. As stated previously, the nature of this effect can be observed at the single cell level, that is, cells have response fields which explicitly encode the difference between current hand and fixation positions. This can be interpreted as encoding the relative location of the hand and eye, that is, hand-in-eye or eye-in-hand coordinates, an efficient and flexible representation whose benefits are described below. In more caudal parts of PMd, the representation of movement goals and state information is not as clear. Though some neurons encode reach goals in eye coordinates, hand/limb coordinates, or both, a substantial number of neurons do not appear to encode goal locations or limb position in any clear reference frame. The fact that more caudal parts of PMd do not use relative position codes makes sense in that such codes must eventually be broken down before individual commands to move the eyes and limbs can be specified and delivered to these effectors. Since caudal PMd lies immediately adjacent to M1 this area could already be specifying information solely about limb movements and in coordinates that are more intrinsic to the arm, that is, joint angles or muscle activity.

Conclusions

Information about the locations of potential goals and the positions and configurations of the various parts of the body are initially encoded in the brain in the natural coordinates of the sensors. However, orienting behavior depends critically on deriving the relative positions of goals and effectors; it is not useful to know only where an object is with respect to your eyes if what you also desire is to reach out and pick that object up. Such relative codes in the arm movement system have been known at least since the work of Georgopoulos and colleagues in the 1980s, who demonstrated that arm movement-related activity in M1 reflects the movement vector, rather than the position of the desired endpoint in space or with respect to the body. The more recent findings discussed here describe how such relative codes may be derived by nodes of the parieto-frontal intention network.

A critical component of the described scheme is the encoding of the relative position of body parts, particularly the relative coding of eye and hand position. These relative codes provide a flexible and efficient mechanism for performing sensorimotor transformations. First, a code for the relative position of the eyes and hand can be interpreted or read out by other brain areas as specifying the position of the hand in eye coordinates and thus can be used to derive a representation of the desired movement vector in this reference frame. However, relative codes allow this process to be inverted as well, for example, the position of a goal location in eye coordinates can be recovered if only the eye-in-hand and reach movement vectors are known. Such computations would be particularly useful during tasks requiring coordinated movements of the eyes and hands and could reduce errors in estimating eye and limb position that normally accumulate over time. The manner of relative coding described in PMd, where an explicit code for the eye–hand vector coexists with codes for the reach goal in both eye and limb coordinates, provides a compact estimation of the entire system state that would allow coordinated hand and eye movements to occur even while the entire body is translating through space.

See also: Brain–Machine Interfaces; Cognition; Learning and Memory: Spatial; Current Models and Assessment of Limb Apraxia; Peripersonal Space and Body Schema; Voluntary Movement: Control, Learning and Memory.

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Neural Substrates of Conditioned Fear and Anxiety

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Glossary

Anxiety – An anticipation of a future threat, which is a normal and adaptive response that ensures dangers are either avoided or reduced through preparation and vigilance. When a threat is poorly defined or temporally remote, the behavioral state it elicits is typically referred to as anxiety. Anxiety is a cognitively mediated anticipatory state activated to potential threats.

Auditory fear conditioning – A form of associative learning in which an initially neutral conditioned stimulus (CS; e.g., a tone) acquires aversive properties following its pairing to an unconditioned stimulus (e.g., a footshock).

Conditioned stimulus – An initially neutral stimulus (e.g., tone) that acquires aversive properties after being paired to an aversive unconditioned stimulus (e.g., a footshock).

Fear – An adaptive response to an immediate threat. It is a normal and adaptive response that ensures dangers are either avoided or reduced through preparation and vigilance. Well-defined threatening stimuli that are immediately present or imminent in the environment elicit a fear response. Fear is thus a sensory-driven and time-limited response to actual adversity.

Long-term potentiation (LTP) – A persistent strengthening of synapses that is generally induced by applying high-frequency stimulation to an afferent pathway. It is thought to be the mechanism underlying memory formation. There is some evidence that LTP-like changes are induced during learning.

Plasticity – The potential for a system or network to be modified. Modifications in synaptic efficacy (e.g., LTP induction) are taken as evidence that a network was plastic.

Unconditioned stimulus – In the context of auditory fear conditioning, the unconditioned stimulus is an aversive stimulus (generally a footshock) that yields fear responses when presented alone. After pairing it to an initially neutral, CS (e.g., a tone), the CS also generates fear responses on its own.

Introduction: From Behavior to Synaptic Plasticity

The acquisition of fear engages a complex neural network that involves a number of structures. However, when both behavior and underlying circuitry are reduced to a more basic form, a powerful framework from which to study fear emerges. This framework can be used to study the neural changes associated with normal fear learning, as well as the mechanisms that underlie many psychiatric disorders that arise from fear dysregulation. Here, we review the basic anatomical circuitry and neurophysiological plasticity associated with auditory fear conditioning, and then conclude by emphasizing the relevance of these models to fear- and anxiety-related plasticity.

Fear Conditioning and Anxiety

Fear is a response to an immediate threat, while anxiety is an anticipation of a future threat. Fear and anxiety are normal and adaptive responses that ensure dangers are either avoided or reduced through preparation and vigilance. Well-defined threatening stimuli that are immediately present or imminent in the environment elicit a fear response. When a threat is poorly defined or temporally remote, the behavioral state it elicits is typically referred to as anxiety. Fear is thus a sensory-driven and time-limited response to actual adversity, whereas anxiety is a cognitively mediated anticipatory state activated to potential threats. When a fear or anxiety response is out of proportion to that warranted by the situation, or surfaces in the absence of threat, a fear or anxiety disorder exists.

Animal models are essential for understanding the neural basis of behavior, and a number of experimental paradigms are used to study fear and anxiety. Fear, which is easier to define and measure, has been most extensively studied using Pavlovian fear conditioning – an experimental procedure in which an initially neutral stimulus (conditioned stimulus, CS; e.g., a tone) acquires the ability to elicit fear responses after being paired with an

unconditioned stimulus (US; e.g., a shock). Much evidence indicates that this type of association requires circuits that transmit information into and out of the amygdala. Although animal models of anxiety have been developed to test for anxiolytic drug efficacy, the neural circuitry that underlies these anxiety-like behaviors is not well understood. Thus, in describing the neural mechanisms that mediate fear and anxiety, we mostly rely on and draw inferences from pathways known to be implicated in fear processing through studies of fear conditioning.

Anatomy of the Fear Circuit: Pathways to, within, and Out of the Amygdala

Most work on fear conditioning has utilized an auditory fear-conditioning paradigm in which an auditory stimulus serves as the CS. The neural network underlying auditory

fear conditioning is very well characterized and involves pathways into, through, and out of the amygdala. In brief, evidence from a variety of different approaches indicates that the physiological basis of fear learning starts with the relay of sensory information to the lateral nucleus of the amygdala (LA), where the CS and US converge and initiate synaptic changes. When the CS is later encountered, it is transmitted via intra-amygdalar pathways to the central nucleus of the amygdala (CE), which controls the expression of behavioral and physiological fear responses by way of projections to brainstem and hypothalamic targets (see **Figure 1**).

Transmission of CS and US Information to the Amygdala

Information about the auditory CS is processed in thalamic areas that transmit the signals to the auditory cortex

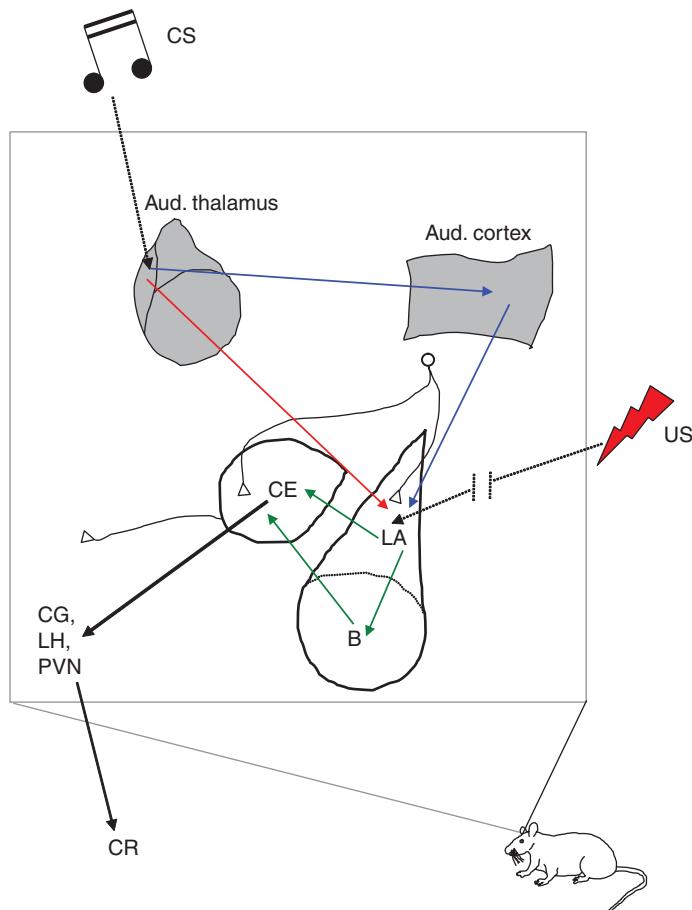


Figure 1 Anatomical circuits of auditory fear conditioning. Auditory fear conditioning is a form of associative learning in which an initially neutral conditioned stimulus (CS; e.g., a tone) acquires aversive properties after its pairing to an unconditioned stimulus (e.g., a footshock). Information about the CS travels through direct (thalamus, shown in red) and indirect (thalamus + cortex, shown in blue) pathways, and inputs from CS and US converge on single neurons in the lateral nucleus of the amygdala (LA). Following intra-amygdalar transfer of information to the central nucleus (CE; shown in green), outputs to the brainstem (central gray (CG)) and hypothalamus (lateral hypothalamus (LH)), paraventricular hypothalamus (PVN) initiate fear expression (conditioned response (CR), shown in black). This is a simplified version of the anatomical circuitry that underlies fear conditioning.

as well as directly to the amygdala. In the thalamus, the medial division of the medial geniculate nucleus and the posterior intralaminar nucleus (MGm/PIN) project directly to the LA, the sensory gateway into the amygdala. All areas of the medial geniculate nucleus also project to the auditory cortex, which, in turn, connects with LA. The US is also transmitted to the amygdala via thalamic and cortical pathways.

Both the thalamic and cortical auditory inputs to the amygdala involve excitatory glutamatergic projections. Both the direct and indirect pathways can support fear learning, but the pathways transmit different kinds of information. The thalamic route carries rapid but crude aspects of the CS; the cortical route reaches the LA more slowly, but transmits more detailed information. The direct, but not the indirect, route is thought to be necessary for auditory fear conditioning – pretraining lesions of the MGm/PIN impair the acquisition of cued fear memories, while those of the auditory cortex do not. The auditory cortex, nevertheless, plays a key role in stimuli discriminations. Effectively, the cortex is necessary for conditioning to a complex stimulus (such as frequency-modulated tones of ultrasonic vocalizations). Furthermore, cell firing is modified in the auditory cortex during conditioning. To date, however, the precise conditions that necessitate the involvement of the cortex in the acquisition of fear are not well understood.

Intra-Amygdalar Circuits and Outputs

The amygdala is composed of many different subnuclei, and these different areas are thought to play different roles in the acquisition of fear. The LA receives the initial inputs from the CS and US and is widely believed to be the key site of plastic events leading to and supporting fear conditioning. The basal (B) and CE nuclei also clearly play a role in fear expression and, possibly, acquisition. Both of these receive connections from the LA. In addition, LA connects with CE via B and the intercalated cells (ICs).

The CE projects to the various brainstem areas that have been shown to mediate the specific behaviors and physiological responses elicited by the CS. In particular, the CE projects to the hypothalamus, brainstem, and the forebrain – regions that control endocrine, autonomic, as well as behavioral responses elicited during fear conditioning. Projections from the CE to the periaqueductal gray are important for behavioral responses; CS-triggered endocrine responses are thought to involve projections to the paraventricular nucleus (PVN) of the hypothalamus; and the connections to the lateral hypothalamus mediate cardiovascular responses. Output cells from the LA are excitatory, whereas the ICs and many CE cells are inhibitory. Thus, increased input from the auditory thalamus leads to an increased glutamatergic projection from the

LA which potentiates CE output. The net result is an enhancement of behavioral and physiological responses to the threat.

The role of the B in fear conditioning is not yet fully elucidated. Pretraining B lesions do not prevent fear conditioning; however, selective lesions of its anterior, but not posterior, region are associated with deficits in both auditory and contextual fear conditioning. The B was also found to be involved in the performance of fear-motivated instrumental responses. Furthermore, post-training lesions of the B impair fear-memory expression, suggesting that though it is not required for the acquisition of fear, if it is intact during acquisition, it becomes a necessary part of the circuit, and is required for retrieval.

In sum, fear conditioning initially involves the relay of sensory information from the auditory thalamus to the LA, either directly or via auditory cortex, and then the transfer of signals over intra-amygdalar pathways to the CE. Fear responses are initiated by way of projections from the CE to various brainstem targets. The manner in which these various circuits to, within, and from the amygdala can be modified by fear conditioning is crucial to our understanding of the acquisition of fear memory.

Neurophysiology of the Fear Circuit: Synaptic Plasticity and Memory in the LA

Activity-dependent modifications in synaptic plasticity occur during learning, and these changes in the circuitry are thought to persist and make memory possible. As the site of CS-US input convergence, the LA is ideally suited as the area of plasticity underlying Pavlovian auditory fear conditioning. Here we review evidence that LA cells are plastic – have the potential to be modified – in response to input from areas implicated in the fear circuit. Converging evidence in this regard comes from a number of analytical levels – from single-cell changes, to broader network connections. Studies in this area are conducted using a variety of preparations – *in vitro* and *in vivo* (acute and freely behaving) – and plasticity is induced via both artificial means (stimulation) and more realistic paradigms (behavioral conditioning). The tremendous impact of fear in an animal's life, coupled with the relatively simple nature of its most basic underlying circuits, boosts our ability to detect changes induced by its acquisition and storage.

Single-Cell and Multiunit Firing

Pairing the CS with the US induces changes to firing properties of LA neurons – stimulus-evoked firing increases after conditioning. Single-unit studies suggest that there are two populations of LA cells in the dorsal half of the LA that undergo plasticity following

conditioning. One group, in the superior portion of the dorsal LA, shows a transient increase in firing in response to the CS. The other group, found in the inferior portion of the dorsal LA, exhibits a persistent increase in firing throughout training and testing. The superior 'transiently plastic cells' exhibit shorter firing latencies (10–15 ms) and are susceptible to extinction, whereas the inferior 'long-term plastic cells' have firing latencies that are likely indicative of a polysynaptic response (30–40 ms after the onset of the tone), and are resistant to extinction. Importantly, electrophysiological studies have shown that the same cells that receive CS inputs from the auditory thalamus and cortex also fire in response to the footshock US. This is reminiscent of the plasticity model proposed by McCullough and Pitts in 1943 and by Hebb in 1949. Hebb's model, for example, states, "when an axon of cell A repeatedly and persistently takes part in firing onto B, some growth or metabolic process takes place such that the efficiency of cell A, as one of the cells firing onto B, is increased."

Integrated networks: Artificially and behaviorally induced potentiation

Arguably the most widely studied physical instantiation of Hebbian plasticity, long-term potentiation (LTP) – an artificial means of inducing synaptic modification – has provided an experimental model that has often substituted for directly assessing the effects of learning on synaptic modification. Generally induced by applying high-frequency stimulation to afferent inputs, LTP leads to an increase in the synaptic population response of a group of cells. If the synaptic efficacy and memory hypothesis accurately reflect the mechanisms initiated in the amygdala during fear conditioning, we should expect: (1) for LTP to be inducible in the amygdala, and be bound by similar rules as fear conditioning; (2) that amygdala-dependent learning should increase synaptic strength in that structure (behaviorally induced potentiation); and (3) that preventing the induction of LTP in the LA should prevent the acquisition of fear conditioning and associated plasticity. Past studies support all of these predictions.

- (1) LTP is inducible in the LA by stimulating thalamo-amygdala and cortico-amygdala projections *in vitro* and *in vivo*. LTP in the LA has been induced both by using high-frequency stimulation (tetanus) to afferent pathways as well as a form of associative LTP, induced by pairing subthreshold presynaptic auditory inputs with postsynaptic depolarization of cells in the LA. The two forms differ mechanistically – tetanus-induced potentiation is *N*-methyl-D-aspartate (NMDA) dependent, whereas associative LTP relies on L-type voltage-dependent calcium channels (L-VDCC). Furthermore, associative LTP in the LA is sensitive to the same stimulus contingencies as fear conditioning. Bauer and colleagues have shown that

potentiation is strong when presynaptic trains precede postsynaptic depolarization 100% of the time; however, when noncontingent depolarizations are interleaved with the same number of contiguous pairings (thus decreasing the percentage of contiguous pairing over the course of an entire session) the resultant potentiation is significantly weaker. This suggests that the strength of association, and related change in synaptic efficacy, depends on the contingency between pre- and postsynaptic activity, rather than simply temporal contiguity. The same principles, described by Rescorla, apply behaviorally – contingency, rather than mere temporal overlap, is critical for fear conditioning.

- (2) Auditory stimuli elicit field potentials in the LA of awake, freely behaving rats, and the coincident induction of LTP from stimulation of the thalamic input to LA leads to an enhancement of these auditory responses (see Figure 2). Importantly, associative fear conditioning itself leads to an increase in synaptic efficacy similar to that seen following LTP induction *in vivo* and *in vitro*. Reactivation of a consolidated fear memory was also recently found to induce potentiation in the LA.
- (3) Genetically modified mice that show specific deficits in amygdala-dependent learning also show deficits in LTP induction in that structure. In addition, drugs that block LTP in the LA also interfere with fear conditioning and associated plasticity. For example, both LTP and fear conditioning are impaired by pharmacological blockade of NMDA receptors or L-type VDCCs. Pharmacologically interfering with mitogen-activated protein kinase (MAPK) signaling in the LA has also been found to interfere with LTP, as well as inhibit long-term memory storage and associated plasticity after conditioning.

Cellular and Molecular Mechanisms of LA Plasticity and Fear Learning

Modifications in synaptic strength occur in the LA during the acquisition of fear, and together, the results from previous studies provide strong support for the notion that synaptic strengthening in the LA is required for the acquisition of emotional memories. For that reason, much work reported in previous studies has focused on the mechanisms underlying LTP induction in that region. Two predominant forms of LTP have been found to occur in the LA – early (E-) and late LTP (L-LTP). The former is dependent on sufficient depolarization through calcium entry via NMDA receptors, and the latter occurs via further depolarization mediated by VDCCs. Intra-amygdalar treatments that impair E- and L-LTP, namely NMDA or VDCC blockade, also impair short- and long-term memory,

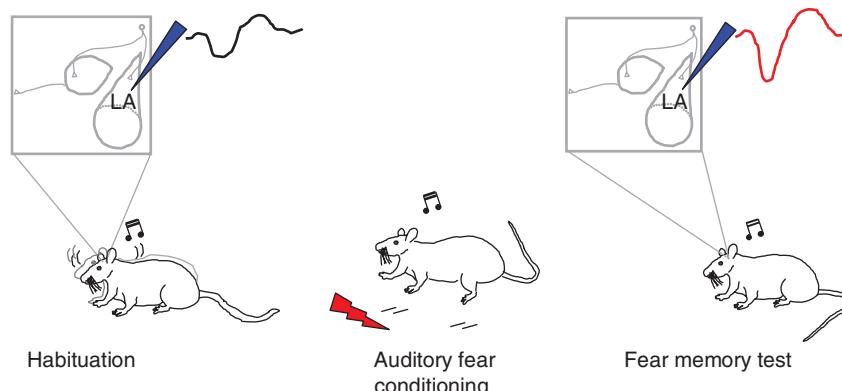


Figure 2 Fear conditioning induces synaptic potentiation at lateral amygdalar synapses. In a chronic recording preparation, the response evoked by the conditioned stimulus (CS; a tone) can be measured before (habituation, CS-evoked field potential shown in black) and after (fear-memory test, CS-evoked field potential shown in red) auditory fear conditioning. When a neutral auditory stimulus is first presented, rats will first show an orienting response, and then ignore it. After its pairing to the unconditioned stimulus (US; a footshock), the CS elicits conditioned responses (e.g., freezing), and a stronger (potentiated) response at lateral amygdalar (LA) synapses. Fear-conditioning-induced potentiation has also been demonstrated *in vitro*.

respectively. Recent studies have shown a role for calcium-regulated intracellular signaling cascades in fear acquisition. For example, both protein kinase A (PKA) and MAPK have been implicated in fear-memory consolidation and associated plasticity. These signaling cascades contribute to long-term memory formation by inducing activation of transcription factors in the nucleus (e.g., cyclic adenosine monophosphate (cAMP)-response-element-binding protein (CREB)). Ou and Gean have also recently shown that calcium influx through NMDA receptors and L-VGCC channels activates PKA and calcium calmodulin-dependent protein kinase IV (CaMKIV), each inducing CREB phosphorylation. In turn, phosphorylated CREB binds to the brain-derived neurotrophic factor (BDNF) promoter, leading to an increase in BDNF expression in the amygdala, and likely contributes to fear-memory consolidation. MAPK signaling in the LA is also necessary for the extinction of fear memories.

Rumpel and colleagues have also shown that fear conditioning drives incorporation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors in LA neurons in a synapse-specific manner. Interfering with AMPA receptor trafficking impairs fear conditioning as well as amygdalar LTP, suggesting that it is necessary for the acquisition of fear and associated plasticity. Plasticity at LA synapses is necessary for fear conditioning; yet, approximately only 20% of these cells show conditioning-induced plasticity. Using a transgenic mouse (TetTag) that allows persistent tagging of neurons that are *c-fos* active during a certain time window, Reijmers and colleagues showed that a portion of basolateral neurons that are active during fear conditioning are also active during memory retrieval. Han, Kushner, and colleagues have shown that approximately 20% of cells showed CREB phosphorylation (at the ser831 site) following fear conditioning, suggesting that

CREB potentially plays a role in dictating which cells are to be recruited into a fear-memory engram. These researchers then examined this idea by conducting a series of experiments in which they manipulated CREB function in a subset of LA neurons. They showed that manipulating CREB function influences the probability that individual neurons from the LA will be recruited into a memory trace. Specifically, increasing CREB function renders the neurons more likely to be recruited into the fear-memory network. The precise mechanism underlying this effect is not yet fully understood.

We should also point out that in a typical auditory fear-conditioning experiment, an animal acquires information not only about the tone and the shock, but also to the context in which this pairing occurs. Learning information about the context can also occur in the absence of the tone, using an experimental paradigm in which a shock is repeatedly presented in the context, and leads to contextual fear conditioning. We chose to focus this review on amygdalar plasticity; yet, it is clear that other structures are involved in the fear circuit. One area in particular – the hippocampus – likely plays a role in contextual fear, although its specific role needs to be studied in further detail. The neurophysiological changes that take place during contextual fear learning are also thought to involve the B and LA, as lesions to those regions generally prevent conditioning to context.

Neural Basis of Anxiety

Less is known about the neural mechanisms of anxiety than fear, largely because most of the current tasks designed to test it are not readily conducive to studying circuitry. However, two approaches are discussed below.

One theory, originally proposed by Jeffrey Gray in 1982 and expanded by Gray and McNaughton in 2000, suggested that the key to understanding anxiety and its underlying circuitry was to study the effects of antianxiety drugs. Gray worked based on the premise that a number of drugs – including alcohol, barbiturates, and benzodiazepines – decreased anxiety, despite the fact that they had different biochemical mechanisms of action. These anxiolytics have a similar effect on behavior in animal models of anxiety, and despite acting through different mechanisms, all of these drugs were found to affect septo-hippocampal theta rhythms. In addition, lesions of the septo-hippocampal circuit had a similar effect on behavior as the anxiolytics. In his initial theory, Gray proposed that septo-hippocampal circuits were at the core of anxiety. In a more recent revision of this theory, Gray and McNaughton have expanded the circuitry to also involve the amygdala and the prefrontal cortex. There is some evidence that the amygdala plays a crucial role in anxiety-related behaviors, since injections of benzodiazepines directly into that structure reduce anxiety phenotypes in animal models.

Another theory about anxiety, put forth by Michael Davis, suggests that anxiety might be a function of the bed nucleus of the stria terminalis (BNST). This brain region is an extension of the amygdala, shares similar outputs, and is involved in contextual fear conditioning. The role of the BNST in some animal models of anxiety (e.g., the elevated plus maze and open-field tests), however, has met with mixed results.

One thread that links both theories is the fact that much of the evidence is based on tasks that crucially involve contextual fear learning (i.e., passive avoidance and light-dark test). Since contextual fear conditioning depends on the hippocampus, one interpretation is that the hippocampus is involved in these tasks because they involve context processing rather than because they involve anxiety itself. On the other hand, the hippocampus may be involved in anxiety because of its role in explicit memory. That is, a large part of anxiety about future threats is based on explicit memory of past experiences with threats or with facts we have learned about threats.

Clearly, more work remains to be done in order to better understand the neural basis of anxiety. Isolating the precise neural mechanisms engaged during anxiety is challenging, yet, similar effects of neuromodulators on fear and anxiety, as discussed next, suggests that they likely engage overlapping systems.

Neuromodulation of Fear and Anxiety

The information acquired during learning is strongly altered by neuromodulatory inputs engaged either around the time of encoding, or during retrieval. We

illustrate this here with examples from the serotonergic and noradrenergic systems. Serotonin receptors are present in the amygdala, and serotonin release is known to modulate neural transmission in that region. Selective serotonin reuptake inhibitors (SSRIs) are routinely prescribed for, and have shown efficacy in, treating a number of fear and anxiety-related disorders. Burghardt and colleagues have recently shown, using fear conditioning in rats, that acute and chronic administration of SSRIs have a differential effect on fear learning. Specifically, acute administration of SSRIs enhances, and chronic treatment reduces, the acquisition of fear memories. These effects are in agreement with evidence from clinical populations that suggests that in the initial weeks of SSRI treatment, anxious patients show increased anxiety, but after several weeks, it significantly decreases.

Norepinephrine (NE) has long been thought to serve as a signal that facilitates synaptic transmission in response to biologically significant events, and thus contribute to learning and memory. Consistent with this view, locus ceruleus (LC) neurons are activated by novel, arousing, and rewarding stimuli. The specific contribution of NE to learning and memory has been studied extensively by manipulation of NE receptor subtypes systemically or within specific brain structures using a variety of different learning tasks. NE has long been implicated as playing a role in fear and anxiety. Increases in NE's metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), are generally found in anxious individuals. In addition, increased plasma levels of NE are also associated with anxiety states.

Debiec and LeDoux have shown that intra-amygdalar or systemic administration of the nonspecific β -adrenergic receptor blocker propranolol at the time of retrieval prevents the reconsolidation of fear memories. This suggests a potential role of the noradrenergic system in modulating the intensity of a previously established memory. In a recent contextual fear-conditioning experiment, in 2007, Hu and colleagues demonstrated that NE enhances memory via phosphorylation of the GluR1 subunit of AMPA receptors (at ser845, and ser831 sites) in the hippocampus, specifically at sites that are critical for synaptic delivery. Phosphorylation of ser845 (PKA site) and ser831 (PKC/CaMKII) is known to regulate glutamate receptor 1 (GluR1) trafficking and function, and may thus be important for structural modification of synapses. Manipulations that persistently alter GluR1 trafficking show promise in attenuating fear memories, and preventing relapse.

Conclusions

Exaggerated or persistent fear is common in psychiatric disorders. The neurophysiological basis of anxiety is not very well defined; however, because anxiety and fear

engage overlapping circuitry, studying the latter provides a useful framework to understand the neural mechanisms that underlie other emotions. This is important, because deliberate actions and thoughts in threatening situations are preceded by automatic adaptive responses, and by understanding these adaptive, phylogenetically conserved reactions, we can be better equipped to keep them in check when they spin out of control. The reductionistic approach of studying a simple behavior in a simple pathway offers many advantages; however, there remains clearly more to uncover. Little is currently known about how emotional reactions transition into actions, but with the study of increasingly complex behavioral tasks and their circuitry, we hope to further our knowledge in that realm. At a minimum, auditory fear conditioning provides a good foundation to work from.

Acknowledgments

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See also: Animal Models of Learning and Memory; Animal Tests for Anxiety; Cognition: Learning and Memory: Pavlovian; Communication of Emotions in Animals; Comorbidity – Depression; Depression; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Effects of Stress on Learning and Memory; Emotion–Cognition Interactions; Emotions; Evolution of Emotions; Fear Conditioning; Fear, Anxiety, and Defensive Behaviors in Animals; Fear: Potentiation and Startle; Human Fear and Anxiety; Implicit Learning and Memory: Psychological and Neural Aspects; Knock-Outs: Learning and Memory; Learning and Memory: Computational Models; Memory Consolidation; Mouse Genetic Approaches to Psychiatric Disorders; Neural Basis of Classical Conditioning; Neuron Excitability and Learning; Neuropsychological Aspects of Anxiety Disorders; Physical and Emotional Pain; Pleasure; Protein

Synthesis and Memory; Psychiatric and Substance Use Disorder Comorbidity; Role of Gene Transcription in Long-Term Memory Storage; Stress and Emotionality; Stress and Energy Homeostasis; Subjective Experience and the Expression of Emotion in Man; Synapse Formation and Memory; Synaptic Mechanisms for Encoding Memory; Transgenic Technologies and Their Application to the Study of Senile Dementia.

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- <http://www.scholarpedia.org> – Scholarpedia; emotional memory.

Neural Substrates of Unconditioned Fear, Defense, and Anxiety

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Glossary

Conditioned behaviors – Behaviors that occur to previously neutral stimuli that have been associated with unconditioned stimuli.

Cytotoxic lesions – Lesions affecting the neuronal cell bodies sparing fibers-of-passage.

Fos protein – The product of the *c-fos* immediate early gene commonly used as a marker of neuronal activity.

Unconditioned behaviors – Innate behavioral responses that do not require previous learning.

defensive behaviors (such as freezing and flight) should occur during the response to either a natural predator or an artificial harmful stimulus. This seemingly limited response repertoire suggested the plausible hypothesis that an animal's defensive behavior system processes cues about predators and an artificial threat in the same way, and this has led to a unitary view of the neural network mediating fear responses, with the central amygdalar nucleus playing a critical role in linking fear processing and defensive responses. However, it is also possible that differentiable mechanisms are engaged. Animals are naturally selected to protect themselves from dangers associated with the presence of a predator or a dominant conspecific, which evoke the sensation of fear and associated behavioral responses.

In this article, we examine the neural system analysis from studies using the unconditioned and conditioned defensive behaviors in response to a natural predator or its odor, and the defensive behaviors in subordinate rats in response to conspecific attack. Next, we provide a brief description on the neural systems related to shock-based fear conditioning, and comment on the validity of this model to predict the neural circuitry underlying defensive behaviors triggered by natural threats (i.e., a predator or a dominant conspecific).

Introduction

Fear is the response to a realistic, often imminent threat, while the term 'anxiety' tends to be applied when the source of threat is uncertain, ambiguous, or unrealistic. In his seminal work, *The Expression of the Emotions in Man and Animals*, Charles Darwin considered that the biological origin of human anxiety is based on the defensive responses that nonhumans show to threatening stimuli. These responses, therefore, can be seen as normal emotional states with an adaptive function aimed at promoting survival across species. Anxiety disorders, on the other hand, can be characterized as extreme manifestations of aspects of normal anxiety. The neural basis of anxiety has been established to a great extent in nonhumans, and recent neuroimaging studies have confirmed that human anxiety is accompanied by changes in neural systems involved in coordinating defensive responses to threats in experimental animals.

Animals exhibit defensive behaviors when they are confronted by any type of threatening stimulus. Predators, attack by conspecifics, and dangerous features of the environment represent three classes of threat stimuli that are found in almost any natural environment. The range and complexity of these stimuli may vary for different species and in different locales, but it is probably safe to say that all these classes of threats are relevant to the overwhelming majority of animal species living now or in the past.

It is generally accepted that animals have a set of several, genetically determined, prepackaged behaviors to solve certain functional problems. This idea has been used in the species-specific defensive reaction (SSDR) theory, suggesting that the same innately determined

Antipredatory Defensive Systems

Studies from the Blanchard laboratory have provided much of the background on defensive responses in rodents to cats and other predators, and to cat fur/skin odor. These studies suggest a much wider array of unconditioned defensive behaviors than measured in aversive learning procedures, and this view is strongly supported by field studies of antipredator defense. Of particular relevance, the study of defense and defensive conditioning mechanisms to nonpainful threat has proved particularly helpful in understanding the neural basis of fear and anxiety. Rodent unconditioned defensive behaviors appear to consist of at least the following: flight, hiding, freezing, defensive threat, defensive attack, and risk assessment. These are species typical (i.e., typically expressed by individuals of a given species under appropriate circumstances), but not species specific, in the sense that they occur in the same form across a variety of mammalian species. The unconditioned defensive behaviors are elicited in wild or laboratory strains without

prior relevant experience. In particular, cat exposure to rats produces in the latter intense freezing, avoidance (and hiding, if a place of concealment is available), and elements of risk assessment, such as orientation to the predator. Responses to cat exposure are very resistant to habituation, and conditioning occurs to the context in which the cat had been encountered.

As an experimental technique, the use of predator odor has recently become more common than direct exposure to a cat because of the difficulties in maintaining cats or other predators in laboratory settings. Cat fur/skin odor elicits some freezing and avoidance, typically less intensely than when a live cat is present, combined with higher levels of risk assessment than are seen when exposed to a live cat. These behaviors show rapid conditioning to the context in which the cat fur/skin odor had been present.

Next, we provide an overview of the neural systems underlying unconditioned and conditioned defensive responses to a cat, as well as cat fur/skin odor exposure.

Neural Systems Underlying Unconditioned Responses to a Predator or Its Odor

Amygdalar systems involved in predator detection

Over the last years, a great deal has been learned about the neural system involved in processing innate defensive behaviors to a predator or its odor. The amygdala occupies a central role in integrating the sensory clues related to the predator. Predator odors may in fact be processed by prey species in the accessory olfactory bulb, rather than in the main olfactory bulb. This suggests that cat odor is processed by rats more as a pheromone than as a conventional odor, and the authors suggested that cat odor may be an example of a 'kairomone' – a semiochemical released by one species that has a favorable adaptive effect on a different 'receiving' species. The accessory olfactory bulb projects principally to the medial amygdala,

and rats exposed to cat odor also show substantial activation in this nucleus, particularly in its posterodorsal part. In line with this view, rats with cytotoxic lesions in the medial nucleus, but not in the central nucleus, exhibited a significant reduction in unconditioned fear responses to cat odor. During exposure to a live predator, in addition to activation of the posterodorsal part of the medial amygdalar nucleus, a distinct activation in two other amygdalar sites, namely, the posterior basomedial amygdalar nucleus and caudal levels of the lateral amygdalar nucleus, has also been observed. Importantly, these amygdalar nuclei receive inputs from visual and auditory association areas, and are likely to integrate predator-derived sensory clues, other than olfactory ones. As shown in **Figure 1**, the amygdalar sites related to predator detection project to the dorsomedial part of the ventromedial nucleus of the hypothalamus, which is also particularly mobilized during exposure to a live predator or its odor, and part of the medial hypothalamic defensive system. Therefore, studies using rats exposed to a live cat or its fur odor suggest an amygdalar–hypothalamic path to detect a live predator or its cues. Recent work testing mice exposed to rats validates the idea of this particular amygdalar–hypothalamic path for predator detection in other prey species.

The hippocampal formation and the contextual analysis for predatory environment

Apart from the well-known hippocampal functions related to mnemonic processing, spatial learning, and navigation, the hippocampus also works as a context analyzer. In this regard, it is relevant to point out that the hippocampal formation receives inputs from the amygdalar sites involved in detecting predator-related cues (**Figure 1**) and, therefore, is likely to be involved in associating predator threats to a given environment.

The septohippocampal system is directly related to the medial hypothalamic defensive system, and, therefore, is

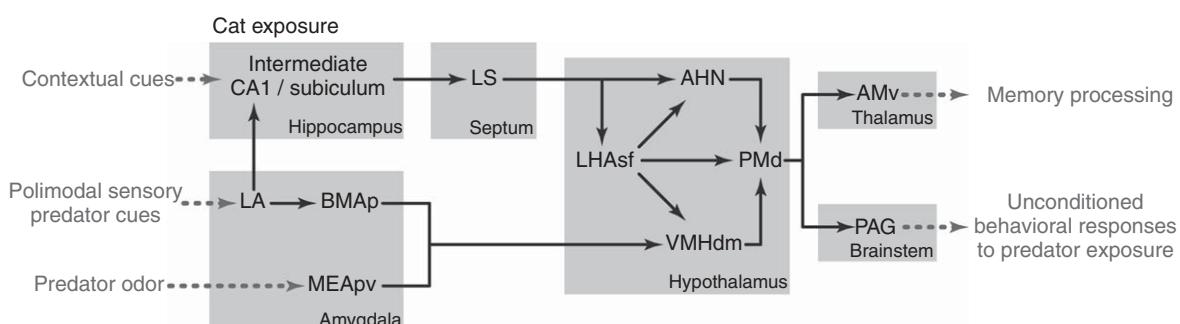


Figure 1 Schematic diagram showing the brain systems involved in processing unconditioned predatory threats, as well as in organizing unconditioned fear responses. AHN, anterior hypothalamic nucleus; AMv, anteromedial nucleus thalamus, ventral part; BMAp, basomedial nucleus amygdala, posterior part; CA1, field CA1; LA, lateral nucleus amygdala; LHAIf, lateral hypothalamic area, subfornical region; LS, lateral septal nucleus; MEApv, medial nucleus amygdala, posteroventral part; PAG, periaqueductal gray; and PMd, dorsal premammillary nucleus; VMHdm, ventromedial hypothalamic nucleus, dorsomedial part. With permission from Cezario AF, Ribeiro-Barbosa ER, Baldo MVC, and Canteras NS (2008) Hypothalamic sites responding to predator threats: The role of the dorsal premammillary nucleus in unconditioned and conditioned anti-predatory defensive behavior. *European Journal of Neuroscience* 28: 1003–1015.

in a position to control antipredatory defensive responses. In line with this view, the intermediate regions of field CA1 and subiculum target distinct septal regions projecting to the anterior hypothalamic nucleus, which is part of the medial hypothalamic defensive system.

The medial hypothalamic defensive system

As shown in **Figure 2**, exposure to a cat or its odor induces in the medial hypothalamic zone a distinct Fos up-regulation in the anterior hypothalamic nucleus, the dorsomedial part of the ventromedial hypothalamic nucleus, and the dorsal premammillary nucleus.

Both the anterior hypothalamic nucleus and the dorsomedial part of the ventromedial nucleus project to the

dorsal premammillary nucleus, which, by far, represents the most sensitive brain region responding to a predator or its clues, and where lesions have been most effective in reducing antipredator defensive responses. In fact, the anterior hypothalamic nucleus, the dorsomedial part of the ventromedial hypothalamic nucleus, and the dorsal premammillary nucleus (PMd) are particularly interconnected, forming a partially segregated circuit in the medial zone of the hypothalamus, the so-called medial hypothalamic defensive circuit. Notably, the dorsal premammillary nucleus appears to work as an amplifier for the neural processing in the medial hypothalamic defensive circuit. This would explain why this region is so responsive to predator threats, and why lesions therein are able to reduce defensive responses so

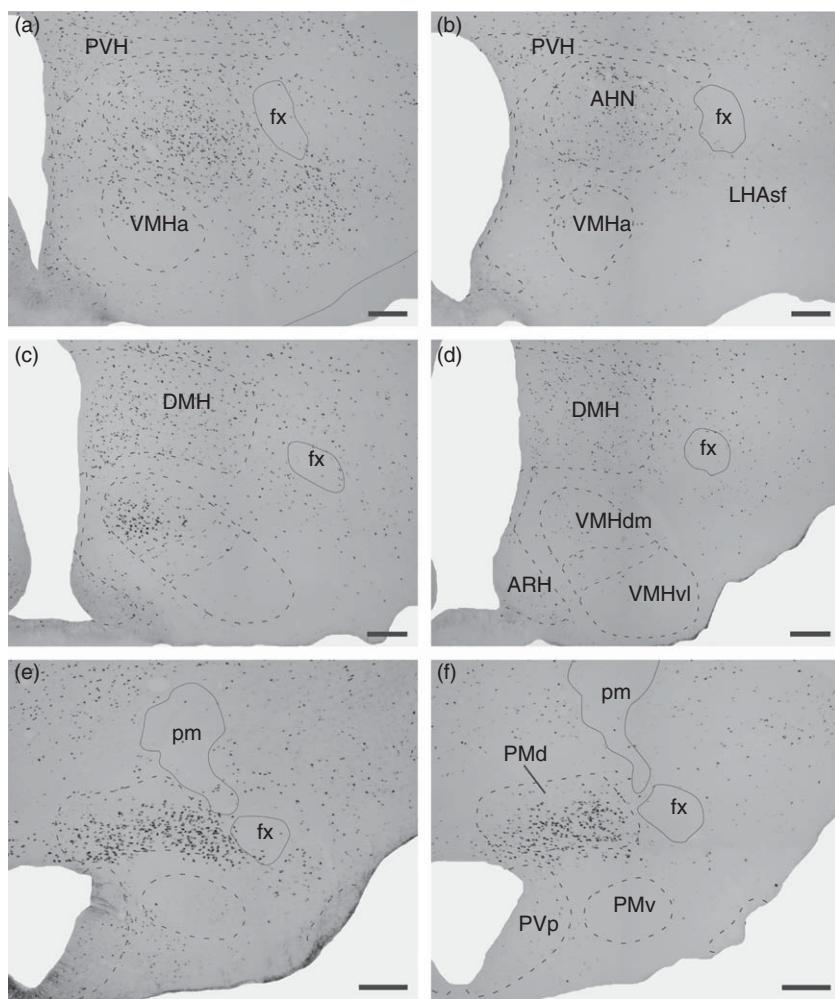


Figure 2 Photomicrographs of transverse Fos-stained sections of the anterior hypothalamic nucleus and subfornical region of the lateral hypothalamic area (a, b), ventromedial hypothalamic nucleus (c, d), and dorsal premammillary nucleus (e, f) from a rat exposed to a cat (left-hand column, a, c, and e) and from an animal exposed to the predatory context (right-hand column, b, d, and f). AHN, anterior hypothalamic nucleus; ARH, arcuate nucleus; DMH, dorsomedial hypothalamic nucleus; fx, fornix; LHASf, lateral hypothalamic area, subfornical region; pm, principal mammillary tract; PMd, dorsal premammillary nucleus; PMv, ventral premammillary nucleus; PVH, paraventricular hypothalamic nucleus; and PVP, periventricular hypothalamic nucleus, posterior part; VMH a, dm, vl, ventromedial hypothalamic nucleus, anterior, dorsomedial and ventrolateral parts. Scale bar = 200 μ m. With permission from Cezario AF, Ribeiro-Barbosa ER, Baldo MVC, and Canteras NS (2008) Hypothalamic sites responding to predator threats: The role of the dorsal premammillary nucleus in unconditioned and conditioned anti-predatory defensive behavior. *European Journal of Neuroscience* 28: 1003–1015.

drastically. However, it is noteworthy that lesions in the PMd have a minimal effect on nonpredator threat stimuli, such as elevated plus maze and postshock contextual cues.

In the lateral hypothalamic zone, the lateral preoptic area and the subfornical region also present a significant Fos increase in response to cat exposure. The lateral preoptic area has been implicated in the modulation of somatomotor responses and general arousal associated with motivated behaviors. Conversely, the subfornical region, similar to the anterior hypothalamic nucleus, also integrates information from the septohippocampal system, and projects to all three medial hypothalamic sites largely activated during cat exposure (see **Figure 1**).

The medial hypothalamic defensive system, in particular the dorsal premammillary nucleus, also receives inputs from another lateral hypothalamic region located immediately dorsal to the supraoptic nucleus, heavily targeted by the lateral component of the retinohypothalamic tract. Retinal ganglion cells projecting to this lateral hypothalamic region are likely to convey information about environmental light intensity. In the context of antipredator defensive responses, it seems plausible to suggest that different behavioral strategies might be expressed depending on environmental luminescence. For example, instead of flight behaviors, freezing immobility seems to be particularly effective as a camouflage in darkness, where freezing greatly reduces prey visibility and noise generation. Moreover, it seems reasonable to believe that this path may, at least in part, mediate the well-documented anxiogenic profile induced by high levels of environmental luminescence.

More recently, an additional prey–predator dyad, mice and rats, respectively, has been used to investigate the hypothalamic systems underlying defensive responses. In the mouse, predator exposure also induces a striking activation of the medial hypothalamic defensive system, giving support to the concept that hypothalamic processing in response to a predator should be preserved across different prey species.

The periaqueductal gray and the integration of antipredatory responses

The periaqueductal gray (PAG) represents the main brainstem target of the medial hypothalamic defensive system, and is critical for the expression of defensive responses. Of special relevance, the pattern of projection from the medial hypothalamic defensive system to the PAG largely overlaps the pattern of PAG activation in animals exposed to a predator or its odor, where Fos expression was mostly seen in the rostral two-thirds of the PAG in the dorsomedial and dorsolateral regions, whereas in the caudal PAG, a less intense, but more widespread activation, was observed. A similar pattern of PAG activation was also described after administration of drugs known to induce panic in humans. In fact,

particularly the dorsolateral PAG appears to play a critical role for integrating forebrain limbic information related to the presence of a natural predator or its odor.

Neural Systems Underlying Conditioned Responses to a Predator or Its Odor

Rats rapidly acquire contextual conditioned defensive responses to the environment where they had previously encountered a predator, or its odor. Thus, long-term risk assessment, as well as freezing and avoidance, are seen in the area where a live cat or its skin/fur odor had been encountered.

The dorsal PMd also seems to be a critical site to influence mnemonic mechanisms linking predatory threats to the associated context. Blocking the N-methyl-D-aspartic acid (NMDA)-mediated neurotransmission of the PMd during cat-odor exposure drastically reduces conditioned defensive responses to the associated environment. This effect in contextual learning may be viewed either as an impairment in the memory consolidation process, or, alternatively, as the result of the decreased emotional component of the aversive event during the learning stage. However, PMd lesions do not seem to affect predator detection, and therefore, PMd-lesioned animals certainly have the aversive experience to the predator presence. The most likely pathway influencing mnemonic mechanisms linking predatory threats to the associated context is the PMd-projecting branch to the ventral anteromedial thalamic nucleus. The anterior thalamic nucleus has been shown to be involved in contextual memory mechanisms. Notably, β -adrenergic blockade of the PMd also produces a deficit in the contextual fear conditioning to cat odor. The effects of β -adrenoceptors on the consolidation processes of aversive memories have been consistently demonstrated in studies using shock-based inhibitory avoidance, as well as shock-based contextual and cued fear conditioning, and have stressed the role of the basolateral amygdala in mediating the effects of β -adrenoceptors on the consolidation processes of aversive memories. Accordingly, an alternative view seems to emerge suggesting a path comprising the PMd and the ventral anteromedial thalamic nucleus as mediating the effects of β -adrenoceptors on the emotional memory processing to predator threats (see **Figure 1**). Notably, in contrast to what has been found using shock-based inhibitory avoidance and fear conditioning, basolateral amygdalar lesion or pharmacological inactivation, appear to produce only a relatively small reduction in cat-odor contextual conditioned responses. This relationship between the PMd and memory processing perhaps helps to explain why exposure to a cat or its odor, which induces a striking PMd activation, produces such a rapid and robust conditioned defensive behavior, while the

trimethylthiazoline (a synthetic compound isolated from fox feces) which does not activate the PMd, fails to support rapid and robust conditioned responses.

Studies examining the neural system responsible for contextual conditioned responses to cat odor observed a robust activation of the PMd in response to cat-odor-associated context, and suggested that there is an overlap between the neural systems associated with the expression of unconditioned and contextual conditioned antipredator defensive responses. In line with this view, rats exposed to a hostile environment, where a live cat had been previously encountered, present a partial activation of the medial hypothalamic defensive system, where increased Fos expression was found in the anterior hypothalamic nucleus and the PMd. As previously noted, the anterior hypothalamic nucleus integrates most of the septohippocampal inputs to the medial hypothalamic defensive system, and lesion studies have shown that damage to the ventral hippocampus significantly attenuated conditioned defensive behaviors following re-exposure to contexts associated with either a live cat or its odor. Moreover, pharmacological PMd inactivation with muscimol drastically reduced conditioned defensive behaviors during exposure to the hostile environment, where a live cat had been previously encountered.

In contrast to the anterior hypothalamic nucleus and the PMd, the dorsomedial part of the ventromedial hypothalamic nucleus and its allied amygdalar paths, which seem particularly involved in detecting the actual predator-related stimuli, either a live cat or its odor, do not respond to predator-related contextual cues. Taken as a whole, the evidence suggests that the medial hypothalamic defensive system, in particular the anterior hypothalamic nucleus and the PMd, should be involved in integrating antipredator contextual conditioning responses as well (Figure 3). Curiously, previous fear-conditioning studies using foot-shock do not identify the involvement of any element of the medial hypothalamic defensive system.

As with unconditioned antipredator defensive responses, the PAG seems to be a critical site for organizing the expression of contextual conditioned antipredator responses. Animals exposed to the environment where a live cat had been previously encountered present a pattern of PAG activation similar to the one seen in response to the actual predator, but considerably less intense.

Neural Systems Underlying Defensive Responses to a Dominant Conspecific

Instinctive responses accompanied by fear have also evolved to protect individuals from opponents of the same species (dominant conspecifics). The neural basis of defensive behavior seen in subordinate rats exposed to dominant males has just begun to be unraveled in studies using the resident-intruder test. For the resident-intruder test, residents are first housed individually with a female rat for several weeks. On the test day, the female is removed and an unfamiliar male intruder is placed in the home chamber. After the first resident attack with a painful experience (i.e., a bite), intruders remain passively frozen in the position they were left by the resident (passive defense) for a long period. However, during the attack, intruders also present an active form of defense by trying to push the resident away, assuming an upright position with sparse boxing, and occasionally dashing away from the resident (active defense). The investigation on Fos-activation pattern in intruder conspecific animals revealed quite a different pattern from that found in rats exposed to a natural predator. Intruder rats exposed to resident males upregulate Fos expression in the medial preoptic area, ventrolateral part of the ventromedial nucleus, ventral premammillary nucleus, and PMd. Careful anatomical inspection indicates, however, that PMd-activation patterns are not identical between intruders and rats exposed to a predator or predatory context. While, in rats exposed to a predator or predatory context, Fos up-regulation is centered in ventrolateral regions of the PMd (Figure 2), up-regulation following exposure to a conspecific resident is centered in dorsomedial regions of the nucleus. However, exactly how information related to cues involving the social recognition of dominant conspecifics reaches the dorsomedial region of the PMd remains to be established neuroanatomically.

Similarly to what was described for predatory threats, PMd lesions also disrupt fear responses during social agonistic encounters. In fact, PMd-lesioned intruders lose passive defensive postures such as freezing and the stereotyped, sustained on-the-back position after resident departure, and do not try to escape from the resident. The

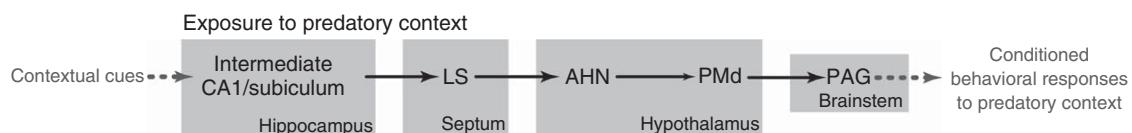


Figure 3 Schematic diagram showing the brain systems involved in processing contextual conditioned predatory threats, as well as in organizing conditioned fear responses to predatory threats. AHN, anterior hypothalamic nucleus; CA1, field CA1; LS, lateral septal nucleus; PAG, periaqueductal gray; and PMd, dorsal premammillary nucleus. With permission from Cezario AF, Ribeiro-Barbosa ER, Baldo MVC, and Canteras NS (2008) Hypothalamic sites responding to predator threats: The role of the dorsal premammillary nucleus in unconditioned and conditioned anti-predatory defensive behavior. *European Journal of Neuroscience* 28: 1003–1015.

behavior of the animals suggests that their fear of the dominant conspecific is drastically reduced while active defense responses are maintained, and they can keep the resident at bay during the attack by standing upright and boxing.

The evidence thus far indicates that PMd lesions profoundly and selectively impair defensive behavioral responses to a predator and a dominant conspecific, and that ventrolateral regions are preferentially activated in the former, whereas dorsomedial regions are preferentially activated in the latter. As noted above, the PMd is a major source of axonal inputs to the PAG, which apparently acts as a downstream mediator of PMd influences on defensive behavioral responses. Complementary to what was observed for predatory threat, the behavioral response to a dominant conspecific is associated with a pattern of Fos up-regulation in the PAG that matches closely the axonal projection pattern from the dorsomedial part of the PMd to PAG, which during conspecific threats, upregulates Fos expression in its lateral and dorsomedial divisions.

The current evidence indicates that the hypothalamus plays a critical role in responses to both predatory and conspecific threats, and that fear reactions evoked by these different natural-threat categories are processed by distinct neural pathways.

in the lateral nucleus. In fact, both acquisition and retention of fear conditioning occur in the lateral nucleus, where electrolytic and excitotoxic lesions, as well as pharmacological blockade, prevent acquisition and expression of fear conditioning. The lateral nucleus presents clear synapse plasticity during fear conditioning, and changes the way a conditioned stimulus is processed after the shock pairing. The lateral nucleus, in turn, projects to the central nucleus. The central nucleus, via projections to the hypothalamus and brainstem, is critical for the expression of fear conditioning. In fact, lesions of the central nucleus disrupt freezing, along with the autonomic reactions observed during fear conditioning. More recent studies, however, have also suggested a role for the central nucleus in learning and storage of fear conditioning.

However, this relatively well-developed neural system model for general fear responses does not apply to innate fear responses elicited by ethologically relevant threats. Thus, lesions of the central nucleus have at best marginal effects on defensive responses to a predator or predator odor, and latent Toxoplasma infection with parasite entry into the rodent brain, and a subsequent loss of innate defensive responses to cat odor has no effect on fear responses to a conditioned stimulus previously paired with a shock.

Pavlovian Fear-Conditioning Studies

The prevailing view of central fear system organization emerged from paradigms using electric shock as a conditioning stimulus. Based on the assumption that animals display an essentially unitary defensive response to any and all types of threats, it has been suggested that a general core circuit or neural system underlies all types of fear.

Studies on the neural basis of Pavlovian conditioned fear indicate the amygdala as a major player in learning, storage, and expression of fear conditioning. Among the amygdalar regions, two nuclei have been particularly focused on the fear-conditioning research, namely, the lateral and central nuclei. As shown in **Figure 4**, associative learning between the conditioned and unconditioned stimuli is likely to occur

Concluding Remarks

Different animal-model approaches have distinct backgrounds, and therefore, they tell us different things. Fear responses to ethologically relevant threats, such as a predator or dominant conspecific, should be comparable to other types of goal-oriented behaviors inasmuch as they are accompanied by powerful motivational components. As is the case for other classes of goal-oriented behaviors like ingestion and reproduction, evidence suggests that the hypothalamus plays an essential role in integrating defensive responses to life-endangering natural threats, and further indicates that different neural circuits mediate hypothalamic processing and behavioral responses to different classes of natural threats.

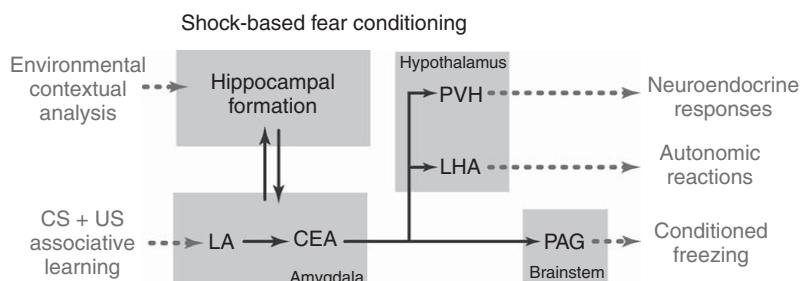


Figure 4 Schematic diagram showing the brain systems involved in processing shock-based fear conditioning. CEA, central nucleus amygdala; LA, lateral nucleus amygdala; LHA, lateral hypothalamic area; PAG, periaqueductal gray; and PVH, paraventricular hypothalamic nucleus.

See also: Animal Tests for Anxiety; Cytokine Effects on Neuronal Processes and on Behavior; Effects of Stress on Learning and Memory; Emotions; Evolution of Emotions; Human Fear and Anxiety; Neural Bases of Defensive Aggression; Neural Substrates of Conditioned Fear and Anxiety; Neuropsychological Aspects of Anxiety Disorders; Stress and Arousal/Sleep.

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Neural Systems of Motivation

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Glossary

- Conditioned reinforcement** – The process by which a Pavlovian conditioned stimulus can act as a reinforcer.
- Discounting** – The reduction in economic utility of a goal as a function of some parameter of that goal (such as the delay to obtaining it, the probability of obtaining it, or its magnitude).
- Goal-directed action** – Behavior that depends upon a joint representation of (1) the instrumental contingency between a subject's actions and the consequences or outcomes of those actions, and (2) the value (instrumental incentive value) of those outcomes.
- Habit (stimulus–response habit)** – Behavior that depends on a direct association between environmental stimuli and behavioral responses.
- Incentive learning** – The process by which actual experience of a reinforcer, in a particular motivational state, leads to learning or modification of the incentive value governing goal-directed action.
- Instrumental (operant) conditioning** – The modification of behavior in response to a causal contingency between a subject's behavior and significant environmental consequences.
- P(A|B)** – Probability of A occurring, given that B has occurred.
- Pavlovian (classical) conditioning** – The association of neutral stimuli with biologically significant events (unconditioned stimuli); as a result of the association, the neutral stimuli become conditioned stimuli.
- Pavlovian-instrumental transfer** – The influence of Pavlovian conditioned stimuli upon the rate of ongoing instrumental responding.
- Reinforcer** – Events whose occurrence or removal strengthens preceding behavioral responses. Those that strengthen preceding responses are positive reinforcers; events whose removal strengthens preceding responses are negative reinforcers.
- Reward** – a term with variable uses, but typically (as here) used to mean an appetitive stimulus and positive reinforcer.

mechanisms for action. Ongoing experimental developments in the field of animal learning theory have elucidated multiple psychological processes contributing to action, and significant progress has been made recently in determining the neural substrates of these processes. Behavior has also been studied in terms of economic decision making, yielding further information about brain function. Contemporary views of motivated action will first be presented briefly, to allow discussion of its neural basis.

Basic Motivational States

Motivational states such as hunger and thirst are intuitively obvious through personal experience, but defining motivational states is instructive. Motivational states are inferred in other animals by observing their behavior, and a circular argument must be avoided. Suppose an experimenter arranges matters so response R produces outcome O, and the subject performs R frequently. It contributes nothing to suggest that the subject lacks O and has an O-seeking drive, which motivates its behavior, as this is circular (R is motivated by O-drive; O-drive is inferred from the performance of R). Likewise, it contributes nothing to suggest that the subject likes performing R: any behavior can be explained by postulating that the behavior itself is the subject's objective. Skinner defined reinforcers by their effect on behavior (events that strengthen preceding responses are positive reinforcers; events whose removal strengthens preceding responses are negative reinforcers) and, to avoid circularity, held that behavior cannot be said to have altered as a consequence of reinforcement.

Nonetheless, it need not be circular to suggest motivational states as 'hidden' explanatory variables. Concepts such as thirst are useful because they are excellent and parsimonious predictors of behavior and are themselves easily predicted. For example, thirst may be elicited by many well-known stimuli (water deprivation, eating dry food, hypertonic saline injection, angiotensin II) and predicts many behaviors (volume of water drunk, speed of drinking, the amount of aversive adulterant required to stop the drinking, and the amount of work the subject will perform to obtain water).

Such ideas lead naturally to concepts of motivation in terms of 'drive reduction' and homeostasis. These concepts provide clear descriptions of many simple consumptive behaviors and remain of interest, for

Psychology of Motivated Action

The study of motivation is the study of the reasons why animals act. Neuroscientific approaches to this question depend on a sound understanding of the psychological

example, in the study of food intake and long-term body weight regulation. Equally, animals' actions are not always directed to a clear physiological need: obvious examples are sexual behavior, the consumption of addictive plant products, and electrical brain self-stimulation, although homeostatic concepts and related ideas such as allostasis (where a homeostatic set point is chronically changed at physiological cost to the organism) have been applied to the regulation of 'reward' intake more generally, including in the context of addiction. However, not all motivated behavior yields to a simple homeostatic analysis. It is useful, therefore, to consider routes to action and economic perspectives on behavior more generally.

Unlearned Responses

Some actions are not motivated in the intuitive sense at all: complex behavioral responses may be innately specified, with specific triggering stimuli (such as swallowing, or the manner in which some geese roll eggs or egg-like objects into their nests, continuing the movement even if the egg is lost or removed by an experimenter).

Pavlovian Conditioning

In a Pavlovian conditioning procedure, an initially neutral stimulus (the (to-be-)conditioned stimulus, or CS) is presented to the subject paired with a motivationally significant unconditioned stimulus (US) that, on its own, elicits an unconditioned behavioral response (UR). This CS-US pairing can create associations between internal representations of the CS and the US (stimulus-stimulus associations); it can also create associations between the CS and the UR (stimulus-response associations), and it can create associations between the CS and a representation of value or affect ('good' or 'bad' emotional states). Pavlovian conditioning therefore creates several psychological routes to action.

Of note, while some conditioned responses are concerned with direct physical responses to a predicted stimulus (e.g. blinking, salivation), other so-called 'preparatory' conditioned responses are directly concerned with motivated action. For example, subjects may approach appetitive CSs; this Pavlovian conditioned approach response brings the subject directly towards stimuli that are likely to be behavioral goals.

Goal-Directed Action

In instrumental conditioning studies, the subject's own behavior influences the environment. For example, an experimenter might arrange a contingency between a laboratory rat pressing a lever (action) and receiving food (outcome). An intuitive view might be that the rat comes to press the lever because it learns that the action

causes the outcome, and because it wants the outcome – that is, its actions are goal-directed. Dickinson, Balleine, and colleagues have shown that rats can indeed represent the *action-outcome contingency* and the *instrumental incentive value* of the food, and put these two psychological representations (which are explicit or declarative) together dynamically to act. Changes to either the contingency (e.g., by reducing $P[\text{outcome} | \text{action}] - P[\text{outcome} | \text{no action}]$) or the value of the food affect responding. Environmental cues can act as *discriminative stimuli* telling the subject which instrumental contingencies are in force.

Some caveats apply. Not all actions can be goal directed in a given species; for example, while rats can represent action-outcome contingencies involving lever pressing with ease, not all actions are represented so readily: it is very hard to train rodents to scratch themselves for reward. More significantly, some actions may be trained easily yet not be under the control of the action-outcome contingency: for example, locomotor behavior can be controlled by Pavlovian contingencies (e.g., when an approach response is elicited directly as a Pavlovian conditioned response, described above), and so the goal-directed nature of a given action cannot be taken for granted in neuroscientific experiments.

Incentive Value, Incentive Learning, and Hedonic Assessment

The instrumental incentive value that governs goal-directed action is responsive to primary motivational state: for example, hungry rats will work more for food than sated rats. Counterintuitively, the representation that relates outcome value to motivational state must be learned. Thus, rats do not know, until they have learned, that food is worth more when they are hungry than when they are sated, or that heroin is worth more when they are in the somewhat artificial motivational state of heroin withdrawal. They learn these relationships through a process termed *incentive learning*. Motivational states (hunger, heroin withdrawal, etc.) directly but covertly 'prime' the brain to deliver a *hedonic experience* of outcomes (food, heroin, etc.) that depends directly and unconditionally upon the motivational state. However, actual experience of this hedonic reaction is necessary to 'teach' the value systems that govern action, so that goal-directed behavior can in the future take appropriate account of the motivational state.

Stimulus-Response Habits

A further mechanism of responding is one envisaged from the earliest days of animal learning theory: that the delivery of reward strengthens a direct association between environmental stimuli and the response that preceded the

reward. These stimulus–response associations are referred to as ‘habits’ because, once learned, the occurrence of the response is triggered by the environmental stimuli without depending on the current value of the outcome to the subject. For example, if a rat has developed a habitual lever-press response that gains it food, then devaluation of the food (by satiating the rat, or pairing the food with nausea) does not prevent performance of the habit. Habits represent ‘procedural’ learning in which the outcome itself is not encoded in the association. The degree to which habits come to control behavior depends upon the schedule of reinforcement on which subjects are trained.

Conditioned Motivation: Pavlovian–Instrumental Transfer

A final well-established psychological phenomenon affecting motivated responding is that stimuli with emotional significance acquired through Pavlovian conditioning can affect the rate of instrumental responding. This is termed Pavlovian–instrumental transfer (PIT). In the simplest form (‘simple PIT’), performance of a lever-press response for food can be elevated by presenting, independently of the subject’s behavior, a CS previously paired with food. The strength of the effect depends on the subject’s current motivational state. The effect of such Pavlovian stimuli may be divided into two. There is a general motivating effect (‘general PIT’), so that a CS for a sucrose solution will enhance lever-pressing for sucrose solution, but also for dry food pellets, when the subject is thirsty. This phenomenon is often described as *conditioned motivation*. In addition, CSs may act selectively to potentiate actions with which they share an outcome (‘outcome-specific PIT’; in this example, potentiating lever-pressing for sucrose more than for food), but this does not depend on current motivational state.

Caveats in the Interpretation of Neuroscientific Studies of Motivated Responding

It will immediately be obvious from the discussion above is that it is not possible to characterize an action as, for example, goal-directed, habitual, or Pavlovian without experimentally testing it. The same actions can be generated by different psychological processes and neural systems (**Figure 1**). Thus, simple studies of lever-pressing, approach behavior, or other tasks in laboratory subjects may in fact contain a mixture of types of action, and this is relevant when brain activity is measured or manipulated.

Economic Approaches to Motivation

Traditional economic analyses of behavior begin by assuming that agents are rational (for example, not possessing intransitive preferences) and somehow implement a ‘utility function’ that quantifies preferences. If these agents then learn action–outcome relationships, they can choose actions to maximize their expected utility. These assumptions may be used to calculate subjects’ preferences and utility functions based on their actions, allowing prediction of future behavior.

Behavioral economics extends this approach by noting nonrational phenomena and utility functions that lead to temporally inconsistent choices. For example, Kahneman and Tversky showed that decisions are affected by the context in which they are presented (framing). Ainslie and others have emphasized that the way in which subjects make decisions about delayed rewards is based on a so-called hyperbolic temporal discounting function, in which value is proportional to $1/(1 + K \times \text{delay})$, where K is the individual’s discounting parameter. This leads to time-inconsistent choices such as preference reversal, in which A (e.g., dieting, drug abstinence) may be preferred to B (e.g., dessert, drug) if the decision is made a long time in advance, but B may be preferred to A when the subject is temporally closer to the outcomes. Exaggerated temporal myopia is associated with drug addiction.

Neuroscientific applications to date have included examining the effects of pharmacological and/or anatomical manipulations on the utility function, along with functional imaging correlates of parameters influencing decisions, discussed below.

It must be acknowledged that although the ‘psychological process’ and ‘behavioral economic’ approaches both analyse the same behavior, they have not been fully unified. For example, although it is often tacitly assumed (particularly in human experiments) that economic utility corresponds to instrumental incentive value, this need not always be the case; it is not known exactly in what way goal-directed action, PIT, and habits are influenced by factors such as delays to reward or probabilities of reward arrival, and how their differential contribution to behavior varies with these parameters.

Neuroscience of Basic Motivational States and Hedonic Reactions

Incentive learning depends upon information about basic motivational states. The hypothalamus serves as the final controller of many bodily homeostatic systems, including endocrine function, thermoregulation, autonomic control, and circadian rhythmicity. It plays a key role in initiating ‘consummatory’ behaviors, such as eating, copulation, and

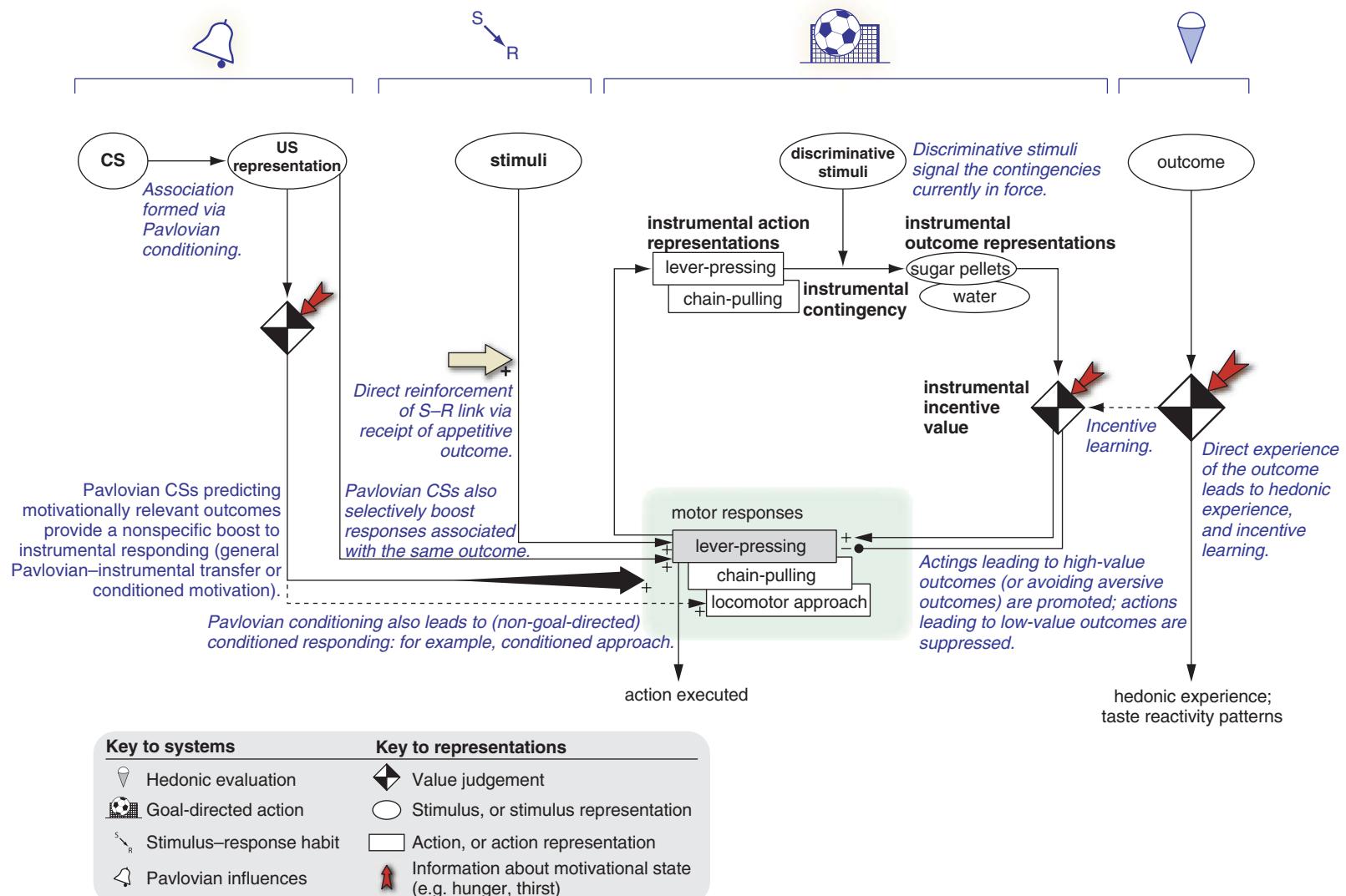


Figure 1 Selected psychological routes to action, including goal-directed action (with examples of actions and goals shown), the learning via hedonic experience of the incentive values governing goal-directed action, stimulus–response habits, Pavlovian influences on instrumental responding (general Pavlovian–instrumental transfer, or conditioned motivation, and outcome-specific Pavlovian–instrumental transfer), and Pavlovian conditioned locomotor approach. See the main text for further explanation. Other aspects of Pavlovian conditioning, and unlearned behaviors, are not shown. (S, stimulus; R, response; CS, conditioned stimulus; US, unconditioned stimulus.)

acts of aggression. It is also responsible for detecting many of the physiological variables relevant to motivational states such as hunger and thirst (such as blood glucose, gut hormones released in response to feeding, tissue osmolality, and hormones released in response to fluid depletion). It is involved in food intake regulation and in the expression of food preferences. The neuroscience of these and related systems is discussed elsewhere in this encyclopedia. A classic example of the distinction between hypothalamic ‘consummatory’ and telencephalic ‘appetitive’ motivation (and hormonal influences thereon) is that lesions of the medial preoptic area of the hypothalamus impair male rats’ ability to copulate with females, but leave their motivation to lever-press for females intact; in contrast, lesions of the basolateral amygdala impair lever-pressing but not copulation, while loss of testosterone impairs both.

As discussed above, hedonic experience triggers incentive learning and is thus required to link basic motivational state information to systems for goal-directed action. Berridge and colleagues have argued that orofacial reactions of several species to food stimuli (taste reactivity patterns) measure hedonic experience; these reactions behave in a manner consistent with the ‘hedonic evaluation’ system, such as tracking motivational state directly. Several neural systems contribute to hedonic experience by this measure, including opioid systems in the nucleus accumbens shell (also shown by Kelley and colleagues to be involved in food preference determination) and in the ventral pallidum, in addition to benzodiazepine- or gamma-aminobutyric acid (GABA)-sensitive systems in the pontine parabrachial nucleus. In keeping with this, incentive learning is affected by benzodiazepines, and by the ‘satiety hormone’ cholecystokinin in interaction with serotonin systems.

Structures and Neuromodulators Supporting Motivated Action

Many neural structures contribute to Pavlovian conditioned responding, and valuation in Pavlovian contexts (such as responding to the value of passively presented stimuli), but not all these routes to action will be discussed. Instead, goal-directed action, habits, and Pavlovian conditioned motivation will be emphasized, and causal (interventional, e.g., lesion) studies of them in particular. These processes rely on a number of valuation systems (discussed above and with neuroeconomics, below). Likewise, appetitive motivation will be emphasized over aversively motivated behavior.

Action–Outcome Contingency and Incentive Learning

Work led by Balleine and others has examined the role of neural structures in goal-directed action. As discussed above, such action depends on the twin representations of the action–outcome (instrumental) contingency and the instrumental incentive value of the outcome. Goal-directed action may be tested for by contingency degradation (typically, two responses that deliver two outcomes are used; one of the outcomes is then also given noncontingently, reducing the contingency for that outcome) and outcome revaluation (in which the value of the outcome, usually a food, is typically reduced by feeding to satiation or associating it with nausea, before responding is retested). These tests of responding are carried out in extinction (without food being delivered) to prevent new learning at the time of testing.

Lesions of the rat medial prefrontal or prelimbic cortex impair the acquisition of goal-directed behavior, as assessed by contingency degradation or outcome devaluation, without preventing subjects from discriminating the actions or the outcomes themselves, or preventing them from acquiring instrumental responding *per se* (the responding such animals do acquire presumably reflecting habitual responding at least in part). However, following training, medial prefrontal cortex lesions do not disrupt sensitivity to outcome devaluation, suggesting that action–outcome associations and incentive values are not permanently stored in this structure. Similar results are seen with lesions of the mediodorsal nucleus of the thalamus.

In contrast, insensitivity to contingency degradation is induced by lesions of the dorsomedial striatum whether those lesions are made before or after training, indicating that this striatal region is involved in action–outcome encoding, and similar effects are seen with temporary N-methyl-D-aspartate glutamate receptor blockade in the dorsomedial striatum at the time of instrumental learning.

Lesions of the basolateral amygdala attenuate the effects of contingency degradation and outcome devaluation in rats, likely by disrupting aspects of the discrimination or encoding of the instrumental outcomes. Lesions of the basolateral amygdala also disrupt Pavlovian motivational processes (discussed below).

The nucleus accumbens makes a complex contribution to motivated action, and is discussed further below. Kelley and colleagues have shown that protein synthesis inhibition within the nucleus accumbens after instrumental training sessions impairs subsequent performance, implying that it contributes to the consolidation of instrumental behavior. However, lesions of the nucleus accumbens do not in general prevent rats from passing the core tests of goal-directed action.

Excitotoxic lesions of the dorsal hippocampus do not affect goal-directed action, but lesions that disrupt the entorhinal cortex and subiculum, or their efferents traversing the hippocampus, impair sensitivity to contingency degradation. This may be due to the involvement of this region in contextual conditioning, since the detection of instrumental contingency requires the comparison of action–outcome relationships, $P(\text{outcome}|\text{action})$, to no-action–outcome or context–outcome relationships, $P(\text{outcome}|\text{no action})$.

Rats with lesions of the insular cortex (insula), the primary gustatory cortex, perform normally on contingency tests, but fail the test of outcome devaluation until the food rewards are actually delivered. This suggests that the insula is involved in storing memories of the incentive value of foods, for use when the foods are not present.

Stimulus–Response Habits

A role for the dorsal striatum in habit learning had been suggested for many years before modern behavioral techniques confirmed this view. Lesions of the dorsolateral striatum abolish habitual responding, leaving actions under the control of the goal-directed system. Killcross and colleagues have demonstrated the same effect after lesions of the rat infralimbic cortex. The role of striatal dopamine in habit learning is discussed below. However, the involvement of other regions of the striatum in goal-directed action (see above) should also be noted.

Conditioned Motivation: Pavlovian–Instrumental Transfer and Related Phenomena

General PIT (conditioned motivation, through which a CS simply energizes behavior) is abolished by lesions of the central nucleus of the amygdala. Such lesions also abolish the acquisition of appetitive Pavlovian conditioned approach, though its role here is confined to learning or teaching this response, since lesions made after training do not affect conditioned approach. The central nucleus of the amygdala may be one controller of the mesolimbic dopamine system, discussed further below, and inactivation of the ventral tegmental area attenuates PIT, as do lesions of the nucleus accumbens shell, one of its targets.

The nucleus accumbens core, which is not required for goal-directed learning, nevertheless strongly influences instrumental responding, including via Pavlovian mechanisms. For example, nucleus accumbens core lesions impair Pavlovian conditioned approach, Pavlovian conditioned place preference, and Pavlovian–instrumental transfer. Conditioned reinforcement is a phenomenon where subjects work for previously learned appetitive Pavlovian CSs. Infusion of amphetamine into the nucleus accumbens enhances the power of

conditioned reinforcement to motivate behavior; the vigor of this effect depends on the nucleus accumbens shell, whilst its direction depends on the nucleus accumbens core.

Lesions of the orbitofrontal cortex, whilst not affecting the outcome devaluation test of goal-directed action described above, disrupt outcome-specific PIT, suggesting that the orbitofrontal cortex is involved in encoding the outcome in Pavlovian but not instrumental conditioning (see also the discussion below). The mediodorsal thalamus is also required for this task.

Similarly, lesions of the basolateral amygdala abolish outcome-specific PIT. Holland, Gallagher, and colleagues have also shown that they abolish the ability of conditioned stimuli to potentiate feeding behavior, an effect that depends upon projections to the lateral hypothalamus. Disconnection (by crossed unilateral lesions) of the basolateral amygdala from the orbitofrontal cortex in macaques impairs choice based upon stimulus–outcome associations when that outcome changes in value. The basolateral amygdala is involved in a host of tasks involving the direction of instrumental (and other) behavior by CS-activated representations of specific and motivationally significant outcomes. This includes conditioned reinforcement, and more complex behavioral tasks such as second-order schedules, in which responding is motivated by an end goal and also by intermittent response-contingent Pavlovian CSs ultimately paired with the final goal. Again, information about conditioned reinforcement is processed through the nucleus accumbens, basolateral amygdala, and orbitofrontal cortex, with the orbitofrontal cortex being important for conditioned reinforcement based on the outcome-specific associations of the conditioned stimulus. In the case of cocaine seeking under second-order schedules, there is evidence that a direct functional connection between the basolateral amygdala and the nucleus accumbens core is required for normal motivated instrumental behavior.

Dopamine Systems and Their Interaction with the Striatum

The midbrain dopamine system, projecting from the substantia nigra pars compacta to the dorsal striatum (nigrostriatal dopamine) and from the ventral tegmental area to the ventral striatum (mesolimbic dopamine) and the prefrontal cortex (mesocortical dopamine), has long been implicated in motivation. The discovery by Olds and Milner that rats would electrically self-stimulate regions of their brain with extreme tenacity and persistence, even crossing electrified floors to reach the stimulating lever, was followed by the observation that many of the ‘hot spots’ for self-stimulation matched the course of axons of mesolimbic dopamine neurons. In recent years, the principle has been used to provide

radio ‘remote control’ of rats by reinforcing directional change with brain microstimulation. Dopamine-releasing drugs including amphetamine and cocaine are powerful reinforcers, and all natural appetitive reinforcers studied increase the release of mesolimbic dopamine.

Dopamine is clearly central to motivational processes. However, dopamine systems appear not to play a role in hedonic experience, instrumental incentive learning, or assessing the magnitude of food rewards. Instead, dopamine plays a major role in Pavlovian conditioned motivation. For example, the strength of PIT, a prototypical measure of conditioned motivation, depends on intact dopamine systems. Dopamine depletion of the nucleus accumbens prevents the acquisition of Pavlovian conditioned approach. As discussed above, amphetamine injection into the nucleus accumbens magnifies the effect of Pavlovian conditioned reinforcers, and this depends on accumbens dopamine. The role of dopamine in response effort is discussed below. Accumbens dopamine is also necessary for ‘preparatory’ behaviors directed at reinforcers themselves: for example, depletion of accumbens dopamine disrupts food hoarding and the locomotor activation induced by food in rats, without disrupting the ‘consummatory’ behavior, eating.

Electrophysiological studies by Schultz and others have shown that midbrain dopamine neurons signal reward prediction error, firing when unpredicted reward is delivered, but transferring this firing to reward-predictive CSs, and decreasing their firing when predicted reward is omitted. A natural idea that follows from this observation and studies of intracranial self-stimulation is that dopamine ‘stamps in’ active patterns of neural activity in its targets, for example consolidating stimulus-response associations in the striatum to become habits. There is evidence in support of this view; for example, dopamine manipulations affect learning rates in humans, amphetamine sensitization (by repeated amphetamine administration) enhances the speed of acquisition of stimulus-response habits, posttraining intra-caudate amphetamine injections enhance learning of stimulus-response tasks, and dorsolateral striatal dopamine depletion impairs habit learning. Likewise, the acquisition of Pavlovian conditioned approach is impaired much more by dopamine depletion of the nucleus accumbens than is its performance, and posttraining dopamine D1 receptor antagonist infusion into the accumbens blocks this acquisition. It is likely that the conjunction of presynaptic (cortical) activity, postsynaptic (striatal) activity, and dopamine release causes long-lasting corticostriatal synaptic strength changes, and this underlies at least part of the process of reinforcement. Dopamine and *N*-methyl-D-aspartate receptor activity in the medial prefrontal cortex is also required for normal acquisition of instrumental responding. However, the precise manner in which such behavior is reinforced, and the anatomical

sites in which habits and other relevant representations are initially formed and eventually reside remains to be determined.

As described above, dopamine is not required for all reinforcement-related learning. Moreover, its effect depends on the target region involved: for example, dopamine in parts of the dorsal striatum may help to consolidate stimulus-response habits, and triggers previously learned behavior, while dopamine in the ventral striatum may help to consolidate and to amplify the impact (and perhaps salience) of conditioned and unconditioned stimuli on instrumental behavior. Differences may exist in responding for drugs of abuse. Established cue-controlled responding for cocaine depends more on dorsal than ventral striatal dopamine; this may reflect habitual or compulsive behavior, which has been shown to dominate responding for some types of drug of abuse. However, this behavior is not free of the influence of the accumbens via other neurochemical systems, and the dorsal and ventral striatum interact (via mechanisms that are still debated) to control responding on cue-controlled schedules of cocaine reinforcement.

The role of dopamine in aversive motivation must not be neglected: for example, accumbens dopamine is elevated in response to conditioned and unconditioned aversive stimuli and is required for normal avoidance behavior; similarly, lesions of the accumbens can impair aversive conditioning tasks, including the modulation of behavioral speed by cues predictive of aversive outcomes, though this topic has not received as much attention as appetitive motivation.

Other Neurochemical Systems

Other neuromodulator systems also play a role in motivated behavior. For example, acetylcholine transmission in the nucleus accumbens is required for normal cue-triggered heroin seeking and acetylcholine influences the ventral tegmental area in simple instrumental acquisition. Serotonin systems modulate the impact of punishment on behavior and promote response inhibition. Other systems (such as hypothalamic orexin neurons) have motivational roles that have yet to be characterized fully. However, the contribution of these systems has been studied less than that of dopamine and space prohibits a full review here.

Neuroeconomic Studies of Motivation

The value of outcomes is a function of their magnitude, the delay to their arrival, and their probability of delivery. The cost of obtaining a desirable outcome, including the effort required, also plays a role in decision making. A number of studies have examined the neural substrates

of complex choice, including abnormalities in executive control and decision making seen in drug addicts, but we will not examine these here. Instead, taking subjects' choices as evidence for their valuation systems (as discussed above), we will consider a number of studies that have examined the neural systems underlying calculations of utility.

Pharmacological Studies

Low levels of serotonin metabolites in the cerebrospinal fluid are correlated with impulsivity and risk taking in several species. Forebrain serotonin depletion tends to steepen the discounting function for delayed rewards, making animals value delayed rewards less. However, the specific effects of serotonin manipulations are complex, varying with receptor subtype and with acute versus chronic manipulations. Forebrain serotonin depletion does not appear to affect the discrimination of reinforcer magnitude, or choice involving reward probability. Richards and colleagues have shown that dopamine D2 receptor activity is necessary for normal choice of delayed rewards, whereas D1 receptor antagonists do not affect such choices. While dopamine neurons may carry information about reward uncertainty in their firing patterns, little is known of the causal role of dopamine in selecting rewards based on their probability.

On the response cost side, Salamone and colleagues have shown that dopamine depletion of the nucleus accumbens substantially reduces the ability of rats to perform instrumental responses when the work requirement is high, but not when it is low, indicating that dopamine here has a significant motivational role. The ventral pallidum is an important downstream target of the nucleus accumbens, and similar 'anergia' can follow pallidal manipulations.

Lesion Studies

Rats' ability to choose large, delayed rewards over small, immediate rewards ('self-controlled' choice) is impaired by lesions of the nucleus accumbens core. Such lesions also impair rats' ability to learn instrumental conditioning tasks with action–outcome delays, and to choose uncertain large rewards over certain small rewards, but do not appear to alter the processing of reward magnitude information.

Bradshaw and colleagues have shown that the orbitofrontal cortex and its dopamine innervation are required for normal valuation of delayed rewards (with lesions inducing steeper discounting) and for the assessment of reward magnitude (with lesions increasing sensitivity to the difference between large and small rewards). The orbitofrontal cortex is also required for normal choice based on reward probability (with lesions steepening

uncertainty discounting and producing risk-averse choice).

Lesions of the basolateral amygdala also impair self-controlled choice, although the specific contribution of this structure to the processing of reward delay and magnitude information is not yet clear. An opposite effect (increased self-control) has been seen after lesions of the subthalamic nucleus, but the same caveats apply.

Excitotoxic lesions of the hippocampus likewise impair self-controlled choice, and the available evidence suggests that such lesions do not disrupt reward magnitude perception. In contrast, hippocampal lesions delay-dependently improve simple instrumental conditioning with delayed reinforcement, perhaps because in this situation the lesions hinder context–outcome associations, promoting response–outcome associations instead.

The anterior cingulate cortex, in contrast, is involved in the assessment of the effort required to obtain a reward; Rushworth and colleagues have shown that rats with lesions here become 'lazy,' or less willing to invest energy for reward. However, the dopaminergic projection to this region is not critical for this.

Correlative Studies

Human functional brain imaging and animal electrophysiology of economic decision making is an expanding field, and has demonstrated neural structures whose activity correlates with reward anticipation and parameters of reward delivery; only a few examples will be given. Many structures thus highlighted accord with the lesion studies summarized above. For example, the nucleus accumbens responds to anticipated rewards in rodents, primates, and birds, and accumbens activation precedes the selection of high-reward, high-risk choices in humans. Theoretical suggestions regarding the observed hyperbolic form of the delay-discounting utility function (whose significance is discussed above) have also been applied to such results. For example, hyperbolic temporal discounting may be due to some combination of a declarative system exhibiting minimal or a simpler form of discounting, reduced associative learning with long delays, and phenomena (perhaps including or related to PIT) that make rewards more salient and promote their choice when they are immediately available. McClure and colleagues have shown that lateral prefrontal and intraparietal cortical regions are activated in a delay-independent manner in temporal discounting tasks, while regions including the ventral striatum and orbitofrontal cortex are preferentially activated by relatively immediate rewards. These results contrast with lesion studies showing that the latter structures promote choice of delayed rewards. An example of the application of economic parameters at high temporal resolution is the demonstration that midbrain

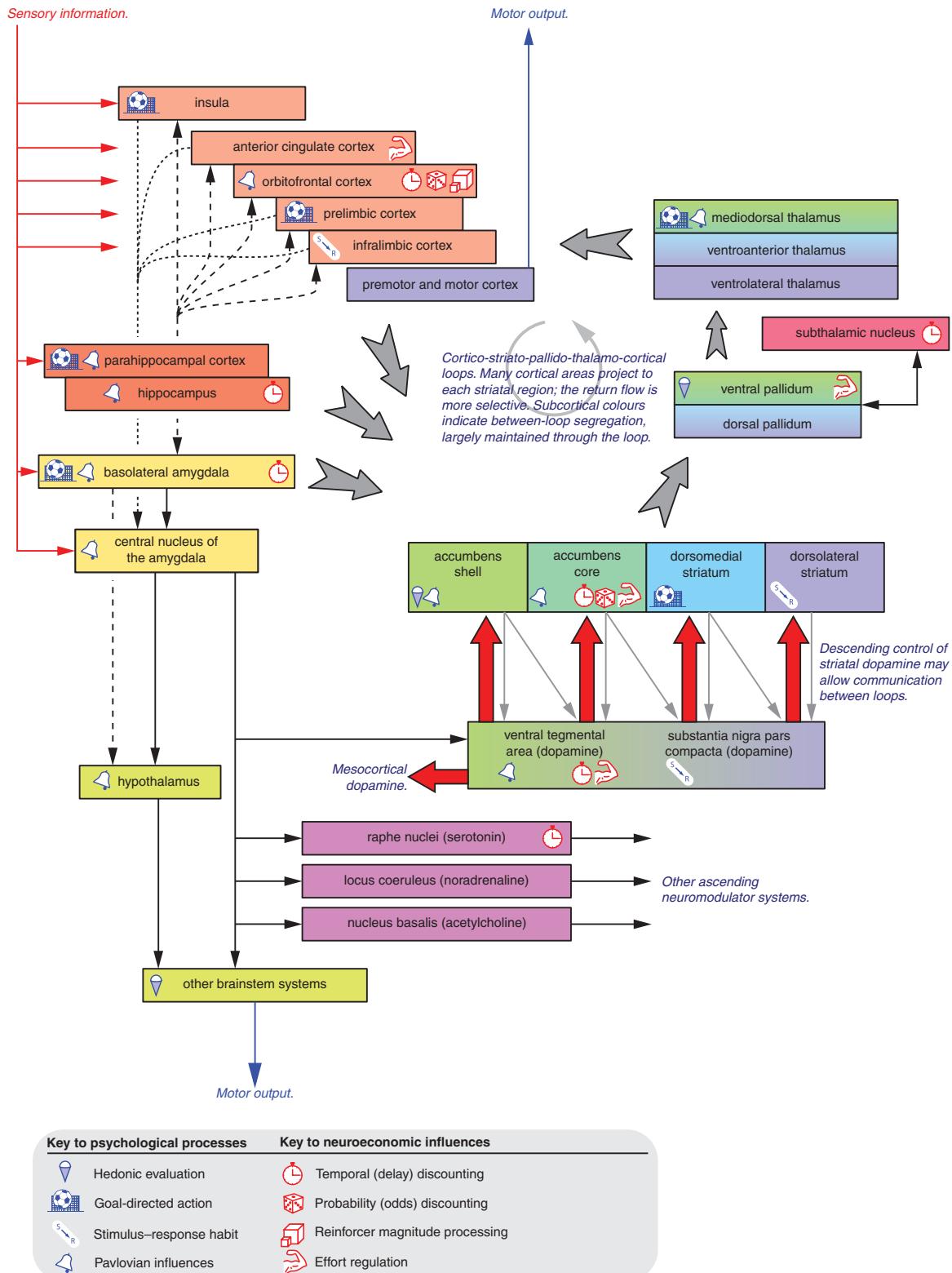


Figure 2 Selected neural structures contributing to motivated action. (The figure is based on the rat brain. The anatomical caudate–putamen distinction in the primate is not as evident in the rat dorsal striatum. The nucleus accumbens is the largest part of the ventral striatum. Prelimbic cortex and infralimbic cortex are regions of rat medial prefrontal cortex; the prelimbic cortex has some functional homology with primate dorsolateral prefrontal cortex.) Many connections are omitted in this figure, and the central corticostriatopallidothalamocortical ('corticostriatal') loops are shown only in outline. Icons on each structure show (in blue) psychological processes subserved by the structure or neuromodulator system, and (in red) neuroeconomic parameters affected by manipulations of the structure. Absence of an icon may indicate either lack of involvement or lack of evidence; space does not permit a complete characterization here. Some structures' involvement may be characterized in alternative ways; for example, one way in which the hippocampus and adjacent cortical areas probably contribute to goal-directed action, Pavlovian conditioning, and responding for delayed reward is by implementing representations of context.

dopamine neuronal responses to CSs exhibit hyperbolic discounting according to reward delay. The causal role of the responses identified by studies such as these has yet to be clarified.

Synthesis and Application to Addiction

The psychological processes outlined in **Figure 1** are implemented by a range of neural structures outlined in **Figure 2**. Systems including the hypothalamus implement basic motivational states and simple ‘consummatory’ behaviors directed at goals that have been achieved. On top of this, several highly complex systems implement behavior to obtain these goals. These include a sophisticated declarative system encoding action–outcome relationships and outcome values, requiring transient involvement of a circuit involving the rat medial prefrontal (prelimbic) cortex and mediodorsal thalamus, and longer-lasting involvement of the dorsomedial striatum; the basolateral amygdala encodes aspects of the predicted goals of behavior, and the memory of the values of foodstuffs also requires gustatory cortex. A simpler habit system depends upon the dorsolateral striatum (likely taught and triggered by dopamine systems) and the rat infralimbic cortex. The direction and invigoration of goal-seeking and preparatory behavior, including by Pavlovian conditioned stimuli, is strongly influenced by information passing through the basolateral amygdala, nucleus accumbens, and in some situations more dorsal regions of the striatum. These and a range of other neurochemical and neuroanatomical systems influence choice between outcomes differing in their probability, delay, and size. The dopamine innervation of the nucleus accumbens, and its pallidal outflow, together with the anterior cingulate cortex, contributes to response effort. In naturalistic settings, goals are frequently available only after a delay, are signaled by environmental stimuli, and require effort to achieve, and a structure such as the nucleus accumbens contributes to motivating behavior in all these situations. Taken as a whole, these systems implement the utility functions that direct behavior.

The concepts outlined here apply as readily to ‘abnormal’ motivations such as drug addiction. Drug seeking may occur for many reasons: drugs are taken for their hedonic value, or their effect on the hedonic value of other rewards, sometimes requiring escalating doses to attain the same effect; users may learn that the drug relieves aversive motivational states such as withdrawal (and the interaction between tolerance to appetitive effects and the avoidance of withdrawal is emphasized in opponent-process theories); drug-associated cues can promote and trigger drug seeking;

drugs may directly amplify the brain’s systems for providing motivation or for learning habits; drugs gain high economic utility to the user through a variety of these processes; drugs alter the normal processing of utility to produce effects such as ‘short-term thinking.’ Current research is elucidating the neural basis of these processes, just as for physiologically necessary motivations, and individual differences in vulnerability to addiction.

See also: Brain Stimulation and Addiction; Cellular Plasticity in Cocaine and Alcohol Addiction; Cognition: Attention and Impulsivity; Cognition: Learning and Memory: Pavlovian; Control of Food Intake; Drug Addiction; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Drug Sensitization and Drug Abuse; Drug Withdrawal – Motivational View; Hormonal Contributions to Arousal and Motivation; Incentive Motivation and Incentive Salience; Motivation; Neural Basis of Classical Conditioning; Neural Substrates of Conditioned Fear and Anxiety; Neurophysiology of Drug Reward; Rewarding Brain Stimulation; Sexual Motivation; Transition to Addiction; Voluntary Movement: Control, Learning and Memory.

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Neurobiology of Offensive Aggression

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Glossary

Autonomic nervous system – It can be subdivided into sympathetic and parasympathetic nervous systems. These systems are involved in the rapid and autonomic control of peripheral organs, including the cardiovascular system required for rest and activity.

c-fos Expression – One of the immediate early genes that become activated after the excitation of a neuron and that remain activated during 1–2 h. The pattern of c-fos expression is used to visualize the neuronal network activated during a certain type of behavior.

Dopamine – An aminergic neurotransmitter system that can be subdivided into two major subdivisions. The nigrostriatal dopaminergic system has the main function of motor control. The mesolimbic dopamine system has its cell bodies in the ventral tegmental area and innervates all neuronal structures of the limbic system.

Gonadal steroids – Steroid hormones produced by the gonads. The male testes produce testosterone and the female ovaries produce estradiol and progesterone. In the brain, testosterone is converted to estradiol by the enzyme aromatase and to dihydrotestosterone by 5- α -reductase.

Offensive aggression – A form of social communication aimed at the active control of the social environment.

Serotonin – An indoleaminergic neurotransmitter whose cell bodies are located in the midline raphe nuclei of the brainstem. Serotonergic neurons innervate virtually every brain structure.

Vasopressin – A peptide produced by magnocellular neurons in the supraoptic and the paraventricular nuclei of the hypothalamus, whose axons project to the posterior pituitary. When released in peripheral circulation, it acts as an antidiuretic hormone, vasoconstrictor, and activator of the hypothalamic–pituitary–adrenal (HPA) axis. Vasopressin is also produced by parvocellular neurons in several distinct brain areas. Within the brain, it acts as a neuropeptide.

aggression is elicited in response to threat or attack by an offensive conspecific, offensive behavior is a form of agonistic behavior initiated by the aggressor and displayed in the context of competition for resources. For example, an offensive male may compete with other males for food, a territory, or females. From a biological point of view, offensive aggressive behavior is a highly functional form of social communication leading to active control of the social environment. It is characterized by a set of species-typical behaviors performed in close interaction with the opponent. Although offensive aggression is highly functional, it is potentially harmful. Therefore, throughout the animal kingdom, strong negative feedback mechanisms have been developed, such as taboos, ritualization, submission, reconciliation, and appeasement, to keep aggression in control and to prevent its potentially adverse consequences. Offensive aggression can thus be defined as a form of social communication aimed at the active control of the social environment. Much of the scientific and public interest in aggression is, however, motivated by the violent, hostile, and presumably less adaptive forms of aggression observed in everyday human life and, clinically, across a wide spectrum of psychiatric and neurological disorders. In this view, violence can be defined as a pathological form of aggressive behavior that is not subjected to inhibitory control mechanisms and that has lost its function in social communication. The relationship between the functional and maladaptive extremes of the offensive aggression spectrum is still far from clear and forms a major obstacle in the integration of animal research with data on human aggression. For the sake of clarity, the neurobiology of violent aggression is not discussed here.

Offensive aggression in animals relates to reactive, hostile, or impulsive aggression in humans. This form of aggression is also called affective aggression and has its strong initiative engagement and autonomic arousal in common with offensive aggression in animals. Moreover, in both animals and humans, this form of aggressive behavior is usually initiated in response to a perceived stress such as the intrusion of an unfamiliar conspecific into the territory. Although both males and females perform offensive aggression, there is a clear gender difference in the frequency and intensity of aggression. Males may perform frequent and fierce offensive aggression in a territorial and sociosexual context. Females show defensive maternal aggression mostly in a maternal context, but low-to-medium levels of offensive aggression can certainly be observed in all female groups in relation to the social hierarchy.

Offensive Aggression

The distinction between offensive and defensive aggression plays a prominent role in understanding the biology and physiology of aggression in animals. While defensive

Neuronal Network

The neuronal network of brain regions involved in offensive aggression has mainly been analyzed in experimental animals such as rats, mice, and hamsters. A network approach in animals is generally based on the pattern of activation of immediate early genes. *c-fos* is such an immediate early gene that shows a prolonged enhanced expression after the excitation of a neuron. Its expression can be visualized using immunohistochemical techniques and is used as a marker of enhanced neuronal activity. Application of this technique in offensive aggression in rats and hamsters reveals a neuronal network that includes the amygdala, the prefrontal cortex, the septal area, several hypothalamic nuclei, and the midbrain periaqueductal gray (see **Figure 1**). This general pattern of activation was confirmed more recently by modern brain imaging techniques such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) that allow the *in vivo* analysis of entire neuronal networks involved in certain kinds of behavior. Presumably, due to their constant high activity, brain structures involved in the direct control of motor output usually do not show up in this type of network analyses. Therefore, to make the picture more complete, the general pathway, including basal ganglia, thalamus, and motor cortex, through which emotion is translated into motion has been included in **Figure 1**. The figure also illustrates the brain structures involved in the neuroendocrine and autonomic support of behavior, including offensive aggression. Comparative research indicates that this highly interconnected network for offensive aggression is remarkably similar in many vertebrate

species, including human beings, indicating that it is evolutionarily ancient and well conserved.

Network Components

Hypothalamus

Classic studies in rats, cats, and monkeys using electrical/chemical stimulation of small populations of neurons have revealed a hypothalamic attack area in which offensive attack can be elicited. This area consists of the intermediate hypothalamic area and the ventral pole of ventromedial hypothalamic nucleus. Upon weak electrical stimulation of this area in male rats, they almost immediately start an aggressive attack that stops at the moment the current is switched off. Neuroanatomical studies showed that the hypothalamic attack area forms a crossroad between input from the medial nucleus of the amygdala and the prefrontal cortex and output to the periaqueductal gray, the mediodorsal thalamus, and the lateral septal area (**Figure 1**). Consistent with the general function of the hypothalamus in behavior, it seems likely that this brain area is involved in the neuroendocrine and autonomic support of offensive aggressive behavior.

A second important hypothalamic structure involved in offensive aggression is the medial preoptic area. This area is characterized by its high density of gonadal steroid receptors and gonadal-steroid-converting enzymes such as aromatase and 5- α -reductase. This hypothalamic structure is sexually dimorphic and its function has mainly been explored in sexual behavior and maternal aggression. A few studies show that it is also involved in offensive aggression. For example, the modulation of

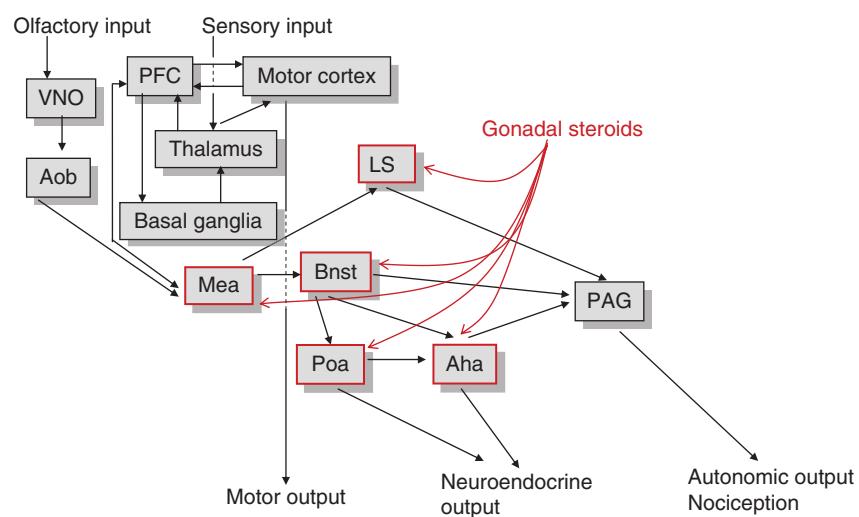


Figure 1 Neuronal network involved in offensive aggressive behavior and the organization of the accompanying neuroendocrine and autonomic activation. VNO, vomeronasal organ; Aob, accessory olfactory bulb; Mea, medial nucleus of the amygdala; PFC, prefrontal cortex; Bnst, bed nucleus of the stria terminalis; Poa, preoptic area; LS, lateral septal area; Aha, anterior hypothalamic nucleus; PAG, periaqueductal gray.

aggressive behavior by seasonal factors in seasonal breeding species is partly mediated by the sensitivity of gonadal steroid receptors in this brain area.

Amygdala

The amygdala consists of a range of interconnected nuclei having a common output through the central nucleus and the bed nucleus of the stria terminalis (Bnst). In particular, the medial amygdala (Mea) and the Bnst have been implicated in offensive aggression. The medial amygdala receives direct input from the accessory olfactory bulb, which, in turn, is the main relay station of olfactory information originating from the vomeronasal organ (**Figure 1**). This part of the olfactory system is specialized in the detecting species-specific chemosensory signals. Hence, olfactory stimuli that are relevant for social behavior in general have a dedicated entrance into the brain, reaching the medial amygdala almost directly. The medial amygdala is characterized by a high density of both estrogen and androgen receptors. These receptors are located, in particular, on neurons that produce the neuropeptide vasopressin (AVP). The synthesis of AVP in these neurons is enhanced by testosterone. This testosterone-dependent vasopressinergic system is sexually dimorphic and projects to the lateral septal area. The medial amygdala has an important function in the modulation of social behavior based on social experience.

Septum

The lateral septum is reciprocally connected to the medial amygdala. It is characterized by a sexual dimorphic density of vasopressinergic fibers originating from the medial amygdala. Males have a higher AVP fiber density than females, and, within the male gender, the density is negatively correlated with offensive aggression. Both in rats and in mice, highly aggressive males have a less dense vasopressinergic innervation of the lateral septum than less aggressive males. Early studies showed that lesions of the septal area in rats induce the septal rage syndrome, which is, in fact, an extremely defensive reaction to both conspecifics and humans. This suggests that the contribution of the septum in offensive behavior might be more anxiety related. Indeed, recent studies using microdialysis of vasopressin during offensive aggression in rats show that male rats characterized by low anxiety and high aggression reduce AVP release in the lateral septum. It seems that septal AVP is involved in the acute modulation of nonaggressive social and anxiety-related behaviors.

Prefrontal Cortex

The prefrontal cortex consists of a number of subregions defined on the basis of their connections with thalamic

nuclei and neuronal cyto-architecture. Although the degree of complexity increases in higher vertebrates, there is a clear homology of prefrontal structures across a wide variety of vertebrate species. The prefrontal cortex is closely connected to both the amygdala and the brain structures involved in motor output, such as the motor cortex and the basal ganglia (see **Figure 1**). In particular, the orbitofrontal cortex has been associated with the inhibitory control of offensive aggression in a number of species. This brain area is more generally involved in behavioral inhibition or impulsivity and the planning of motor output. Indeed, measures of impulsivity in male hamsters are positively correlated with offensive aggression and prefrontal cortex activity measured by c-fos expression. Moreover, reduced prefrontal cortex functioning has been associated with impulsive and violent forms of aggression in animals and humans.

Periaqueductal Gray

c-fos studies show that the periaqueductal gray is activated during offensive aggression. It is therefore generally considered to be part of the neuronal network of offensive aggression. However electrical and neurochemical manipulation of this brainstem structure mainly affects defensive behavior and many of the accompanying sympathetic and parasympathetic responses rather than true offense. Moreover, the structure is involved in the control of pain. Hence, it is conceivable that the periaqueductal gray has a role in the network of offensive aggression mainly by controlling autonomic arousal and antinociception that accompany offensive aggression.

Modulation of the Neuronal Network

Serotonin

All components of the neuronal network for offensive aggression are substantially innervated by serotonergic (5-HT) neurons originating in the dorsal and median raphe nuclei in the brainstem. More than any other neurochemical system, this evolutionary ancient and very well conserved neurotransmitter system is considered the primary molecular modulator of aggression in a wide variety of animal species. However, the nature of this linkage is complex, and it has proven difficult to unravel the precise role of this amine in the predisposition for and execution of offensive aggressive behavior in both its normal and pathological forms. For decades, high levels of aggressive behavior were associated with low serotonergic neurotransmission. This serotonin deficiency hypothesis seems consistent with the fact that serotonergic receptor agonists used to mimic higher serotonergic activity generally reduce aggressive behavior. However, recent studies of the functional status of the

5-HT system before, during, and after the execution of normal adaptive and abnormal pathological forms of aggression have led to a somewhat different view. Display of normal, adaptive offensive aggressive behavior aimed at territorial control and social dominance is associated with a higher 5-HT neuronal activity. A negative correlation between aggression and 5-HT as expressed in the deficiency hypothesis seems to be a trait-like characteristic of pathological forms of aggression (e.g., violence). For example, a clear positive correlation was found between the level of offensive aggression and basal CSF concentrations of 5-HT and/or its metabolite 5-HIAA. A highly significant negative correlation between aggression and 5-HT levels was found only upon inclusion of samples from abnormal and excessively aggressive trained fighter animals. A critical evaluation of the csf 5-HIAA data in aggressive humans confirms the idea that the serotonergic deficiency appears to hold, in particular, in specific groups of individuals who engage in impulsive and violent forms of aggressive behavior rather than in individuals with instrumental (functional) forms of offensive aggression.

Acute treatment with 5-HT_{1A} and 5-HT_{1B} receptor agonists is known to potently and selectively suppress aggressive behavior in a variety of species. Apart from acting on receptors at postsynaptic sites, these two receptor subtypes are the two main serotonergic autoreceptors involved in the negative feedback control of the 5-HT neuron at the level of the synapse (5-HT_{1B}) and at the level of the cell soma (5-HT_{1A}). Activation of these receptors by agonists will potently activate the negative feedback and thereby reduce 5-HT neurotransmission. It appears that the anti-aggressive effects of these compounds are largely expressed through their action on the inhibitory autoreceptors located at the cell soma and the nerve terminal, presumably attenuating intruder-activated 5-HT neurotransmission.

Dopamine

Dopamine has several important functions in the general control of behavior. The nigrostriatal dopaminergic system has a central function in the control of motor output, which is obviously important for offensive aggression as well. The involvement of the mesolimbic dopaminergic system in behavior is only partially understood. The balance between the dopaminergic and serotonergic innervation of the prefrontal cortex is an important determining factor in prefrontal cortex functioning and hence in impulsivity and control of goal-directed behavior, including offensive aggression. The dopaminergic projection from the ventral tegmental area to the nucleus accumbens is generally considered to be involved in reward and the development of addiction. Several studies show that offensive aggression can be highly rewarding, which is consistent with the release of dopamine in the

nucleus accumbens in the offensive male during an aggressive interaction. Moreover, the aggressive phenotype in rats and mice is more generally characterized by a highly sensitive dopaminergic system, which is consistent with a higher vulnerability to substance abuse, as demonstrated in animal models of cocaine self-administration.

Gonadal hormones

Testosterone is the gonadal steroid hormone that has been traditionally associated with male offensive aggression. Indeed, castration in adulthood may reduce aggression, but the correlation between levels of circulating testosterone and aggression is often low or even absent. The behavioral effects of testosterone are partly due to its action on peripheral secondary sex characteristics, changing the stimulus characteristics of the animal. Equally important, however, is its action on the brain. During development, testosterone plays an important role organizing brain and behavior into a more masculine direction. Characteristic of the neuronal network for offensive behavior is that the forebrain structures, in particular, are sensitive to gonadal steroids. The medial amygdala, the Bnst, and the preoptic area are characterized by a high density of estrogen and androgen receptors. Moreover, these structures contain high amounts of aromatase and 5- α reductase, enzymes that convert testosterone into estradiol and the androgen dihydro-testosterone, respectively. Both metabolites of testosterone play a distinct role in the modulation of offensive aggression through their respective action on estrogen and androgen receptors. In particular, the ER α receptor has been implicated in the modulation of offensive aggression. Hence, individual variation in offensive aggression may depend on the density of ER α and the amount of aromatase present in various brain areas. Indeed, aggressive males show higher numbers of ER- α -expressing cells in the lateral septal area, the Bnst, and the preoptic area, and the number of ER α receptors increase with aggression in seasonal reproducing animals. Some evidence suggests that aromatase, and hence the conversion of testosterone into estradiol, depends on experiential factors independent of the level of circulating testosterone. This might explain the absence of a clear correlation between testosterone and aggression in many species.

Vasopressin

The neuropeptide vasopressin plays an important role in the regulation of offensive aggression. Its role in aggression seems to be highly conserved throughout the animal kingdom. The cell bodies of neurons producing the neuropeptide vasopressin are present in a number of brain areas such as the medial amygdala, the Bnst, the supraoptic nucleus, and the paraventricular nucleus of the hypothalamus. These latter two structures are involved

in the release of vasopressin as a hormone in the general circulation through the portal system of the pituitary.

The AVP neurons originating from the Mea and the Bnst are sensitive to the circulating levels of testosterone through both ER α and the androgen receptor. Moreover, the density of AVP neurons in these brain areas and their projection to the lateral septal area are sexually dimorphic. Males have more AVP-producing neurons and nerve terminals than females do. AVP receptors are widely distributed in the brain, but present in higher densities in the lateral septum, anterior hypothalamus, and Bnst. When administered locally in, for example, the anterior hypothalamus, vasopressin generally facilitates offensive aggression through its action on the AVP1a receptor subtype. Studies in hamsters indicate that not only AVP synthesis is dependent on circulating gonadal steroids, but also AVP binding to its receptor in certain brain areas. This strongly suggests that this neuropeptide is involved in the orchestration of brain processes and behavior in response to fluctuations in gonadal hormones. The modulation of offensive behavior is a clear example of this. In addition to this gonadal-steroid-dependent modulation, AVP is also involved in the social-memory-dependent modulation of offensive aggression. For example, repeated winning experience and social dominance enhances AVP1a receptor binding independent of the levels of gonadal steroids.

Neurogenetics

The era of molecular genetics has generated a wealth of transgenic animals, some of which were specifically targeted at genes related to offensive aggression. Others were developed for different purposes, but appeared to differ from their controls in the level of aggressive behavior. Hence, analysis of the neurobiological systems affected in these transgenic animals may elucidate additional components of the neurobiology of offensive aggression.

Manipulation of genes involved in the molecular cascade of serotonergic, dopaminergic, and vasopressinergic neurotransmission clearly affect offensive behavior as predicted from the current knowledge of the involvement of these systems in offensive aggression. An example of this is given in **Table 1** which summarizes the effects on offensive aggression of genetic modification in various components of the serotonergic system.

Currently, there are over 70 genetically modified mouse lines known that show increased or decreased levels of offensive aggression. Several of these transgenic animals show changes in aggressive behavior that could not be predicted from the existing knowledge of the molecular neurobiology of aggression. This holds, in particular, for nitric oxide (NO), which is involved in the modulation of neuronal activity in the cortex and medulla. Mice that lack the gene nitric oxide synthase (nNOS), required for the synthesis of NO, are extremely aggressive if not violent. It is not known how NO fits into the general neurobiological network of aggression as outlined in **Figure 1**. However, changes in serotonergic neurotransmission through unknown molecular pathways seem to be a common factor in the increased level of aggression in many transgenic mouse lines.

Concluding Remarks

Several studies indicate that the neuronal network of offensive behavior as described above is not exclusively involved in this type of behavior. In particular, the medial amygdala and the caudal medial preoptic area are also involved in sexual behavior. It seems that the different forms of social behavior are only partially separated by hard-wired neural systems in the brain and share several higher-order brain areas as well. Several of these areas bind gonadal steroids, and it is suggested that the different forms of social behavior are associated with a differential activation pattern of the various components of the neuronal

Table 1 Genetic modification of serotonergic signaling and its effects on offensive aggression

Gene (chromosome)	Coding Protein product	Method	Effects on aggression
<i>Tph</i> (11)	Tryptophan hydroxylase	Polymorphism	↑
<i>Tph2</i> (10)	Tryptophan hydroxylase isoform 2	Polymorphism	↑
<i>Htr1a</i> (13)	R441H mutation 5-HT1A receptor	tph2 knock-in Knock out Conditional overexpression in 5-HT neurons	↑ ↓ ↑
<i>Htr1b</i> (9)	5-HT1B receptor	Knock out	↑
<i>HT2a</i> (14)	5-HT2A receptor	Polymorphism	↑
<i>HT6</i> (4)	5-HT6 receptor	Polymorphism	↑
<i>HT3A</i> (9)	5-HT3 receptor	Polymorphism	↓
<i>Maoa</i> (X)	Monoamine oxidase A	Knock out	↑
<i>SERT</i> (11)	5-HT transporter	Knock out	↓

network. Hence, one has to consider the possibility that a large part of the brain is not organized along the lines of behavioral systems such as offensive aggression. Indeed, a number of studies in a variety of species show the multi-dimensional nature of offensive aggression. For example, a high level of offensive aggression is correlated with high impulsivity, indicating low inhibitory control by the pre-frontal cortex. It is likely that a low prefrontal cortex function will also affect impulse control in the context of sexual behavior or food intake. Offensive aggression is also correlated with a general tendency to actively cope with different kinds of environmental challenges. It can be considered more generally as proactive coping with any kind of environmental stressor. The antipode of this proactive coping is a reactive coping style. This is expressed as the absence of self-initiated aggressive behavior in a social situation and passivity in other challenging environmental conditions. These divergent coping styles have now been observed in a wide variety of species and determine adaptive capacity and vulnerability to stress-related pathologies, including disorders characterized by outbursts of intense aggression and violence. This leads to the general view that part of the neuronal network for offensive aggression is not specifically involved in this type of behavior, but may control more generally the aspects of proactive coping and/or social behavior.

See also: Effects of Stress on Learning and Memory; Fear, Anxiety, and Defensive Behaviors in Animals; Hormones and Female Sexual Behavior; Human Fear and Anxiety; Measuring Stress; Neural and Pharmacological Substrates of Aggression; Neural Bases of Defensive Aggression; Neural Substrates of Conditioned Fear and

Anxiety; Neural Substrates of Unconditioned Fear, Defense, and Anxiety; Offensive and Defensive Aggression; Psychoneuroendocrinology of Stress; Regulation of the HPA Axis by Acute and Chronic Stress; Sex Hormones, Mood, and Cognition; Stress and Arousal/Sleep; Stress and Brain Morphology; Stress and Emotionality; Stress and Energy Homeostasis; Stress and Reward; Stress and Social Behavior.

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Place Cells

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Glossary

Backward expansion – The center of mass of a place field's firing rate distribution will shift in a direction opposite to the trajectory of the subject such that the field becomes skewed.

Context discrimination hypothesis (CDH) – Hippocampus contributes to complex forms of learning and memory by discriminating meaningful contexts.

Grid cell – A neuron that exhibits elevated firing in a repetitive, triangular, grid-like pattern within an environment.

Pattern completion – The ability of a network to retrieve a stored representation of information to generate a whole representation from degraded or sparse inputs.

Pattern separation – The ability of a network to disambiguate similar input patterns.

Phase precession – Place cell discharges are phase-locked to theta rhythm oscillations such that the first spike of successive spike bursts begins at progressively earlier phases of the theta cycle.

Place cell – A neuron that exhibits location specific elevations in firing rate.

Theta rhythm – 4–12 Hertz oscillations generated by the activation of excitatory and inhibitory neuronal populations.

neuron will increase its firing rate when the rat is in a particular location of the environment (Figure 1). HPC place cells have been studied at length to determine how they contribute to mnemonic processes ascribed to HPC. It is speculated that population activity from place cells provides a spatial framework within which information is encoded that can be used to guide spatial navigation and direct learning and memory processes.

Quantification of Place Field Properties

HPC place fields exhibit properties that can be used to quantify the quality of the place representation. The reliability of the field is often a measure that determines how consistently the cell fires when an animal passes through the field of the cell. The specificity of the field is a measure of the sharpness of the place field. For example, place fields in the CA1 region of HPC (Figure 2) have been found to be broader or larger than those in CA3. Thus, fields in CA1 are thought to be less spatially specific than those in CA3. Spatial correlation measures are used to quantify the degree to which the location of a field shifts or the within-field firing rate changes. In general, these properties illustrate how rate coding can represent the stability of a place field.

Place fields can also be studied in terms of the temporal properties of the firing rate, and these are reflected in phenomena called 'phase precession' and 'backward expansion.' Phase precession is related to the fact that the occurrences of place cell discharges are phase-locked to the theta rhythm (4–12-Hz network oscillations generated by activation of excitatory and inhibitory neuronal populations). That is, spike timing relative to theta is not random and tends to occur during a specific phase of the 360° theta cycle. When an animal travels through a place field, the first spike of successive spike bursts occurs at progressively earlier phases of the theta cycle, a phenomenon referred to as 'phase precession' (Figure 3). In other words, as a rat enters a place field the first spikes occur late in the theta cycle and as a rat leaves the field the last spikes occur early in the theta cycle. Furthermore, as an animal passes through a field several times within a behavioral epoch, the center of mass of the place field's firing-rate distribution will shift in a direction opposite to the trajectory of the rat such that the field becomes skewed. This phenomenon is known as 'backward expansion' (Figure 3). These basic temporal properties are

Introduction

The role of hippocampus (HPC) in learning and memory has been studied extensively since the discovery of severe memory impairments in patient HM after bilateral removal of his HPC. Over the years, HPC has been found to be involved in multiple forms of learning and memory. Lesion and pharmacological manipulations of HPC have revealed its role in spatial processing, relational learning, declarative learning, episodic memory, and context processing. Given the major role that HPC plays in spatial learning and memory, and the ease with which rats can learn and be tested on spatial tasks, electrophysiologists sought to determine HPC neural correlates of spatial processing in behaving rats (Figure 1). When rats actively move through extended environmental space, the most predominant behavioral correlate of individual HPC neurons is location-selective firing; that is, an individual

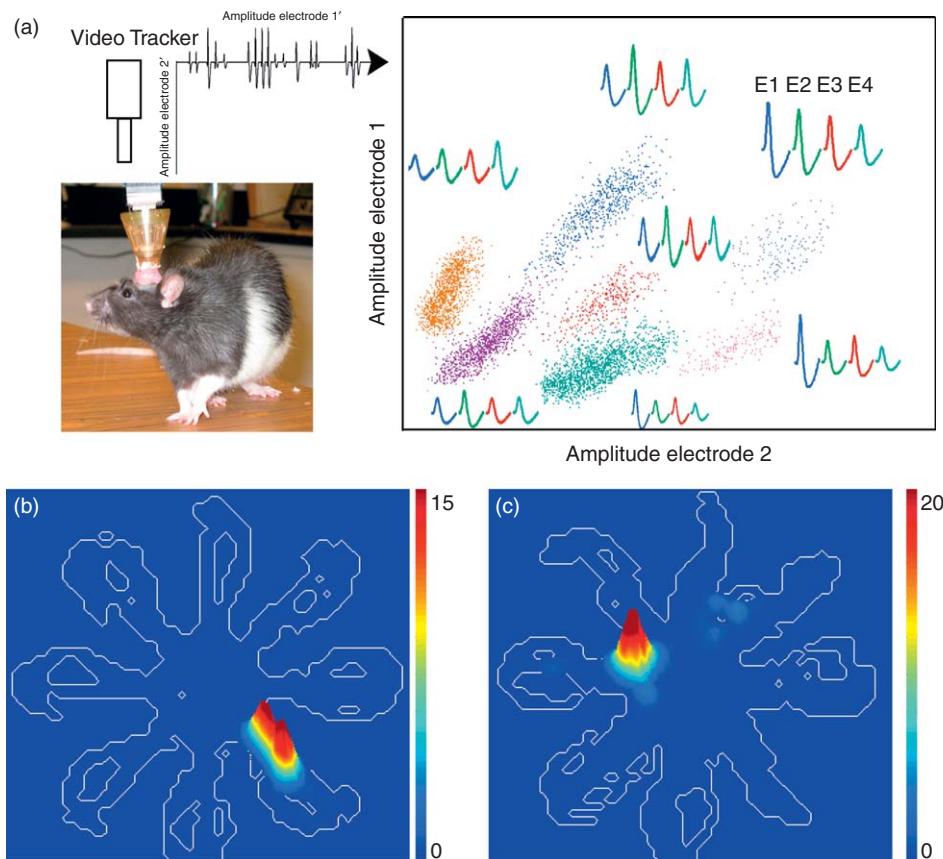


Figure 1 (a) An illustration of recordings from freely behaving rats. Position information recorded from a video tracker is synchronized with neural signals recorded from tetrodes. The neural data are separated into signals from individual cells using a cluster analysis that compares values for spike parameters across four electrodes of a tetrode. Spike-amplitude comparisons across electrode 1 (E1) and electrode 2 (E2) are illustrated and seven clusters are isolated as signals from individual neurons. (b, c) Overlay of video tracker data and neural activity from two individual place cells recorded while rats navigated an eight-arm radial maze. White lines represent the areas of the maze the rat visited and the neural firing pattern is overlaid onto these areas. The maximum firing rate for the cell in (b) is 15 Hz and occurred when the rat visited the maze arm on the lower right. On the other hand, when the rat visited other maze arms the firing rate of the cell was close to zero. Similar patterns of neural activity are illustrated in (c) except the place field is in a different location and has a higher firing rate.

thought to result from inherent Hebbian synaptic plasticity mechanisms that ultimately strengthen existing associative connections. What follows is a discussion of how place field characteristics are related to HPC-dependent learning and memory.

Effects of Sensory Manipulations on Place Fields

Relationships between Place Fields and External Sensory Stimuli

Place fields respond to changes in the visual environment. The fields expressed while rats are navigating in an environment surrounded by familiar distinct visual cues will remap or change field locations in accordance with the rotation of visual cues. For example, when a constellation of cues is rotated by 45°, some place fields will shift their locations by 45°. In addition, place fields respond to

changes in the geometric features of the environment as well as to changes in other nonvisual features of the external environment such as olfactory, somatosensory, and auditory cues. This suggests that place fields provide a representation of the general external sensory environment that is not limited to spatial information. However, place fields also endure after the removal of external sensory cues and exist in blind rats, suggesting that other variables, such as internal sensory information, contribute to the place field when external sensory cues are not available.

Relationships between Place Fields and Internal Sensory Stimuli

Place fields are sensitive to idiothetic (or self-motion) information. For example, the firing rates of place fields are correlated with the animal's velocity and place fields are influenced by vestibular input. In addition, place fields

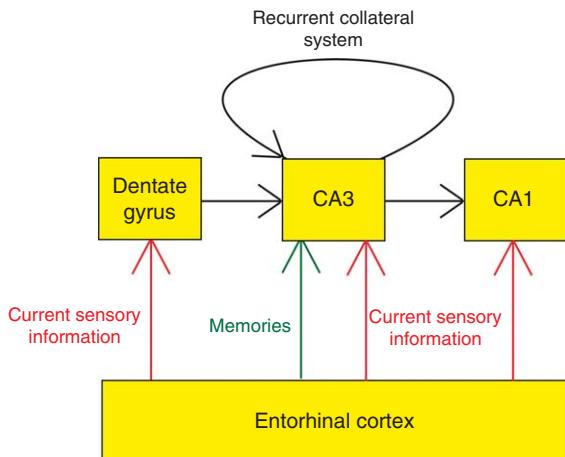


Figure 2 Schematic of HPC circuitry. Entorhinal cortex input to dentate gyrus is comprised of current sensory information. This information is orthonormalized by pattern separation processes that dominate dentate gyrus function. CA3 receives current sensory information and already encoded memory representations from neocortical structures via entorhinal cortex. The CA3 recurrent collateral system is thought to sustain memory representations. Both pattern completion and pattern separation processes in CA3 are involved in determining whether current features of the environment provided by dentate gyrus and entorhinal cortex match expected features of the environment that are maintained by the recurrent collateral system. CA1 is also in a position to mediate pattern completion and separation using current sensory information from entorhinal cortex and information from CA3.

tend to be less specific and reliable when rats are passively moved through a place field either by being held by the experimenter or placed on an electronic moveable device. Under normal circumstances, these data indicate that idiothetic information serves to enhance external representations of the environment. The combination of external and internal cues represented by place fields suggests that place cells contribute to both egocentric and allocentric information processing. Idiothetic information represented by place fields can be used to facilitate path integration processes, that is, the integration of linear and angular motion to keep track of spatial location within an environment, and this allows rats to navigate environments in which external cues are sparse or become unreliable.

Effects of Learning-Related Manipulations and Conditions on Place Fields

Relationships between Place Fields and Synaptic Plasticity

Long-term potentiation (LTP) and long-term depression (LTD) are predominant models of HPC-dependent learning-induced synaptic changes. LTP and LTD

inductions modify synaptic strengths, which should produce changes in spatial representations of place cells. Indeed, induction of LTP produces increases in in-field firing rates and remapping. LTP is dependent on N-methyl-D-aspartic acid (NMDA) receptors and NMDA receptor antagonists impair spatial navigation. In agreement with behavioral deficits, NMDA receptor antagonists also decrease the stability of place fields. Furthermore, genetically engineered mice that lack NMDA receptors exhibit poor spatial learning and have less specific place fields. Temporal properties of place fields are also influenced by NMDA-mediated plasticity mechanisms. Backward expansion is disrupted by NMDA receptor antagonism, suggesting that this place field property is mediated by LTP-like mechanisms. These data provide compelling evidence for similar underlying biological mechanisms that support both HPC-dependent learning and stable place fields.

Place Fields and Sequence Learning

Sequence learning has been shown to be dependent on HPC and may be an integral component of episodic memory formation. As rats traverse a portion of an environment, populations of place cells will fire in a temporal order that reflects the trajectory of the animal. This temporal sequence of place field expression may contribute to HPC-dependent sequence learning since place cells recorded in rats traversing a linear track replay the order in which they fired along a trajectory during subsequent rapid eye movement (REM) sleep. In addition, when a rat reaches the goal location on a linear track, place cells will fire in a reverse temporal order relative to the order in which they fired on the linear track. These replay mechanisms may be related to memory consolidation of the temporal components of the behavioral experience.

Backward expansion and phase precession are two mechanisms that are thought to contribute to encoding and recall of temporal aspects of memory by taking advantage of plasticity mechanisms that are considered necessary for HPC-dependent learning. Backward expansion of place fields may reflect asymmetric Hebbian strengthening of synapses (the presynaptic cell must fire before the postsynaptic cell in order for plasticity to take place) and computational models suggest that backward expansion leads to predictive firing of place cells that can enhance recall of sequences of information. Phase precession can lead to more efficient sequence encoding because it generates temporally compressed sequences of partially overlapping place fields within individual theta cycles (**Figure 3**). This sequence of overlapping representations is also repeated during successive theta cycles. The compression allows the spiking activity of cells, with overlapping fields, to occur within an optimal time

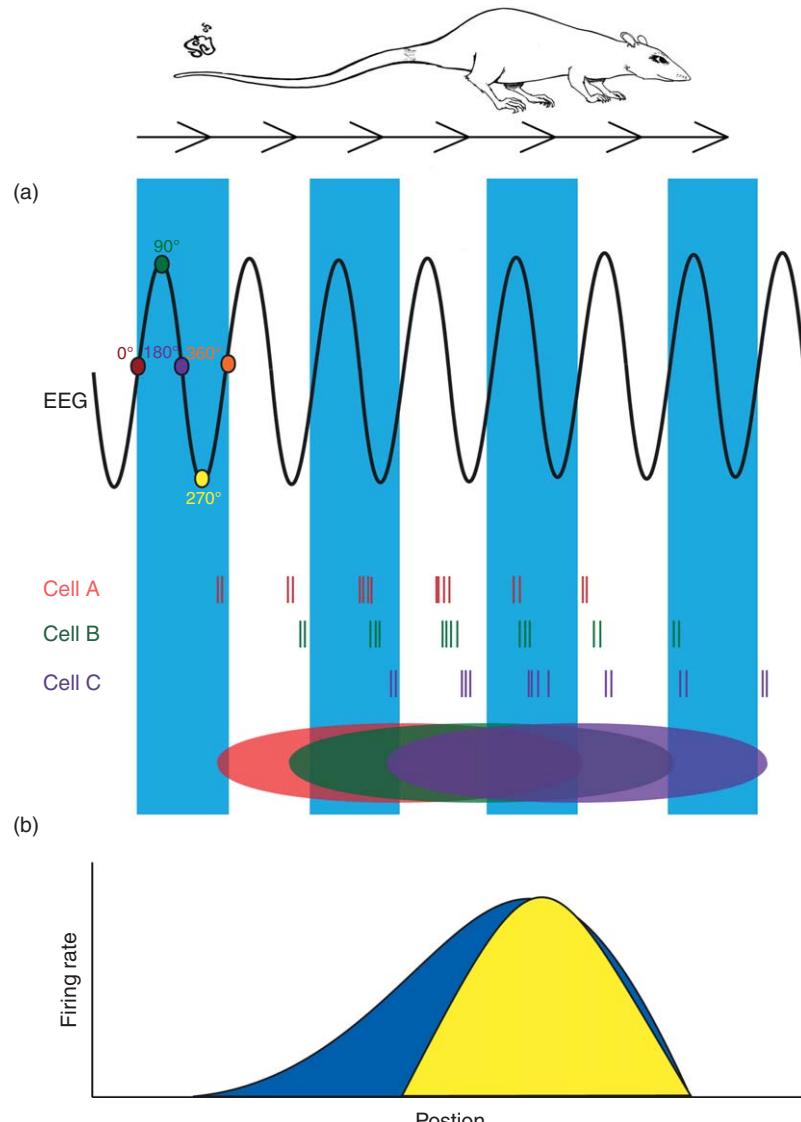


Figure 3 (a) Illustration of theta phase precession. The EEG theta signal is represented by the black sinusoidal function and a complete theta cycle is highlighted in blue. Each short vertical line below the theta waveform represents individual spikes from three place cells with overlapping fields. As the rat enters a place field from left to right the first spike of the first burst occurs late in the theta cycle, as the rat leaves the field the first spike of the last burst occurs early in the theta cycle. Information from the three overlapping place fields is temporally compressed within individual theta cycles such that the ABC sequence is repeated several times across multiple theta cycles during a single pass through the location. (b) Illustration of backward expansion of a place field as a rat makes several passes through the field. The firing-rate distribution of a place field as a rat is moving from left to right during the first pass through the field is shown in yellow. The distribution during the last pass through the field is shown in blue. After the rat passes through the field several times, the place field expands backward and becomes negatively skewed.

window for synaptic plasticity, and repetition could be used to reinforce the associations.

Computational models also suggest that theta rhythm regulates memory encoding and retrieval. The trough and peak phases of theta rhythm are thought to enhance memory encoding and retrieval, respectively. During trough phases of theta, input from entorhinal cortex is strong and HPC synapses show LTP, which allows for encoding of entorhinal representations of the current environment. During peak phases of theta, CA3 activity

(representing retrieval of previously encoded memories) is strong while entorhinal input is weak and synapses show strong LTD, ensuring that the retrieved memory is not encoded as a new memory.

Place Field Comparisons across Animals with Different Learning Capabilities

Place fields have been compared across groups of young and aged rats. Aged rats are often impaired in

their spatial memory abilities and show deficits in LTP induction. To date, the effects of aging on place field properties, such as reliability and specificity, are unclear and somewhat contradictory. Place fields can be just as reliable and specific in aged rats as they are in young rats, but this result can differ depending on the task used at the time of recording. However, place fields tend to be smaller in size and the magnitude of backward expansion is attenuated in aged rats. These results indicate that synaptic plasticity mechanisms are impaired in aged animals, and this impairment may contribute to findings of poor stability of place fields and poor spatial memories of old rats.

Place Field Changes during New Learning

Spatial learning is presumed to occur when an animal is exposed to a novel environment and HPC is known for its involvement in rapid encoding of new information. Disruption of HPC activity results in deficits in one-trial learning, and HPC lesions made immediately after acquisition, but not later, impair contextual fear conditioning. Place-specific firing is evident upon initial exposure to a novel environment or novel portions of a familiar environment. The in-field firing rate and stability of place fields initially increase over time but, as more time is spent in the novel environment, these changes cease such that field properties eventually resemble those found in familiar environments. Place cells have also been recorded while rats are learning HPC-dependent tasks. Under these conditions, place fields become more specific as learning progresses. These fields also tend to move toward new goal locations when rats are tested in familiar environments. In addition to changes observed in spatial tasks, place field responses change after fear conditioning. More specifically, HPC-dependent contextual fear conditioning leads to more place field remapping than cued fear conditioning, which is not dependent on an intact HPC. These results indicate that the stability of the place field and the degree of remapping are related to learning new spatial information. However, these changes may also be related to changes in the behavior of the animal such as movement, emotion, and attention that are associated with new learning.

Place Field Responses to Changes in Cognitive Demand

A different approach to elucidating the role of place cells in learning and memory is to examine the place field when an animal is exposed to changes in cognitive demand. In this behavioral paradigm, sensory, movement, and motivational variables are held constant across

conditions, thus eliminating these explanations for differential responses of place cells. Using this approach, place cells have been recorded while animals perform a HPC-dependent plus-maze task during which animals learn that reward is always located on a particular arm of the plus maze. When the well-learned reward location is relocated to a different area of the maze, place fields undergo remapping, reflecting a memory influence for the previous goal location on the place cell. Place cells have also been recorded while rats are required to shift from an HPC-dependent strategy (e.g., always go to the same location for reward) to a striatum-dependent strategy (e.g., always turn right for reward) to solve a plus-maze task. This explicit manipulation of cognitive demand induces a new place field organization that presumably reflects the newly activated memory that underlies the use of a different strategy to solve the task.

Place cells also exhibit conditional firing that depends on the impending or previous behavior of the animal. Place cells recorded during asymptotic performance on a plus-maze task will fire differentially depending on the trajectory the animal must take to reach the goal location (prospective coding) or depending on what trajectory the animal took to reach the goal location (retrospective coding). In addition, the prospective signal is severely degraded during error trials, suggesting an important role for this signal in accurate performance of a HPC-dependent task. In sum, these signals illustrate a strong memory influence on place fields. They also provide a link between information represented by place cells and episodic memory functions attributed to HPC by illustrating a temporal component in place cell rate coding that is required in defining episodic memory formation.

Effects of Context Manipulations on Place Fields

Although the described features of place fields are consistent with current views of HPC-dependent learning and memory functions, their precise contribution is still far from being understood. Until very recently, many of the behavioral paradigms did not permit assessment of place fields during the performance of HPC-dependent tasks, and they did not consider the responses of place cells within a context-processing theoretical framework. The following describes recent attempts to consider the various dynamic place field responses within a broader context-processing view.

Context Discrimination Hypothesis

The context discrimination hypothesis (CDH) proposes that HPC contributes to complex forms of learning and memory by discriminating meaningful contexts. HPC

place fields are thought to represent spatial contexts and perform match–mismatch comparisons to determine the extent to which a particular context has changed. The results of these comparisons provide a context code propagated by the combined activity of a population of place cells that can be used to distinguish distinct contexts in which significant events or episodes occur. The context code includes several types of contextual information, including spatial information, reward, movement, external nonspatial information, motivation, and memory. What follows is a discussion of how place fields represent contextual information and how this information is relevant to learning and memory.

Place Field Responses to Changes in Context

Some place fields remap in response to changes in context, while other place fields remain stable (Figure 4). The fields that reorganize are thought to represent current contextual features of the environment, while the

fields that remain stable may represent expected contextual features. If there is a mismatch between the current and expected contextual features, then an appropriate message signaling the change may be sent out of HPC to update cortical memory structures. Detected mismatch signals are sent to cortical structures to be used to alter active memory systems and the subsequent selection of behaviors. If the context does not change, the stable place fields can be used as a signal to reinforce ongoing behavior and add to the persistence of the currently engaged memory system. Next is an account of computational models and empirical studies of context processing that reveal the importance of the HPC network in match–mismatch processing.

Pattern Separation and Pattern Completion in HPC Subfields

According to computational theories, HPC function can be characterized as an interaction between pattern

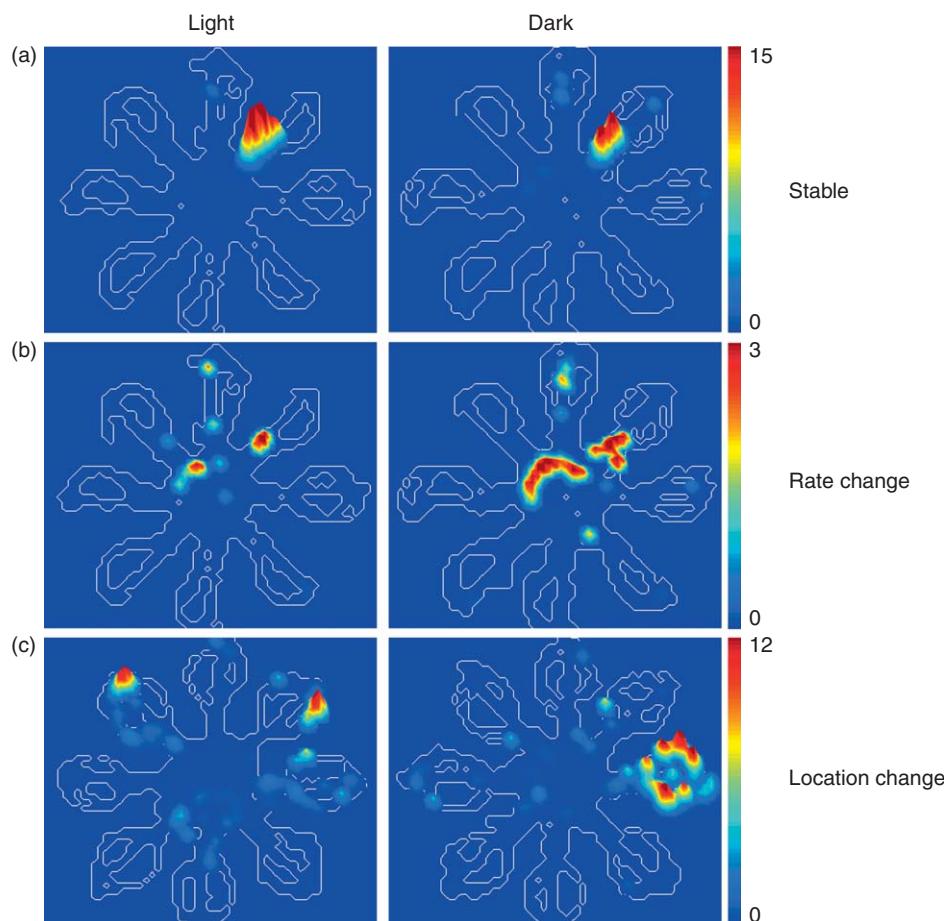


Figure 4 Place field remapping to changes in context. (a, b) Two simultaneously recorded place cells before and after maze lights were extinguished. The place field in (a) did not remap during darkness, whereas the place field in (b) showed robust rate remapping in that the location of the field was still stable but the in-field firing rate increased. (c) A place cell recorded during a different behavioral epoch shows both rate and global remapping in response to imposed darkness. The in-field firing rate on the right maze arm increased and two fields disappeared during darkness.

separation and pattern completion processes. Pattern separation refers to the ability of a network to disambiguate similar input patterns. Pattern completion refers to the ability of a network to retrieve a stored representation of information to generate a whole representation from degraded or sparse inputs. Based on anatomical differences, the dentate gyrus subfield of HPC (**Figure 2**) is proposed to provide a mechanism for pattern separation, whereas the CA1 and CA3 subfields mediate both pattern completion and pattern separation processes. Cells in layer II of entorhinal cortex project directly to the granule cells of dentate gyrus via the perforant path. This pathway is highly plastic and computational models propose that dentate gyrus orthogonalizes (i.e., pattern separates) inputs from cortex. CA3 receives direct projections from layer III of entorhinal cortex as well as strong synaptic connections from the granule cells of dentate gyrus. CA3 is well known for its recurrent collateral fiber system through which the principal cells of CA3 project back onto themselves. The recurrent collateral system is thought to sustain memory representations of the environment, while input from the entorhinal cortex and dentate represent features of the current environment. Interactions between the recurrent collateral fiber system, entorhinal cortex, and dentate gyrus are thought to mediate competition between pattern completion and pattern separation processes in CA3. CA1 receives projections from CA3 via the Schaffer collaterals and projections from layer III of entorhinal cortex. Thus, CA1 is also in a position to mediate pattern completion and separation processes using direct input from entorhinal cortex and CA3.

Comparisons between CA1 and CA3 Place Field Responses to Changes in Context

Based on the unique patterns of connectivity across CA1 and CA3, HPC models propose that CA1 is more involved in novelty detection given its strong direct connections with current environmental information relayed from layer III of entorhinal cortex and memory representations from CA3, whereas CA3 is more involved in rapid encoding (regardless of novelty) via dentate gyrus connections and retrieval of memory-based associations as a result of the recurrent collateral system. Lesion and pharmacological manipulations of CA1 and CA3 have confirmed these predictions, and electrophysiological studies reveal differences in firing patterns of CA1 and CA3 place cells in response to changes in context that support this view.

Place fields in CA3 tend to reorganize more readily during large changes in context, such as placing an animal in a novel testing room, than place fields in CA1. These

results persist regardless of the type of task the animal is performing, although different results can be found depending on the extent to which the context is changed. Slight changes in the expected context, such as rotation of distal cues, cause place fields in CA1 to reorganize more extensively relative to those in CA3. In addition, fields that reorganize in CA3 tend to be more consistent with cue rotations, whereas the location of the fields that change in CA1 cannot be predicted by the cue rotation. The differential responses by CA3 and CA1 place fields during exposure to varying levels of contextual manipulations can be explained by competition between pattern completion and pattern separation processes. When the environment is changed dramatically, pattern separation processes dominate CA3 responses and more remapping is seen in CA3 relative to CA1. When there are subtler changes in the environment, place field responses in CA3 are directed by pattern completion mechanisms and less reorganization is seen in CA3 relative to CA1. It appears that competition between pattern separation and pattern completion in CA3 is mediated by a threshold mechanism such that the CA3 network is very tolerant to small changes in input patterns; however, if the change in input patterns is great enough, the system will switch from expressing information relayed by the recurrent collateral system to signals relayed by entorhinal cortex. In contrast, CA1 place fields may reorganize in response to changes in context in a more linear fashion. More significant reorganizing by CA1 place fields relative to those of CA3 in situations where there are small changes in context indicates that some degree of pattern separation may be occurring in CA1, regardless of the level of change in context. Together, these findings indicate that CA1 is more optimally suited for novelty detection, whereas CA3 may be more involved in determining whether the current features of the context match memory stores of expected contextual features. The combination of output from these two areas is thought to inform neocortical algorithms that select adaptive behaviors.

A Systems Perspective of the Contribution of Place Cells to Learning and Memory

In order to fully understand HPC place cell contributions to learning and memory, one must also consider the role of place cells in other neural systems that underlie learning and memory. Studying afferent and efferent systems associated with HPC has made significant contributions to our understanding of mnemonic functions of place cells.

HPC Interactions with Afferent Cortical Structures

HPC receives a large number of inputs from entorhinal cortex. Grid cells in dorsal medial entorhinal cortex are thought to make a significant contribution to the spatial firing properties of place cells in HPC. These cells show elevated firing at regularly spaced locations within an environment. The grid fields of these cells are arranged in a repetitive, triangular, grid-like pattern. Grid fields are thought to be established by motion-derived information as they maintain their spacing frequency in new environments and in the dark. Unlike place fields, grid fields do not remap in response to contextual variables; instead, they appear to code a more strict spatial representation of the environment. In familiar environments, grid fields will rotate in the direction of cue rotations and if a familiar environment is made to be larger or smaller (by widening or narrowing walls), their spacing frequency will resize in accordance with the environmental change. These small shifts in firing patterns may contribute to some of the remapping seen in place cells to changes in familiar contexts. Therefore, a primary role of entorhinal cortex may be to pass on a spatial framework to HPC within which contextual information can be organized.

HPC also receives input from perirhinal cortex. Following lesions to perirhinal cortex, HPC place fields have been found to be more likely to remap after being returned to familiar environments and are more likely to maintain their positions when visual cues are rotated. Lesions to perirhinal cortex impair context discrimination; therefore, lesioned rats may rely more on olfactory and local cues than on global spatial cues, and slight changes to a familiar environment, such as cleaning, may cause HPC place fields to inappropriately remap, whereas movement of spatial cues would no longer be processed. Given the role of perirhinal cortex in context processing and its direct connections with HPC and entorhinal cortex, this brain region is thought to be a primary source of context-related information to HPC.

HPC Interactions with Efferent Cortical Structures

Although HPC is necessary for establishing new memories, its involvement is temporary and consolidation processes gradually establish memories in cortical areas outside of HPC. HPC is therefore thought to prepare information for integration into neocortical circuits. HPC has a strong monosynaptic projection to medial prefrontal cortex (mPFC) and disruption of this connection produces deficits in spatial learning and memory. More specifically, lesions of HPC produce deficits early on in learning, while lesions of mPFC produce deficits in later learning stages. Activity of individual mPFC

neurons is phase-locked with HPC theta rhythm, and phase precession in mPFC neurons is observed when the local field potential between HPC and mPFC is more coherent. In addition, mPFC phase precession is most robustly observed when rats are making decisions during spatial working memory tasks. Phase locking to HPC theta rhythm has also been observed in cingulate cortex, entorhinal cortex, and visual cortex. Together, this suggests that theta rhythm may be a predominant mechanism by which HPC information becomes incorporated into cortical networks. Future studies need to elucidate how these efferent systems use contextual information from HPC to facilitate the selection of behaviors appropriate for a given context and how HPC place cells contribute to these processes.

Conclusions

The study of place cells in HPC illustrates the importance of studying neural correlates of learning and memory from a broad perspective. One has to consider a broad range of neural responses with a broad range of behaviors that tap into HPC function. Place cells contribute to learning and memory by utilizing a variety of signals, from fine detailed temporal information embedded within the dynamic responses of place fields to more global signals such as local field potentials. The output of these place cell signals can also change depending on what behavior the animal is engaged in. Therefore, interpretations of the significance of these signals are complex, as they should be. With more refinement and expansion of levels of analysis, our understanding of the mnemonic contribution of HPC place cells will become clearer.

Acknowledgments

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See also: Animal Models of Learning and Memory; Basal Ganglia; Cognition: Learning and Memory: Spatial; Declarative Memory; Genetics of Memory in *Drosophila*; Implicit Learning and Memory: Psychological and Neural Aspects; Learning and Memory: Computational Models; Navigation in Virtual Space: Psychological and Neural Aspects; Neural Basis of Classical Conditioning; Neural Basis of Recognition Memory in Nonhuman Primates; Neural Systems of Motivation; Orientation and Navigation; Protein Synthesis and Memory; Role of Gene Transcription in Long-Term Memory Storage; Short-Term Memory: Psychological and Neural Aspects; Transgenic

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Role of Neuronal Synchrony in Normal and Pathological Brain Functions

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Glossary

Dysconnection syndrome – A hypothesis suggesting that cognitive dysfunctions and clinical symptoms of brain disorders arise out of a disconnection between brain regions.

Frequency bands – Neural oscillations have been classified into different frequency bands (delta, 1–3 Hz; theta, 4–7 Hz; alpha, 8–13 Hz; beta, 14–30 Hz; gamma, 30–80 Hz; fast, 80–200 Hz; ultra fast, 200–600 Hz).

Neural oscillations – Refer to periodic variations in the recordings of neural activity. Activity measures are related to the membrane potential of single neurons or populations of neurons and thereby encompass action potentials (APs) and local field potentials (LFPs).

Phase locking – Time locking of brain oscillations to (sensory) stimuli or (motor) responses.

Psychosis – Refers to distortions in inferential thinking, such as delusions (fixed, false beliefs that are firmly held in the face of contradictory evidence), and perceptual disturbances, such as hallucinations. Auditory hallucinations, usually experienced as voices distinct from one's own thoughts, are most common in schizophrenia.

We focus this review on these diseases and exclude epilepsy and Parkinson's disease because several recent reviews have covered the obvious involvement of abnormal rhythms and synchronization in these disorders.

Neural Synchrony during Normal Brain Functioning

Transient synchronization of neuronal discharges has been proposed as one possible mechanism to dynamically bind widely distributed sets of neurons into functionally coherent ensembles that represent the neural correlates of a cognitive content or a motor program. The first indications for a functional role of gamma-band oscillations for information processing in cortical networks was obtained by Gray and Singer in studies investigating the relations between stimulus-induced synchronization of gamma-oscillations and feature binding in cat primary visual cortex (V1) at the Max-Planck Institute for Brain Research in 1987. This relationship was then confirmed in a series of studies with electroencephalography (EEG) and magnetoencephalography (MEG) that found robust support for the role of gamma oscillations in the grouping of stimulus elements into coherent object representations. Synchronization in these studies was consistently associated with an oscillatory patterning of neuronal responses, most often in the beta (15–30 Hz) and gamma (30–80 Hz) frequency range. Subsequent research has indicated that such high-frequency oscillations are particularly effective in supporting precise synchronization of neuronal discharges.

Further research has found a close relationship among attention, oscillations, and synchrony in the gamma-band range. Recordings of neurons in the cortical area V4 while macaque monkeys attended to behaviorally relevant stimuli and ignored distractors revealed increased gamma synchronization (35–90 Hz) compared with neurons at the nearby V4 sites activated by distractors. Consistent evidence for the relationship between attention and gamma-band activity during both visual and auditory perception has been also found in human EEG- and MEG-recordings. Taken together, these findings suggest that gamma-band oscillations have a general computational role in dynamically selecting neurons that

Introduction

Most of the brain's cognitive and executive functions are based on the coordinated interactions of large numbers of neurons that are distributed within and across different specialized brain areas. A fundamental yet unresolved problem of modern neuroscience is how this coordination is achieved. Integration and segregation of neural activity need to occur at various spatial and temporal scales and these scales must be dynamically adjusted depending on the nature of the respective cognitive tasks.

In contrast to the large number of studies that investigated the role of synchrony in a wide range of cognitive and executive processes, relatively few investigations have examined the possible relevance of neural synchrony in pathological brain states, such as schizophrenia, autism, and Alzheimer's disease.

communicate information about sensory inputs effectively.

Gamma oscillations have been also implicated in higher cognitive functions, such as working memory. In EEG-studies, induced gamma-band activity was observed during the delay over frontal and parietal electrodes, indicating that gamma oscillations are involved in the maintenance of information in working memory. Additional evidence for the role of gamma oscillations during working memory has been obtained from intracranial recordings as well as from studies that examined the relationship between auditory working memory and gamma oscillations in MEG-data. Finally, gamma oscillations may also be involved in long-term memory as there is a relationship between the amount of gamma-band activity during the encoding of information and the subsequent recall.

Recent data suggest that synchronized gamma-band activity may also be related to consciousness as a large body of evidence suggest that consciousness has to be understood as a function of numerous interacting systems that require a mechanism that transiently synchronizes a number of widely distributed neural assemblies.

Measures of Oscillatory Activity and Synchrony

Recording methods that assess the activity of large populations of neurons such as microelectrode recordings of local field potentials (LFPs) or EEG and MEG registrations can only detect neuronal activity if it exhibits some degree of synchrony. Entirely uncoordinated activity would not be detectable because the currents of synaptic events, which are the major sources of the measured signals, would cancel out.

In most cases, the signals recorded from neuron populations consist of oscillations that cover a broad-frequency spectrum and are usually quantified by computing the relative power in distinct frequency bands. Until a decade ago, the most frequently applied technique for this spectral decomposition was the Fourier analysis. This classic method has recently been complemented by wavelet-based techniques and multitaper analyses, which are better adapted for the spectral decomposition of nonstationary time series.

In addition to analyzing the frequency spectrum of spontaneous oscillations, it is of interest to determine the time course of stimulus- or task-related oscillations. Two forms of stimulus-related oscillatory activity need to be distinguished: (1) evoked and (2) induced oscillations. Evoked oscillations are strictly phase-locked to the onset of a stimulus and, therefore, can be measured by stimulus-triggered averaging of responses. By contrast, induced oscillations appear in association with stimulus triggered cognitive processes but reflect self-paced temporal coordination of neuronal responses. They are not phase-

locked with external events, and therefore abolished by averaging. These induced oscillations typically occur in the beta- and gamma-frequency range and appear in association with a large variety of cognitive and executive processes.

Although the amplitude of LFP, EEG, or MEG signals correlates with the degree of synchrony of neuronal responses, there are numerous confounding variables that make it difficult to draw firm conclusions on synchrony by considering only amplitude measures. Among these are the size and the alignment of the dipole fields of the contributing neurons, the fraction of synchronously active neurons in the population of cells contributing to the signal, and, above all, the degree of precision with which the neuronal discharges are synchronized. The latter variable is particularly critical when neurons engage in high-frequency oscillatory activity. In this case, the precision of synchrony needs to be in the millisecond range in order to permit effective summation of synaptic currents and to yield a measurable signal. Therefore, methods have been developed which permit assessment of synchrony independently of amplitude.

These measures need to be distinguished from measures of coherence, which determine the covariance of the amplitude of oscillations recorded from different sites for the various frequency bands. Both phase synchronization and coherence have been used to assess functional coupling among distributed neuronal populations.

Neuronal Synchrony: Anatomical Substrate and Neurotransmitters

Studies based on lesions, developmental manipulations, and simulations indicate that neuronal synchronization in the high-frequency range (beta- and gamma-band) is mainly mediated by cortico-cortical connections that link, reciprocally, not only cell populations situated in the same cortical area but also cells distributed across different areas and even across the two hemispheres. Accordingly, synchronization probability between neurons partly reflects the anatomical layout of excitatory cortico-cortical connections.

The generation and synchronization of cortical beta and gamma oscillations involves several neurotransmitter systems. The network of reciprocally coupled γ -aminobutyric acid (GABA)ergic neurons plays a pivotal role in the primary generation of high-frequency oscillations and their local synchronization, whereas glutamatergic connections appear to control their strength, duration, and long-range synchronization. Recent evidence indicates that cholinergic modulation plays a crucial role in the fast, state-dependent facilitation of high-frequency oscillations and the associated response synchronization. In addition to chemical synaptic transmission, direct

electrotonic coupling through gap junctions between inhibitory neurons also contributes to the temporal patterning of population activity and, in particular, to the precise synchronization of oscillatory activity.

Neuronal Synchrony in Brain Disorders

Schizophrenia

Schizophrenia is a severe mental disorder with an estimated lifetime prevalence of 1%. The disorder is characterized by psychotic symptoms (e.g., delusions and hallucinations), negative symptoms (e.g., flattening of affect and apathy) and disorganization of thought and behavior. Cognitive dysfunctions are prominent throughout the course of schizophrenia and have been shown to be a better predictor for outcome than the overt symptoms, suggesting that cognitive deficits represent the core pathology of the disorder.

Current theories of schizophrenia emphasize that core aspects of the pathophysiology are due to deficits in the coordination of distributed processes that involve multiple cortical areas and that are associated with specific cognitive deficits. Some of the deficits concern functions such as working memory, attention, and perceptual organization that have been proposed to involve synchronization of oscillatory activity in the high-frequency band (beta and gamma).

A substantial body of EEG studies supports the hypothesis that schizophrenia is related to impaired neural synchrony. Examination of auditory and visual steady-state responses to repetitive stimulation has revealed a specific reduction in the power of the stimulus-locked response (entrained oscillations) in the beta- and gamma-frequency range but not for the lower frequencies. This could be due to reduced synchronization of stimulus-evoked high-frequency oscillations and/or to an inability of neurons to follow high stimulation rates. Moreover, there is evidence for a reduction of evoked stimulus-locked oscillatory activity, again in the high-frequency range, following auditory and visual stimuli. Reductions in evoked oscillatory activity have been reported for tasks involving visual binding, for backward masking, and an auditory oddball paradigm. Furthermore, there is also preliminary evidence for a reduction of induced non-stimulus-locked oscillations in the gamma-band range during the processing of visual stimuli. These results suggest selective deficiencies in the ability of cortical networks or cortico-thalamo-cortical loops to engage in precisely synchronized high-frequency oscillations.

In addition to these analyses of spectral power of evoked oscillatory activity, several studies have examined phase-synchrony between distributed neuronal populations while patients performed cognitive tasks. In a recent study, we provided evidence for a close relation between

impaired neural synchrony in schizophrenia and specific cognitive deficits using Mooney faces as stimuli (see Figure 1). Schizophrenia patients exhibited a deficit in the perception of Mooney faces and reduced phase-synchrony in the beta-band while the power of induced gamma-band oscillations was in the normal range. This suggests that large-scale synchronization is crucially impaired in patients with schizophrenia while local synchrony in the gamma band is largely intact.

Impairments in the ability of distributed networks to establish precise synchronization of neuronal assemblies oscillating at high frequencies can have many reasons. These comprise both a host of local factors that determine the time constants of interactions within the oscillating microcircuits as well as the properties of long-distance connections that mediate interareal synchronization. Abnormalities have been identified for some of these candidate mechanisms in schizophrenia patients. *In vivo* anatomical examination with diffusion tensor imaging (DTI) has revealed white-matter anomalies that might be related to deficiencies in long-range synchronization. Cortico-cortical connections were reduced in the frontal, temporal, and parietal lobes and between the two hemispheres. However, there is also evidence for locally increased connectivity that is related to productive symptoms, such as auditory hallucinations. One interpretation of these seemingly paradoxical findings is that hyperconnectivity between higher- and lower-order cortical areas favors back-propagation of oscillatory activity generated in higher sensory areas during visual and auditory imagery to the respective primary sensory cortices, thus generating activation patterns that resemble those induced by sensory stimulation. This interpretation receives some support by the finding that hallucinations are associated with increased gamma oscillations and enhanced hemodynamic responses (blood-oxygen-level-dependent (BOLD) signals) in the corresponding sensory areas of the cerebral cortex. For several reasons, this increased BOLD signal is likely to reflect the entrainment of neurons in the primary areas into synchronized, high-frequency oscillations: first, it is improbable that neurons in primary sensory areas exhibit major increases in discharge rates in the absence of sensory stimulation. Second, top-down effects, such as those associated with focused attention, cause an entrainment of selected neuronal populations into well-synchronized gamma oscillations without enhancing the discharge rates. Third, increases of the BOLD signal correlate well with the entrainment of neurons into synchronized high-frequency gamma oscillations.

Further candidate mechanisms for deficient synchronization in the high-frequency range are abnormalities in the rhythm-generating networks of inhibitory interneurons and in the glutamatergic neurons mediating long-distance synchronization. Abnormalities in te

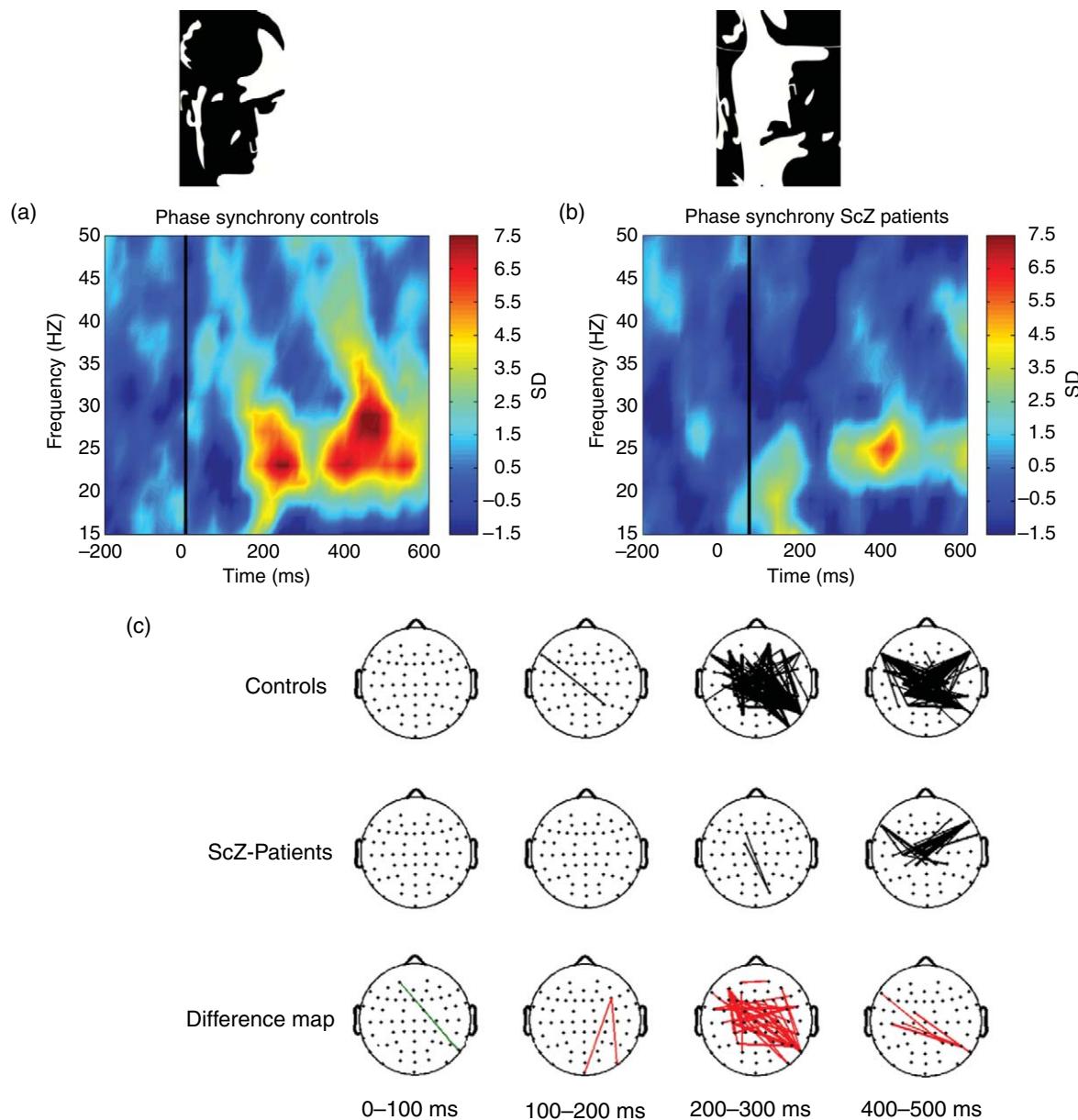


Figure 1 Neural synchrony during Gestalt perception in schizophrenia. Group average of phase synchrony for all electrodes and for correct trials during a Gestalt perception task in controls ((a) and (b)). Phase synchrony during Gestalt perception in controls exhibited two maxima over an average frequency range of 20–30 Hz (a). The increase in phase synchrony between 200 and 300 ms has been related to the construction of coherent object representations, whereas the second peak indexes the preparation and execution of the motor response. In patients with schizophrenia (b), the onset of the first peak in the face condition was delayed and occurred between 350 and 400 ms in the frequency range of 20–25 Hz (Figure 1c). In addition, a second, weaker peak was found around 600 ms. Compared with controls, the reduction in phase synchrony in the frequency range of 20–30 Hz was significant (frequency range: 20–30 Hz, time interval: 200–280 ms, $t(36) = 2.96$, $p = 0.005$). (c) The topography of phase synchrony between 20 and 30 Hz. Synchrony between electrodes is indicated by lines, which are drawn only if the synchrony value is beyond a two-tailed probability of $p < 0.0005$. Differences between groups are displayed in the bottom row. Black lines indicate a decrease in synchrony in schizophrenia patients compared with controls. Green lines indicate increase in synchrony for patients with schizophrenia relative to controls. The decrease in phase synchrony between 200 and 300 ms indexes a deficit in the long-range synchronization during Gestalt perception in schizophrenia. Reproduced with permission from Uhlhaas PJ, Linden DEJ, Singer W, Haenschel C, Lindner M, Maurer K, and Rodriguez E (2006) Dysfunctional long-range coordination of neural activity during Gestalt perception in schizophrenia. *Journal of Neuroscience* 26: 8168–8175.

GABAergic inhibitory neurons and *N*-methyl-d-aspartic acid (NMDA) receptor dysregulation have both been found in patients with schizophrenia. The possible role

of NMDA receptors in the pathophysiology of schizophrenia is supported by the acute effects of NMDA antagonists, such as ketamine or phencyclidine (PCP),

on healthy volunteers. For example, subanesthetic doses of ketamine produce an acute psychosis that includes many of the symptoms and characteristic cognitive dysfunctions of schizophrenia. Because the typical and atypical neuroleptics interfere with dopaminergic and serotonergic neurotransmission, respectively, abnormalities in these transmitter systems are thought to play a central role in the pathophysiology of schizophrenia. How these systems modulate neural synchrony has not been investigated yet.

In Parkinson's disease, reduced dopamine leads to enhanced beta oscillations in the subthalamic nucleus (STN) and decreased gamma-band oscillations in cortical and subcortical networks. In schizophrenia, dopaminergic dysfunctions could affect neural synchronization through established effects on GABAergic interneurons.

So far, direct evidence for a link between high-frequency oscillations and neuromodulators is available only for the cholinergic system. Cortical networks can only engage in synchronized, high-frequency oscillations when muscarinic receptors are activated. Further studies need to clarify whether this finding can be related to the emerging evidence that deficits in cholinergic transmission may be involved in abnormal cortical information processing in schizophrenia.

In summary, there is consistent evidence that neural synchrony is impaired in patients with schizophrenia. This impairment is particularly pronounced for oscillatory activity in the beta- and gamma-frequency ranges and for the synchronization of these high-frequency oscillations over longer distances. Because synchronization of oscillatory activity in this frequency range is associated with cognitive functions that are disturbed in schizophrenia patients, it is conceivable that the relation between impaired synchrony and the symptomatology of schizophrenia is not merely correlative. Data on anatomical connectivity and neurotransmitter systems in schizophrenia suggest several potential causes for impaired neural synchrony, but more focused studies are required to distinguish between cause and effect. Synchronized oscillator activity plays a crucial role in guiding the development of neuronal networks. Therefore, abnormal connectivity could be both cause and effect of abnormal synchrony.

Neuronal Synchrony in Autism

Autism is a developmental brain disorder characterized by a triad of impairments that affect social interaction, verbal and nonverbal communication, and the repertoire of interests and activities. Similar to recent work in schizophrenia, theories that account for the pervasive cognitive dysfunctions associated with autism have highlighted a deficit in the integration of cognitive mechanisms. A number of studies have demonstrated superior performance in tasks requiring recognition of

details and directing attention to small elements as, for example, in visual search and in the identification of hidden figures. This reduced ability to integrate components into coherent representations is not only confined to visual perception but has also been found in the processing of auditory information, linguistic context, and social cues.

Current theories and experimental data converge on the notion that dysfunctional integrative mechanisms in autism may be the result of reduced neural synchronization. Recent functional magnetic resonance imaging (fMRI) and EEG studies have supported this view through demonstrating reduced connectivity during sentence comprehension in high-functioning individuals with autism. Compared with controls, subjects with autism are characterized by a marked reduction in functional connectivity throughout the cortical language system that was most pronounced during comprehension of sentences.

In analogy to the findings in schizophrenia patients, these data predict that autism should be associated with reduced neural synchrony. However, so far, only few studies have examined this possibility. Induced gamma-band activity was examined in EEG data in individuals with autism and in a matched control group during the perception of face stimuli. In controls, an increase in induced gamma power differentiated responses to face from no-face stimuli, while subjects with autism showed no difference between the two experimental conditions. Analysis of auditory steady-state responses indicates that, similar to patients with schizophrenia, there is a reduction in the power of the stimulus-locked responses in the gamma-band range.

Several authors have recently proposed that in autism cortical networks may be characterized by an imbalance between excitation and inhibition, which leads to hyperexcitability and unstable cortical networks. This hypothesis is consistent with abnormalities in GABAergic- and glutamatergic-transmitter systems. Indications for reduced GABAergic inhibition have been derived from the evidence that autism is associated with mutations of genes encoding subunits of the GABA(A) receptor, reduced expression of GAD 65 and GAD 67 and synthesis of abnormal isoforms of these enzymes. Abnormal glutamatergic neurotransmission is supported by polymorphisms in genes that encode both metabotropic and ionotropic glutamate receptors, and a postmortem study has reported reduced alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors in the cerebellum. In addition, the serotonergic system may be dysregulated in autism. To date, it is unclear how these abnormalities relate to the cognitive deficits in autism, whether they play a role in the hypothesized disruption of integrative processes and whether there are electrographic correlates of reduced large-scale synchronization in this disorder.

Neuronal Synchrony in Alzheimer's Disease

Alzheimer's disease (AD) is the most common form of dementia that affects approximately 11% of the world population older than 65 years. AD is associated with a wide range of cognitive dysfunctions that typically start with characteristic memory impairment, followed by deficits in visuo-spatial and executive processes. These differential impairments in cognitive domains reflect the spread of cortical pathology from medial-temporal to parietal association areas. Patients with AD show pronounced deficits while performing tasks that require interhemispheric transfer of information, executive processing, and episodic memory consistent with a disconnection syndrome.

A hallmark of the resting-state EEG in patients with AD is a relative increase in the theta- and delta-band activity that co-occurs with a reduction in activity in the alpha and beta band. The reduction in alpha-band activity correlates well with the severity of the disease and the cognitive deficits. These power changes in distinct frequency bands are associated with impaired synchrony. Patients with AD show reduced coherence of oscillations in the alpha- and beta-frequency band both for distant and nearby recording sites.

Supportive evidence comes from studies that have utilized direct measures of synchrony, such as synchronization likelihood (SL) that is sensitive to linear and nonlinear interdependencies between EEG channels. Topographically, the reduction of synchrony is particularly pronounced for long-range synchronization. In addition to lowered synchronization in the alpha and beta bands, patients with AD are also characterized by a reduction in gamma-band synchronization in the resting state. Thus, there is substantial evidence for reduced neural synchrony during the resting state, but relatively little research has been performed so far to link reductions in neural synchrony directly to impaired cognition by analyzing task- and performance-related changes of synchronization.

The hypothesis that impaired neural synchrony underlies some of the cognitive deficits in AD is compatible with data suggesting that the degenerative processes caused by AD lead to a neocortical disconnection syndrome. Neurofibrillary tangles (NFTs) and neuritic plaques (NPs) are particularly prominent in brain areas that give rise to long cortico-cortical tracts. Accordingly, DTI studies disclosed disintegration of white-matter fiber tracts. Furthermore, neural synchrony in the high-frequency range is expected to be reduced because AD leads to a pronounced degeneration of the cholinergic projections to the cerebral cortex that originate in the basal forebrain and have been shown to be a necessary prerequisite for the generation of beta- and gamma-band oscillations and response synchronization in this

frequency range. The evidence that muscarinic antagonists, such as scopolamine, induce a pattern of memory and cognitive deficits characteristic of elderly subjects and shift EEG power toward lower frequencies is compatible with this hypothesis.

Finally, there is evidence for alterations in glutamatergic neurotransmission that may also effect neuronal synchronization in AD. It has been demonstrated that NPs produce a persistent depression of NMDA-evoked currents in cortical neurons. Moreover, neurons from a genetically modified mouse model of AD expressed reduced amounts of NMDA receptors. These findings suggest that AD-related alterations of cellular functions can cause depression of NMDA-receptor-mediated synaptic transmission.

Taken together, these data suggest that the cognitive disturbances associated with AD may not solely be due to the loss of neurons but also to impairments in the temporal coordination of distributed neuronal activity. So far, studies have concentrated on neural synchrony in the lower frequency bands, especially in the alpha band, and more investigations are required to examine the expected deficits of long-range synchrony in the higher frequency bands.

Conclusion

The evidence reviewed suggests that schizophrenia, autism, and AD are characterized by changes in neural synchrony that are likely to play an important role in the pathophysiology of the disorders. There is consistent evidence across studies that these disorders are associated with a reduction of both local as well as long-range synchronization. In addition, the cognitive functions that are impaired have all been shown to be associated with neural synchronization, suggesting that abnormal synchrony could be one of the causes of the cognitive dysfunctions.

The impairments of neural synchrony in schizophrenia, autism, and AD are consistent with current theories that emphasize a disconnection syndrome as the underlying pathophysiological mechanism. Cognitive dysfunctions are thought to arise from a dysfunction in the coordination of distributed neural activity between and within functionally specialized regions of the cerebral cortex. Reduced neural synchronization can be a consequence of disconnection, but it can also be the cause of impaired coupling between brain areas because synchronization of neural responses is essential for their propagation across sparsely connected networks. At present, it is difficult to differentiate between these possibilities.

The pathophysiological role of neural synchrony in schizophrenia, autism, and AD is likely to be different

however. Both schizophrenia and autism are developmental disorders whose symptoms manifest early during development, whereas AD is a neurodegenerative disease that typically begins after >60 years of age. Thus, neural synchrony is likely to be causally involved in the early developmental alterations in autism and schizophrenia whereas in AD, changes in neural synchrony result from progressive neurodegeneration that are a core feature of the disease.

Evidence suggests that the cognitive deficits correlate particularly well with disturbances of long-range synchrony, in agreement with the hypothesis that higher cognitive functions require large-scale integration of distributed neural activity. In schizophrenia, AD, and autism, large-scale integration was found to be more impaired than was local synchronization, as reflected by the amplitude of local oscillatory activity and BOLD activation. Furthermore, cognitive dysfunctions were particularly pronounced for tasks requiring interactions between widely distributed brain areas, such as integration of polymodal stimulus attributes, dynamic perceptual grouping, working memory, and executive processes. This agrees with the proposals of several authors that complex cognitive processes, such as attention, memory, dynamic grouping, and awareness, require large-scale integration of activity.

Future Perspectives

The data reviewed here suggest that measures of neural synchronization may be of importance for the diagnosis of neuropsychiatric disorders. As measurements of neural synchrony are noninvasive and quantifiable in an objective way that is largely immune against observer bias, advanced methods of time series analysis may provide valuable diagnostic tools for the assessment of disease progression and the efficiency of therapeutic interventions. For example, aberrant large-scale integration of neural activity may also turn out to be a predictor of incipient AD. A recent study examined patients with mild cognitive impairment and showed that the covariance of BOLD responses during a face-matching task was a more sensitive measure for impaired functions than behavioral performance or the amplitude of regional brain activation, suggesting that reduced functional connectivity might represent one of the earliest markers of changes in brain functioning in AD. Prospective longitudinal studies of phase synchronization with EEG and preferably MEG methodology are required to examine whether changes of synchrony in the high-frequency bands can be used as an early predictor of AD.

Impaired neural synchrony may also guide further research into the pathophysiological mechanisms underlying neuropsychiatric disorders. For example, there is increasing interest in the role of GABAergic

neurotransmission in schizophrenia and autism. These efforts have already led to the investigation of therapeutic effects of GABAergic modulators in these disorders. We believe that further research into neurotransmitter systems and other mechanisms involved in the generation of oscillatory activity and its synchronization could ultimately help develop more precise pharmacological interventions for these disorders.

Neural synchrony is also of relevance for several other disorders not reviewed here such as epilepsy, Parkinson's disease, and most likely also multiple sclerosis. In the latter case, it is to be expected that axonal damage and demyelination interfere with the temporal coordination of neuronal activity. In particular, long-distance synchronization is likely to be impaired by prolongation of conduction times.

The possibility may also be considered to use measures of neural synchrony rather than just the power of EEG or MEG signals, in particular, frequency bands as biofeedback signals. Evidence indicates that biofeedback can be used to modify brain states in neuropsychiatric disorders. So far, the therapeutic effects of this approach have been variable, but it is conceivable that more advanced measures of the temporal coordination of distributed activity will be more effective in helping the patients to bring aberrant activity under voluntary control.

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See also: Brain Aging: Structural Imaging Biomarkers of Risk of Alzheimer's Disease; Brain Imaging; Conscious and the Unconscious; From Sensation to Perception; Schizophrenia.

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Short-Term Memory: Psychological and Neural Aspects

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Glossary

Capacity – The limit on the amount of information or the number of items that may be processed, encoded, or maintained in working memory simultaneously.

Chunking – The process of grouping items in working memory according to presented or discovered relationships, resulting in a more efficient representation and the ability to remember more items.

Interference – Any irrelevant information that overwrites or degrades the relevant information being maintained in short-term memory, or reduces the effectiveness of the relevant information in driving behavioral response.

Memory load – The number of items or amount of information currently being maintained in short-term memory.

Recoding – The process of changing information that is to be remembered into a form which is easier to hold in memory. Chunking is one type of recoding, but there are many other ways that information can be recoded, such as using verbal descriptions or remembering only certain features of an image.

Rehearsal – The process of actively and repeatedly thinking about the information in short-term memory in order to protect it from decay and interference. The most common example is silently saying a list of words over and over during the memory period, but other kinds of information can be rehearsed in other ways, such as repeatedly imagining making a series of eye movements to the remembered locations.

Reverberating circuit – Reciprocally connected neurons that form a loop providing positive feedback, which enables their neural signals to maintain their level of activity without additional input from sensory or other sources.

Sustained activity – Neural activity containing information about an item held in short-term memory that persists for several seconds after the item to be remembered is no longer available in the sensory input.

active rehearsal and sustained attention, but will eventually succumb to decay and interference. Short-term memory is often studied with either change detection or delayed recognition tasks. In a change detection task, a display with multiple items is presented briefly and, then a short time later, another display is presented in which one or more of the items may have changed according to one of its features, such as its color. The research participant's task is to indicate whether anything in the display changed from the first to the second presentation. To succeed in the task, short-term memory must maintain a representation of the first display during the delay until the second display is presented. Delayed recognition is a similar task, but the test after the memory delay is usually a single item and the participant is to indicate whether or not this test item matches one of the items in the preceding display. The memory delays in these tasks usually vary from 1 to 20 s.

The concept of short-term memory lies between those of iconic memory and working memory. Iconic memory is an automatic, very brief (less than 1 s) memory for a sensory stimulus, presumably due to persistence of the neural activity arising from that stimulus. Working memory is a combination of short-term memory and the goal-driven manipulation and use of the information maintained in short-term memory. The manipulation and use of the remembered information is often considered to be a process, referred to as 'the central executive' or 'cognitive control,' that is psychologically and neurally distinct from the storage and maintenance of the information. However, even without explicit manipulation and use of the information, maintenance in short-term memory requires executive-like control processes, such as selecting the most important information to be maintained and protecting it from interference. Thus, the distinction between short-term memory and working memory is often blurred.

Behavioral Properties

Capacity

Unlike long-term memory, which given sufficient conditions seems to have unlimited capacity, short-term memory can hold a relatively very small amount of information at one time. This is often looked upon as a problem to be overcome. However, the limited capacity of short-term memory is actually beneficial to the individual. The information maintained in short-term memory

Definition of Short-Term Memory

Short-term memory involves the maintenance of a limited amount of currently relevant information. This information may be maintained indefinitely through

influences sensory attention and goal-directed behavior. Without this influence, sensory processing is driven by stimulus salience (the loudest or brightest), while behavior is driven by habit. Flexible, goal-driven behavior depends on the ability of short-term memory to select information for the task at hand and to allow that information to have the greatest influence on perception and action. Thus, a limited capacity imposes priority so that only the most important information is maintained, and therefore only the most important information drives perception and action.

While it is universally agreed that short-term memory is capacity limited (only a small amount of information may be maintained at any one time), there is much disagreement regarding what the capacity is and the units by which capacity should be measured. The amount of information that may be maintained in short-term memory ultimately depends on several factors, and therefore the apparent capacity can vary greatly, according to the parameters of the task used to measure it. As the definitions of short-term memory and capacity have evolved, the ways of measuring capacity have changed. Answers to the question "How much can be stored in short-term memory?" depend on (1) how the task parameters influence the constraints, (2) how much information can be encoded into short-term memory, and (3) how much information can subsequently be maintained.

Number of items

Estimates of the number of items that may be stored in working memory have ranged from 1 to 7, depending on the type of information (such as auditorily presented words vs. visually presented objects) and the demands of the task. Researchers who use tasks that involve very brief visual displays to present the information to be remembered almost always conclude that short-term memory in healthy adults is approximately three or four objects. When people are given ample time to encode the items, however, estimates of capacity increase. This increase has been attributed to peoples' ability to recode information into a different form that may be easier to remember and to rehearse. People may use inherent relational structure in the presented information, or draw on long-term memory knowledge to recognize or impose structure on the information. This process has been called 'chunking.' For example, the string of numbers 945826734 can be remembered more accurately if one groups these numbers as $(9-4=5)$, $(8-2=6)$, and $(7-3=4)$. Nine items become three chunks. If these chunks can be considered as items, then the capacity of short-term memory again appears to be approximately 3.

Amount of information

While short-term memory capacity appears to be limited to approximately three items (or chunks), there has been

debate over whether the information complexity of those items further limits capacity. In other words, can we remember three simple items but only two complex items? If so, then short-term memory capacity might better be measured in terms of amount of information, rather than number of items, in that the total amount of information to be remembered would be the amount of information per item times the number of items. Again, the answer seems to depend on the nature of the task and the type of information that must be maintained. If the item is complex by way of having multiple low-level visual features (such as color and orientation of an object) that can be quickly and easily bound together, then it does not seem to matter if the items contain a single feature or multiple features. If items are complex in ways other than the combination of low-level features, such as tasks that require memory for abstract shapes, then the increasing complexity of the individual items does decrease the number of items that can be maintained in short-term memory. These constraints on capacity come into play primarily in tasks that involve memory for briefly presented visual material.

Maintenance, Decay and Interference

When the task allows for longer encoding time and performance is more dependent on sustained attention and rehearsal of the information in order to maintain it in memory for several seconds, a different set of factors affects how many items can be maintained. It is these constraints that have led to estimates of approximately seven items for short-term memory or working memory capacity. Most of the research on this aspect of short-term memory has used verbal information, usually lists of words. The number of words that can be maintained in short-term memory depends on, among other factors, the word lengths and how similar they are to each other.

Theories on the word length effect generally posit that there is a fixed amount of time that a given item can be maintained in short-term memory unless its representation is refreshed through rehearsal. In the case of verbal information, it is thought that this rehearsal is achieved through covert speech (i.e., saying the list of words silently, one at a time, and repeating the list as long as required by the task). Thus, the longer it takes to say each word in the list, the longer will be the time to say the entire list before you get back to repeating a word. If too much time elapses between repetitions, the representation of that word would have decayed while you were saying the rest of the list. Researchers have debated as to whether time alone is sufficient to cause decay or if it is only when other information interferes with the representation of the remembered information that decay occurs. However, since other information is always present and the more time passes, the more interfering

information accumulates, it is difficult to behaviorally isolate the effects of the passage of time from that of interference from other information.

Interference comes from multiple sources. The interference that usually is confounded with the delay effect is the presence of completely irrelevant information during the memory delay that can cause distraction from and interruption of the rehearsal process. Sometimes, this information is purposefully introduced by the experimenter to study its effect, but distracting information is present even when not explicitly introduced, such as the presence of an itch during performance of a visual memory task. Thus, it may be more useful to think of the amount of known sources of distracting information rather than the presence or absence of distraction from interfering information.

The item similarity effect is another type of interference. It is more difficult to remember the letter string BDCV than XCKH, because the sounds we use to name B, D, C, and V all have the same ‘ee’ end sound. This effect is most consistent regarding phonological similarity of items in verbal memory. Semantic or visual similarity can sometimes increase short-term memory performance, perhaps because of facilitation of chunking or other organizational strategies. Representations of items in short-term memory are flexible and appear to depend on the representations of other items simultaneously held in memory and the expectations for the upcoming test items. At least regarding phonological rehearsal of verbal information, however, the more overlap there is in the long-term memory representation of the items to be held in short-term memory, the more difficult it is to keep them distinct from one another. Likewise, the more similar a nonmatching test item is to the remembered items, the more difficult it will be to reject that item as a potential match. Similarity between items necessitates a more finely tuned representation of the items to be remembered.

‘Proactive interference’ is a term used to refer to the detriment to performance on the current memory test caused by information that was recently held in short-term memory but is now no longer relevant. For example, you initially try to remember and are tested on a list of five words: apple, banana, pear, cherry, and lemon. Now you are given a new list of words: mango, orange, plum, strawberry, and grape. After a few seconds I give you a test that asks “Was lemon a word on the most recent list?” You will be slower and less accurate in responding “No, it was not on the list,” than if you had not just recently been trying to remember lemon as part of the previous list. Previous experience with an item will facilitate processing of that item when it is presented at test for the new list, resulting in a sense of familiarity and thus a tendency to give a match response rather than the correct nonmatch response. If that particular item was not only on the

previous memory list, but was also a previous test item that required a match response, this previous stimulus-response association will add to the difficulty in now responding nonmatch to the same item.

Neural Basis

Sustained Activity

Long-term memory is instantiated by structural changes in the brain that change how information is processed. Short-term memory, on the other hand, is instantiated by spatiotemporal patterns of neural activity, as is perception of current stimulus information. The key property of neural activity that enables short-term memory is the ability to maintain sustained patterns of activity in the absence of any current, related stimulus input. Sustained activity during the delay period of short-term memory tasks has been observed through single cell recordings in primates and other animals and through functional magnetic resonance imaging (fMRI) and other neuroimaging techniques in humans. Such sustained activity is present in many different brain areas. Different brain areas and different populations of cells within those brain areas demonstrate sustained activity that differs in its robustness to interference and its selectivity for different types of information required for the task. These differences suggest different roles for these brain areas and cell populations for the different components of short-term memory tasks, such as encoding the items, remembering those particular items, remembering the task rules, and preparing to process and respond to an expected test stimulus.

Prefrontal Cortex

The prefrontal cortex is defined as all of the cortex that lies anterior to the premotor cortex in the precentral sulcus. Many of the cells in the prefrontal cortex demonstrate sustained activity during the delay period of short-term memory tasks. Unlike sustained activity observed in perceptual processing areas, in prefrontal cortex it is generally resistant to interference from irrelevant perceptual stimuli and correlates strongly with performance on the task.

Functional organization of prefrontal cortex

The prefrontal cortex contains multiple functionally dissociable subareas. The boundaries between these areas, however, are less distinct in terms of the differences in cellular anatomy across areas and the functional distinctions among these areas have been the subject of much debate. The clearest distinction in humans is between medial and orbital frontal areas versus the lateral prefrontal cortex. Medial and orbital frontal areas appear to be

more involved in the reward, motivation, and social aspects of short-term memory tasks, whereas the lateral frontal areas are more involved in memory for the specific item and rule information for the particular task at hand.

The lateral prefrontal cortex contains further subdivisions that appear to process and maintain different types of information. Within the posterior part of lateral prefrontal cortex, the dorsal areas seem to be more involved in spatial information, such as the posterior part of the superior frontal sulcus in humans playing a role in short-term memory for spatial locations. The ventral areas of posterior lateral prefrontal cortex, on the other hand, appear to be more involved in remembering nonspatial information, such as verbal information and many stimulus aspects related to object identity. The types of information represented by sustained activity in more anterior parts of prefrontal cortex are less well understood, but appear to involve memory for task rules, relationships among items to be remembered, and relationships between items and anticipated responses. It is thought that the patterns of activity in all of these regions interact, perhaps in a hierarchically organized network. Thus, sustained activity representing short-term memory for task rules influences which particular objects or locations will be held in short-term memory, and ultimately these remembered items provide specific feedback to other brain areas that influence perception and behavior.

Interactions between prefrontal cortex and other brain areas

While the prefrontal cortex plays a vital role in short-term memory behavior, it can do so only by interacting with other brain areas. Short-term memory is not a process or a storage area localized to any particular brain area. It is an ability of the animal that arises from the properties of the entire neural system. Sustained activity is thought to be made possible through reverberating circuits in which two populations of cells reinforce the activation patterns in each other through rapid, cyclical interactions. These circuits may exist for two populations of cells within an area of the prefrontal cortex, across separate areas within the prefrontal cortex, or they may exist for interactions between a cell population in the prefrontal cortex and one in a different part of the brain.

Basal ganglia

The neural mechanisms of short-term memory must be able to sustain patterns of activity related to the items currently held in short-term memory and protect them from all sources of interference for as long as those items are important for the current task. At the same time, these mechanisms must be able to very quickly change those patterns of activity to represent new items when the new information becomes more important than the previously remembered information. This balance

between maintenance and updating appears to be controlled in part by interactions between the prefrontal cortex and the basal ganglia, a group of subcortical structures that appear to be also involved in changing current patterns of neural activity governing motor behavior.

Motor and premotor cortex

Early studies of sustained activity in frontal cortex used delayed response tasks in which an animal made an eye movement toward a remembered location after a delay. This task confounds neural activity related to short-term memory for the spatial location of a previously viewed stimulus and neural activity related to anticipating a future motor response directed toward that location. Recent research, however, has demonstrated that both pieces of information are reflected in sustained neural activity and the patterns relating to each are dissociable, with separate neural populations and different time courses. Recordings from cells in prefrontal and premotor cortices suggest that interactions between prefrontal cortex and premotor cortex transform the pattern of sustained activity from a representation of the stimulus-to-be-remembered to the anticipated motor response. Of course, this can happen only when the task demands are such that a particular anticipated motor response can be deduced from the nature of the remembered stimulus and the task rules.

Perceptual cortical areas

Similar interactions are thought to occur between prefrontal cortex and perceptual cortical areas. In this case, the prefrontal cortex appears to enhance the activity or sensitivity of cells in perceptual areas that are selective for the anticipated stimulus that would be a match-to-the-remembered information. This interaction results in a greater neural activation in both prefrontal and perceptual areas for a test stimulus that matches the remembered stimulus than for a test stimulus that does not match.

Relationship to Attention

This interaction between prefrontal cortex and perceptual areas in order to maintain stimulus-specific information in short-term memory and influence processing of anticipated matching stimuli is one way in which short-term memory and attention interact. Perceptual selective attention is the ability to enhance the neural activity of one stimulus, spatial location, or stimulus feature over others. Short-term memory and perceptual selective attention interact when the information being attended is selected based on remembered task rules and short-term memory for the particular stimulus item or feature information to which those rules apply. For example, a classic task used to test selective attention is the visual search task in which the participant is instructed to

respond according to whether a particular item is present in a scene or within an array of multiple other items. The target of the search must be maintained in short-term memory. Attention and eye movements are drawn earlier and more often to items in the array that are most similar to the remembered target item. The contents of working memory can also influence the locations or objects that are selectively attended even when the contents of working memory are irrelevant for the perceptual selective attention task.

The neural mechanisms of perceptual selective attention may apply more generally to enhance the neural activity related to one item held in short-term memory over the neural activity of another remembered item. Thus, both items continue to be maintained in memory, but one is prioritized for more immediate manipulation, response, or comparison to anticipated stimuli. Thus, there appear to be two levels of prioritization. First, there is the limited capacity of short-term memory which dictates that only the top few pieces of information are actively maintained. Second, only the most important of these maintained pieces of information will have an immediate impact on perceptual selective attention and behavioral choice.

Neural Basis Helps Explain Behavioral Properties

As with all psychological abilities and behaviors, what is observed during measurements of short-term memory is the result of the entire, interactive, and interdependent neural system. Constraints on behavior may arise from the properties of any one or a combination of brain areas that comprise the neural system responsible for that behavior. Understanding how each cognitive component of a task depends differentially on various levels or aspects of the neural system makes the complex properties of short-term memory behavior more tractable.

Population Coding and Capacity

There are three components of most short-term memory tasks: (1) encoding of the material to be remembered, (2) maintenance of that information, and (3) comparison of the remembered information to the information presented during the test. Each of these stages can have its own constraints and properties. All three stages require representation of information, which must be accomplished by the spatiotemporal patterns of activity in populations of neurons. The information representation capacity of a population of neurons depends on the number of patterns of activity that sufficiently represents the relevant properties of the items in such a way that each can be distinguished from the other items when all are

simultaneously present in short-term memory. In other words, if the activity of a population of neurons is representing one item and then the activity representing a second item is superimposed on the activity of the first, can the system decode the independent properties of both items? The more the activity patterns of different items overlap and interfere with one another, the fewer items will be accurately represented. If each stage of processing for a task depends on a different population of neurons (potentially in different parts of the brain), then it is possible for each stage to have a different information representation capacity. The nature of the task will determine which of these stages has the dominant effect on behavioral measures of total short-term memory capacity. Whichever stage has the smallest capacity will set the upper limit for the system.

Visual Attention and the Processing Bottleneck

In tasks where the information to be encoded and remembered is presented very briefly, the capacity of sensory processing and perceptual selective attention serves as a bottleneck for the rest of the short-term memory system. It appears that the limit for this perceptual encoding into short-term memory is approximately three items, where all of the task-relevant features of those items must be capable of being bound together to form an integrated representation of each item at the earliest stages of sensory processing. When items can be encoded into short-term memory serially rather than simultaneously, with sufficient time for detailed encoding of each item, then the capacity of the system will be governed more by the representational capacity of the sustained activity in the reverberating circuits of prefrontal cortex.

Reverberating Circuits Are Susceptible to Decay and Interference

The neural populations of the prefrontal cortex may have a representational capacity that is different from that of the perceptual processing and attention neural populations. In addition, the effective capacity of short-term memory representations in the prefrontal cortex is influenced by the ability of these cortical regions to reorganize and transform the information into more efficient and effective representations. This transformation is primarily achieved through the use of relationships among the items to be remembered or through their relationship to items stored in long-term memory.

The capacity of short-term memory representations in the reverberating circuits of prefrontal cortex is dependent on the ability of these circuits to continue refreshing the representations without degradation. If the activity loses a little bit of signal and/or gains a little bit of noise each time it goes through this cycle, the information will

eventually decay to the point where it will no longer be able to support successful memory task performance. Signal transmission between the two cell populations in the circuit will naturally be imperfect, particularly for circuits that depend on long-range white matter pathways, such as between prefrontal cortex and perceptual areas. Noise can contaminate these representations from multiple sources, including interactions between multiple item representations in short-term memory and input from irrelevant sensory stimuli during the memory delay.

Dependence of Short-Term Memory on Long-Term Memory

The behavioral effects of short-term memory maintenance are most apparent when responses dictated by the contents of short-term memory conflict with those made prepotent from previous experience. Short-term memory enables flexible behaviors that are optimal for the current goals and context. The feedback signals provided by sustained patterns of neural activity, however, are acting on a system whose neural structure has been shaped by a lifetime of experience. These neural structures are influenced by many factors, including both long-term habitual stimulus-response associations and priming of circuits from recent experience. These structural changes provide their own bias that affects the individual's response to a given test stimulus. The biasing of behavioral and perceptual selection by short-term memory neural activity patterns must be able to override these structural influences on perceptual processing and responses. This constraint on the influence of short-term memory on behavior helps explain some of the apparent interference effects in short-term memory, including proactive interference and observations that short-term memory capacity for familiar stimuli is greater than for unfamiliar stimuli.

Summary

Short-term memory is an ability of the mind that is both enabled and constrained by the function and structure of neural circuits and systems. Prefrontal cortex plays a vital role in establishing the sustained patterns of neural activity that represent information in short-term memory, and the functional organization of prefrontal cortex provides

valuable information regarding the nature of those representations. However, short-term memory is not localized to the prefrontal cortex. Many of the psychological aspects of short-term memory, including capacity limits, effects of interference, and its relationship to attention and long-term memory, can be best understood by considering the properties of the multiple, interacting components of these circuits and systems.

See also: Attention and Speed of Information Processing; Brain Imaging; Conscious and the Unconscious; Declarative Memory; Emotion–Cognition Interactions; Episodic and Autobiographical Memory: Psychological and Neural Aspects; Evolutionary and Developmental Issues in Cognitive Neuroscience; From Sensation to Perception; Neural Basis of Working Memory; Vision.

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Stress and Brain Morphology

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Glossary

Adrenocorticotrophic hormone (ACTH) – A peptide hormone released from the anterior pituitary gland that mediates the production and secretion of hormones of the adrenal cortex.

Allostasis – The process of maintaining homeostasis through active means, such as the secretion of stress-related hormones in response to a stressor.

Allostatic overload – The wear and tear on the brain and body produced by chronic engagement of allostatic mediators, such as stress-related hormones.

Corticosterone – A steroid hormone released by the adrenal cortex of rodents that plays a major role in homeostatic/allostasic processes and acts throughout the body, including the brain.

Corticotropin-releasing hormone (CRH) – A peptide hormone that participates in the release of stress-related hormones, such as ACTH. This hormone is also referred to as ‘corticotropin-releasing factor (CRF).’

Cortisol – A steroid hormone released by the adrenal cortex of primates that plays a major role in homeostatic/allostasic processes and acts throughout the body, including the brain.

Dendrite – Branching neurites that emanate from the neuronal cell body.

Glucocorticoid receptor (GR) – A low-affinity steroid hormone receptor for the glucocorticoids, such as corticosterone or cortisol.

Hypothalamic–pituitary–adrenal axis (HPA axis) – The major neuroendocrine axis that mediates the hormonal stress response.

Mineralocorticoid receptor (MR) – A high-affinity steroid hormone receptor for the glucocorticoids, such as corticosterone or cortisol.

Paraventricular nucleus of the hypothalamus (PVN) – A nucleus in the hypothalamus that contains CRH neurosecretory cells that regulate the release of stress-related hormones.

with the immediate internal and external demands imposed by the stressful event. This response attempts to restore homeostasis, a process termed ‘allostasis.’ However, prolonged or more chronic exposures to stress and stress-related hormones can lead to allostatic overload, resulting in a number of negative effects, particularly in regard to neurobiological and behavioral function. This article highlights the effects of chronic stress on the structure and function of the hippocampus, prefrontal cortex (PFC), and amygdala, brain areas vitally important in various cognitive abilities, and emotional reactivity.

Stress and the Hypothalamic–Pituitary–Adrenal (HPA) Axis

The hypothalamic–pituitary–adrenal (HPA) axis is the major neuroendocrine axis that controls the hormonal stress response. In response to a stressor, neurosecretory cells in the paraventricular nucleus of the hypothalamus (PVN) release corticotropin-releasing hormone (CRH) into the hypophyseal portal system, which in turn causes the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland. ACTH then induces the secretion of the glucocorticoids, such as cortisol in primates and corticosterone in many rodent species, from the cortex of the adrenal gland. The hormones secreted by the HPA axis control their own release through a negative-feedback loop, specifically the glucocorticoids feedback on the PVN and pituitary to inhibit the release of CRH and ACTH, respectively ([Figure 1](#)).

In addition to the hypothalamus and pituitary, the glucocorticoids also regulate their secretion by acting on the hippocampus, PFC, and amygdala. Through projections to the PVN, the hippocampus and PFC largely contribute to the glucocorticoid-dependent negative feedback on the HPA axis. Conversely, the amygdala can activate the PVN, initiating the hormonal cascade that leads to glucocorticoid release. It is important to note that the balance between each part of this neural–pituitary network that regulates HPA function is dependent upon many modulatory factors, such as age, sex, time of day, and experience.

The mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) mediate the actions of the glucocorticoids. The MR has a high affinity for the glucocorticoids and thus is typically saturated at basal

Introduction

Stress induces a host of physiological and behavioral responses. The hormones released in response to stress are essential for survival, as they allow an animal to cope

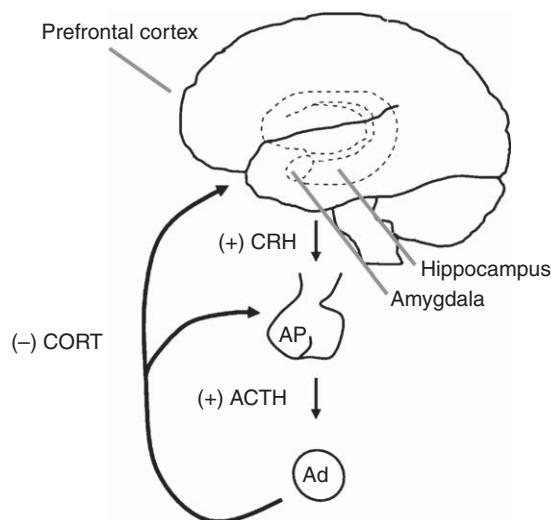


Figure 1 Schematic of the hypothalamic–pituitary–adrenal (HPA) axis and the hormonal cascade induced by stress and the relative anatomical position of the prefrontal cortex (PFC), amygdala, and hippocampus in the human brain. The dotted lines represent the approximate anatomical location of the amygdala and hippocampus within the brain. Adrenocorticotropic hormone (ACTH); adrenal gland (Ad); anterior pituitary (AP); corticosterone or cortisol (CORT); corticotropin-releasing hormone (CRH); positive drive (+); negative feedback (-).

glucocorticoid levels. Conversely, the low-affinity GR is primarily occupied only when elevated concentrations of glucocorticoids are present, such as in response to stress. In the brain, these receptors are found in the highest concentrations in areas associated with HPA regulation and cognitive and emotional functions. These areas, such as the hippocampus, PFC, and amygdala (Figure 1), also show substantial stress-induced structural remodeling, which is the focus of the next section.

Stress and the Brain

Chronic exposure to stress and stress-related hormones has been shown to affect the gross morphology and size of the brain. For instance, it has been reported that humans exposed to recurring physical and mental abuse demonstrate significant cortical thinning, while animals treated neonatally with high doses of corticosterone show reduced brain weight. Though the brain is clearly sensitive to the glucocorticoids, some regions of the brain appear to be more sensitive to the effects of stress than others, that is, stress does not necessarily affect the structure of the brain uniformly. In fact, different regions of the brain can show diametrically opposed responses to prolonged exposure to stress, with some areas showing stress-induced atrophy, while others hypertrophy.

Hippocampus

The effects of stress on the structure and function of the hippocampal formation have been well studied. The hippocampal formation includes both the hippocampus and dentate gyrus, areas critically important in spatial and episodic learning and memory. The hippocampus contains a prominent layer of pyramidal cells called ‘Ammon’s horn’ (*cornu ammonis* or CA), which is usually subdivided into CA1, CA2, and CA3, while the dentate gyrus is composed of granule cells. The dentate gyrus is also one of the two major neurogenic zones in the brain and is capable of producing new neurons throughout an organism’s lifespan. The hippocampal formation expresses very high levels of both MR and GR, and thus is exquisitely sensitive to the effects of the glucocorticoids.

In humans, magnetic resonance imaging (MRI) studies have demonstrated that individuals reporting high levels of perceived chronic stress show reduced hippocampal volumes. A decrease in hippocampal volume is also observed in patients with Cushing’s syndrome, an endocrine disorder marked by high cortisol secretion (e.g., hypercortisolism). Interestingly, hippocampal volumes return to normal after successful treatment of Cushing’s syndrome that reduces the abnormally high cortisol levels. Together, these data suggest that prolonged exposure to the glucocorticoids can lead to hippocampal atrophy in humans.

Not only have animal studies produced similar results, but they have also been able to delve deeper into the regional specificity of the effects of stress on the structure of the hippocampus and the cellular mechanisms that may mediate stress-induced hippocampal atrophy. For instance, in adult male rats, chronic restraint (6 h of restraint stress per day for 3 weeks) or social stress significantly reduce branching of the dendrites of the CA3 pyramidal cells (Figure 2), and to a lesser extent the CA1 pyramidal cells and granule cells of the dentate gyrus. This stress-induced remodeling of the hippocampus is dependent on corticosterone as administration of cyano-ketone, a corticosterone synthesis inhibitor, blocks the stress-induced atrophy of the hippocampal dendrites, while chronic injections of corticosterone mimic the stress-induced atrophy. Activation of the N-methyl-D-aspartic acid (NMDA) receptor also plays a role in these effects of stress, as NMDA receptor antagonists block stress- and corticosterone-induced dendritic atrophy.

It is important to note that these effects of stress on hippocampal morphology are reversible. Specifically, if animals are allowed to recover from chronic physical and/or social stress, then neuronal branching patterns can return to prestress levels within 10 days (Figure 2). Thus, similar to the human imaging studies with

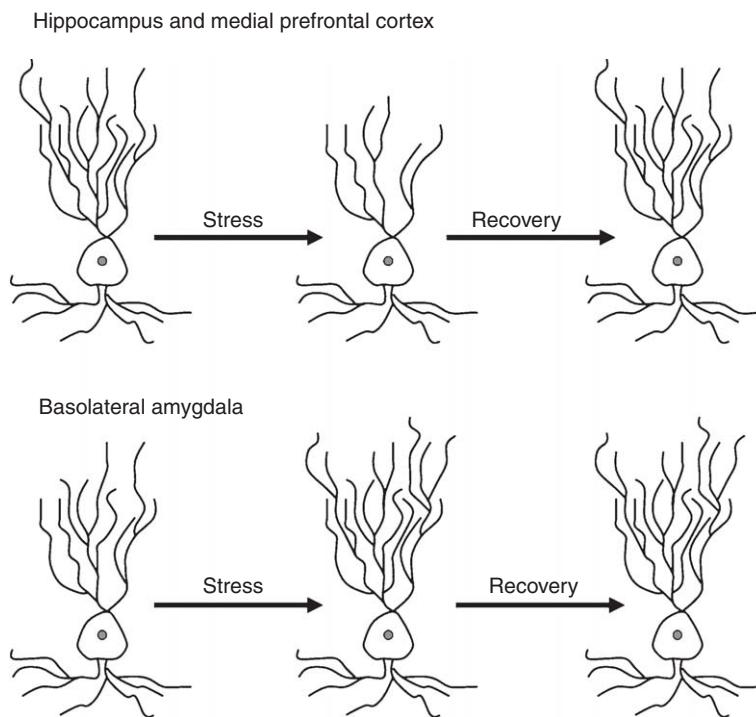


Figure 2 Diagrams of dendritic remodeling in the hippocampus, medial prefrontal cortex (mPFC), and basolateral nucleus of the amygdala in adult male rats. Note that the dendritic atrophy of the hippocampus and mPFC is reversible, while the basolateral amygdala dendritic hypertrophy is longer lasting, and perhaps permanent.

Cushing's patients, it appears that once glucocorticoid levels are reduced, hippocampal atrophy can be reversed.

As mentioned above, the hippocampal formation is integral in many types of learning and memory. In both human and animal studies, chronic stress is associated with impairment of spatial and episodic memory. Interestingly, studies have shown that if stress-induced CA3 dendritic atrophy is blocked pharmacologically, chronic stress does not result in deficits in spatial memory. For example, chronically stressed animals pretreated with tianeptine, an antidepressant that blocks stress-induced hippocampal atrophy, show spatial abilities similar to nonstressed controls. These data suggest that dendritic remodeling of hippocampal pyramidal cells may mediate the decrease in learning and memory observed during extended periods of stress.

It should be noted that chronic stress has also been shown to inhibit neurogenesis in the dentate gyrus of adult animals. Similar to the morphological changes, this suppressive effect of stress on neurogenesis appears to be reversible. Although methods are in development, it is presently difficult to measure hippocampal neurogenesis in living humans. It is therefore not possible to follow men and women in a longitudinal fashion to establish the role of, or recovery from, chronic stress on neurogenesis. Regardless of the unknown relationship between stress and human neurogenesis, it is possible that the stress-induced reduction in hippocampal volume observed in

both human and animal studies is due, at least in part, to the decreases in the birth of new neurons in this brain area.

Prefrontal Cortex

The PFC is essential in the regulation of emotional behaviors, executive function, and fear extinction. Though reports on the effects of chronic stress on the adult PFC are more limited than those which exist for the hippocampus, an emerging literature suggests that the PFC, especially the medial portion of the PFC (e.g., anterior cingulate and prelimbic cortices), is very sensitive to stress-induced neuronal remodeling.

Neuroimaging studies on the volume of the PFC in humans reporting chronic stress are lacking. However, human studies have shown reduced PFC volumes in depressed individuals. As depression can be associated with elevated cortisol levels, these data suggest that the human PFC may demonstrate glucocorticoid-dependent atrophy. Again, animal studies have greatly contributed to our understanding of stress-induced structural remodeling in the PFC. Recent experiments have shown that chronic restraint stress (6 h of restraint stress per day for 3 weeks) or even shorter periods of restraint stress (1 week), lead to significant reductions in dendritic branching of medial prefrontal cortex (mPFC) neurons (**Figure 2**). As chronic exposure to corticosterone mimics

the effects of chronic stress, it appears that stress-induced release of the glucocorticoids is involved in the mechanism(s) that lead to these morphological changes in the mPFC. Analogous to the hippocampus, the remodeling of the mPFC in response to chronic stress is reversible, such that animals allowed to recover for 3 weeks after exposure to stress show a dendritic morphology similar to unstressed controls (**Figure 2**).

In addition to these morphological changes, behaviors mediated by the PFC are also compromised in response to chronic stress. For example, tasks that require an animal to shift its attention, which is dependent on an intact mPFC, are greatly impaired in chronically stressed rats that demonstrate dendritic atrophy in the mPFC. It is presently unknown whether these changes in behavior show similar reversibility as the stress-induced structural remodeling of the mPFC.

Though more research is clearly needed, a growing body of literature suggests that the mPFC and hippocampus are similarly affected by chronic stress in the context of dendritic atrophy and its reversibility. As mentioned earlier, however, the brain is not uniformly affected by stress. Next, we turn to the amygdala, a brain region that shows a very disparate pattern of stress-induced morphological plasticity compared to the mPFC and hippocampus.

Amygdala

The amygdala is composed of many subregions, including the medial, central, and basolateral nuclei, all of which have relatively high GR (but lower MR) expression levels in the rat. Generally, the amygdala plays an important role in emotional memory and fear conditioning. Among other projections, the medial nucleus projects to hypothalamic nuclei relevant to reproductive behavior, the central nucleus (via the bed nucleus of the stria terminalis) to the PVN to mediate HPA activity, and the basolateral to the caudate, accumbens, and cortex to modulate the encoding of emotionally arousing events. Similar to the mPFC, a nascent literature has begun to emerge regarding the structural remodeling of the amygdala in response to long-term stress exposure.

In humans, overall amygdalar volume is reduced in individuals receiving chronic corticosteroid treatment for asthma or rheumatic illnesses compared to unmedicated controls. It has also been reported that people suffering from major depressive disorder have smaller amygdalar volumes than nondepressed individuals. Animal studies, however, indicate that exposure to chronic stress (2 h of immobilization stress per day for 10 days) can lead to increases in neuronal branching, specifically within the basolateral nucleus (**Figure 2**). It is unclear why the human studies report decreases in amygdala volume in individuals experiencing a chronic stress condition or

long-term corticosteroid therapy, whereas animal studies report stress-induced increases in neuronal elaboration. However, as the amygdala is such a complex and heterogeneous structure, it is possible that the human neuroimaging data, which quantify the volume of the entire amygdala, are missing morphological changes in the discrete subnuclei of the amygdala.

Animal studies have shown that behaviors known to be mediated, at least in part, by the amygdala are affected by chronic stress. For example, anxiety-like behaviors are augmented after prolonged exposure to stress, and fear conditioning has been reported to increase following glucocorticoid treatment. Unlike the reversibility of the effects of chronic stress on the dendritic structure of hippocampal and mPFC neurons, chronic stress-induced changes in the amygdala appear to be long lasting. That is, the dendritic hypertrophy in the basolateral amygdala and increases in anxiety-like behaviors remain even after 3 weeks of recovery from chronic stress (**Figure 2**). Although future studies will need to determine whether these stress-induced changes in the structure and function of the amygdala are permanent, it is clear that the neurons within the basolateral nucleus of the amygdala respond very differently to stress than the pyramidal cells of both the hippocampus and PFC.

Stress-Induced Neuronal Remodeling and Psychological and Physiological Dysfunction

Numerous studies have reported that exposure to chronic stress is correlated with the development and exacerbation of various psychological and physiological dysfunctions. It is presently uncertain how stress leads to these dysfunctions. However, stress-induced neuronal remodeling in the hippocampus, PFC, and/or amygdala may be a mechanism through which stress affects these aspects of mental and physical health. Though stress has profound influences on myriad psychological disorders and physiological functions, this section focuses specifically on the role that stress-induced neuronal remodeling in the hippocampal formation may play in major depressive disorder and HPA dysregulation.

Major Depressive Disorder

In addition to severe changes in mood and anhedonia, major depressive disorder is typically marked by a hyperactive HPA axis. For instance, individuals suffering from depression have higher concentrations of CRH in their cerebrospinal fluid, increased production of CRH in the PVN, and higher levels of circulating cortisol, compared to healthy controls. As alluded to in the section above (see sections ‘Prefrontal cortex’ and ‘Amygdala’), major

depression is also associated with changes in the structure and function of key brain regions involved in emotionality, which may be related to this HPA hyperfunction (see section ‘HPA dysregulation’).

Numerous empirical studies and meta-analyses have found a significant decrease in hippocampal, PFC, and/or amygdala volume in depressed men and women. Prospective studies are lacking, and thus, it is difficult to establish whether the depressive episode(s) result in these structural changes in the brain, or whether differences in brain structure lead to depression. Interestingly, upon successful treatment of major depressive disorder some, but not all, studies have found a reversal of these volumetric reductions. As successful treatment of depression is often associated with a normalization of HPA function, these data suggest an important interactive relationship between HPA reactivity, structural changes in brain, and major depression.

Along with structural changes, major depression is also associated with functional alterations in the brain. Depending on both the stimulus used and brain region examined, fMRI studies examining subjects with major depression have reported altered brain activity in hippocampus, PFC, and/or amygdala compared to non-depressed subjects. For example, depressed subjects presented with pictures of sad facial expressions or pictures that evoke negative emotions show higher activity in the amygdala and mPFC compared to control subjects. Again, whether these functional differences exist prior to depression, or conversely, are the result of depression, is uncertain.

HPA Dysregulation

The hippocampal formation is not only a target of the glucocorticoids, but is also intimately involved in modulating the output of the HPA axis (see section ‘Stress and the hypothalamic–pituitary–adrenal (HPA) axis’). Thus, it is not surprising that stress-induced alterations in the hippocampus can lead to changes in HPA reactivity. In addition to dendritic atrophy and suppressed neurogenesis, long-term exposure to high levels of glucocorticoids (e.g., chronic stress or exogenous application) can lead to hippocampal cell death, particularly in the CA3 pyramidal layer. Together, these structural insults and cell loss can compromise the ability of the hippocampus to participate in glucocorticoid-dependent negative feedback on the HPA axis. This reduction in negative feedback then sets the stage for greater secretion of the glucocorticoids, which in turn may result in further hippocampal damage.

The feedforward nature of compromised negative feedback makes it difficult to discern whether increased HPA function in situations such as major depression (see above) is the cause or the effect of elevated glucocorticoid levels. Moreover, it is unclear whether the neuronal

remodeling associated with chronic stress in areas, such as the mPFC and amygdala, contribute directly to HPA dysregulation. It is becoming more clear, however, that stress-induced changes in the morphology of cortical and limbic brain regions can affect the balance between HPA output and negative feedback.

As the effects of chronic stress on the hippocampal formation can have such profound effects on psychological disorders and physiological functions, it would be helpful to determine what factors may insulate, or even prevent, stress-induced changes in the hippocampus. It appears that if the hippocampus is continually engaged, such as in complex spatial situations or enriched environments, then structural and functional parameters of the hippocampus can be enhanced. For instance, imaging studies have demonstrated that taxi cab drivers in London, who have to frequently navigate the labyrinth-like streets of London, have larger hippocampal volumes than their nontaxi driving counterparts. Animal experiments have also shown that voluntary exercise and being exposed to environmental enrichment (e.g., larger housing, toys, and cage mates) can lead to enhanced neurogenesis in the dentate gyrus. Taken together, these data suggest that actively engaging with the environment prior to, or concomitant with, stress exposure may mitigate some of the adverse effects of chronic stress on the structure and function of the hippocampus.

Conclusions and Future Directions

Though a substantial amount of progress has been made regarding the effects of stress on the brain, many questions remain unanswered. Majority of animal studies addressing the role of stress in altered hippocampal, prefrontal cortical, and amygdalar morphology have been conducted on adult male rats. However, several stress-induced physiological and psychological dysfunctions show sex differences in their onset, severity, and/or recovery. For instance, depression occurs at nearly twice the rate in women compared to men, and heightened stress sensitivity in females has been posited as a key underlying factor. Therefore, it is imperative that future studies include females in experimental designs to assess the role of sex in the neurobehavioral consequences of chronic stress. Experiments have already begun to show significant differences in how the male and female brain responds to chronic stress. For example, when male and female rats are exposed to chronic restraint stress, only male rats show dendritic atrophy of hippocampal CA3 pyramidal neurons, though both sexes demonstrate stress-induced decrements in spatial memory.

In addition to sex differences, the stage of an organism’s development also appears to significantly alter the effects of stress on the brain and an organism’s sensitivity

to allostatic overload. For example, an interesting and emerging body of data suggests that the effects of stress during pubertal development may be longer lasting or more dramatic compared to the effects of stress on the adult brain. Recent studies have shown that adolescent animals exposed to chronic physical or psychosocial stress display long-term changes in the size of hippocampal cell layers and decreases in structural proteins (e.g., synaptophysin) in the mPFC well into adulthood. This developmental shift in stress sensitivity of the brain is not just limited to adolescence. In fact, studies have demonstrated differential stress sensitivity in the fetal, neonatal, and aged brain.

Obviously, additional research is needed to more fully appreciate the role of factors, such as sex and development in modulating stress-induced changes in brain structure and function. However, our understanding of stress and brain morphology continues to grow. As reviewed above, numerous studies have convincingly shown that chronic stress, or long-term exposure to stress-related hormones, has significant effects on the brain, particularly in areas critical in cognitive and emotional functions. It is also clear that the stress-induced changes in neuronal structure and function are brain-region dependent. Though the exact relationship between stress, brain morphology, and psychological dysfunction remains to be elucidated, it is encouraging to know that despite the pervasiveness of stress in modern life, many aspects of stress-induced changes in brain structure are reversible, and perhaps even preventable.

See also: Animal Models of Learning and Memory; Animal Tests for Anxiety; Cholinergic Systems in Aging and Alzheimer's Disease: Neurotrophic Molecular Analysis; Cognition: Learning and Memory: Spatial; Depression; Developmental Neurogenesis; Effects of Stress on Learning and Memory; Environmental Influences on Adult Neurogenesis; Hormonal Contributions to Arousal and Motivation; Hormones and Memory; Measuring Stress;

Neurogenesis and Exercise; Neurogenesis and Memory; Perinatal Influences on Behavior and Neuroendocrine Functions; Regulation of the HPA Axis by Acute and Chronic Stress; Stress and Emotionality; Stress and Reward; Stress and Social Behavior.

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Temporal Lobe and Object Recognition

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Glossary

Conditioned taste aversion – A form of associative learning in which a neutral or palatable taste becomes an aversive stimulus as a result of its association with the symptoms of a malaise-inducing drug.

Coolidge effect – Restoration of mating behavior in males that have reached satiety with one female, but show renewed mating behavior when presented with a new female.

Delayed non-matching-to-sample – A visual object-recognition task. First, a subject is presented a sample object and is trained to pick it up and reveal the reward. After a delay, a new object and the sample object are presented simultaneously. The subject is reinforced for picking up the new object. Performance depends on object-recognition memory.

Face recognition – The process by which a subject can recall that a face has been previously encountered.

Object recognition – Successfully remembering that an object has been previously encountered.

Spontaneous object recognition – A nonappetitive object-recognition task developed for rodents. In the first part of each trial, the animal is allowed to explore two identical sample objects, while in the second part the animal is presented one sample and one new object. Rats with intact object-recognition memory spend more time exploring the novel object.

Introduction

Impressive progress has been made towards understanding the neurobiology of object-recognition memory – a cognitive function that is absolutely essential to animal survival. Object recognition is now known to depend critically on temporal-lobe function. This article focuses on four parts of the temporal cortex that have been implicated in object-recognition memory. These include the inferotemporal cortex (IT), perirhinal cortex (PR), insular cortex (IC), and the hippocampus (HC). The role of these structures is shown to depend partly on the type of measurement of object recognition, which in turn depends on the definition of objecthood. Irrespective of these considerations, the PR consistently emerges as a key player in object-recognition memory.

Expanded Concept of Objecthood

Traditionally, the explicit or implicit understanding of objecthood has tended to be restricted to entities that have certain visual-stimulus attributes. According to one definition, an object is a visible entity that is relatively stable in form. However, it is possible to challenge this visuo-centric perspective. Some investigators have expanded the concept of objecthood to include perceptual entities in the auditory modality. The list might be enlarged to include tactile, olfactory, or gustatory, and multimodal objects. The article skirts the interesting issue of the concept of objecthood by defining object recognition based on tasks that might be used to measure it. This approach leaves the reader free to accept or reject any particular task as being pertinent to object-recognition memory. Finally, certain parts of the temporal lobes turn out to be consistently important across several tasks.

Measurements of Object Recognition

Among the following tasks, some depend entirely or partly on vision and some depend on nonvisual-stimulus attributes.

Delayed (Non)-Matching-to-Sample

In the delayed-matching-to-sample (DMS) paradigm the subject is first presented with a sample object that is covering a baited food well. The subject is trained to pick up the sample object to reveal and obtain the reward. Later, a delay interval is introduced between the visual observation of the object and the opportunity to make a choice between the observed object and a novel one. In the DMS version of this task, the novel object always covers the food reward (hence, ‘non-matching-to-sample’). The DMS task varies this rule by always hiding the food reward under the sample object so that the subject must learn to ignore the novel object. The location of the sample and novel objects is always counterbalanced to ensure that the subject does not perform based on the location of the reward. In addition, each trial contains new pairs of sample and novel objects. In primate versions of this task, performance depends on visual recognition of the sample object. In another version of this task, developed for rodents, performance depends on olfactory-recognition memory.

Spontaneous Object Recognition

The spontaneous object-recognition (SOR) task is a non-appetitive variation of the DMS task. When rats are presented with a novel and familiar physical object, their natural tendency is to spend more time exploring the novel one. The SOR task takes advantage of this tendency by measuring the time spent exploring the novel object. This task eliminates the need for a food reward. In the first stage, a rat encounters and explores two novel objects. After a delay period – during which the objects cannot be seen or touched – the rat is presented with one of the former objects plus a novel object. The rat demonstrates object-recognition memory by spending more time exploring the novel object. Depending on the details of this task, it is possible that both visual- and tactile-recognition memory control performance.

Face Recognition

Face recognition has been studied exclusively in humans and other primates. It is unclear whether rodents can visually recognize faces. In the human, face-recognition studies are often carried out using brain-imaging techniques such as functional magnetic resonance imaging (fMRI). Participants are shown images of faces and non-faces such as simple shapes or complex scenes. The dependent variable is activation in parts of the brain as measured, for example, by a blood-oxygen-level-dependent response in the case of fMRI. In monkeys, single-unit recordings commonly serve as the dependent variable. A subject is presented with conspecific faces that are varied in size or orientation as well as nonface stimuli such as bars or textures. The goal is to identify brain regions in which cells change their firing pattern or firing frequency in response to the presentation of faces.

Coolidge Effect and Conspecific Recognition

The so-called Coolidge effect is defined as the restoration of mating behavior in males that have reached sexual satiation with one female but show a restoration of mating behavior if presented with a novel female. A typical paradigm begins by allowing a male to mate with a female to satiety, as indicated by the absence of mating behavior for several minutes. Next, the familiar female is removed, and a novel female is introduced. Mating behavior is measured by counting the number of mounts, intromissions, and/or ejaculations until satiation. The Coolidge effect has been demonstrated in rats, sheep, rams, guinea pigs, and golden hamsters, and is starkly absent in monogamous species such as prairie voles and old-field mice. Since rats are nocturnal, live in burrows, and have relatively poor vision, one expects olfaction, not vision, to play a prominent role in the Coolidge effect. In fact,

conspecific recognition in rats is known to depend on olfaction.

Taste Recognition

There are two common measures of taste-recognition memory in rodents. Both are based on strong biological predispositions toward food. The first measure, called conditioned taste aversion (CTA), is a type of classical conditioning in which a neutral or palatable taste becomes aversive as a result of its temporal association with subsequent symptoms of malaise. The latter can be experimentally induced by a prior injection of lithium chloride. If the food-to-malaise timing is right, the animal will develop an aversion to the taste. Interestingly, rats do not learn to associate malaise with stimuli in other modalities. CTA is highly adaptive because it trains the organism to avoid potentially life-threatening substances within one trial and because it is long lasting. The second measure of taste recognition, called attenuation of neophobia (AN), relies on the fact that rats are innately neophobic toward food. With repeated exposures, neophobia toward food normally decreases, provided that the food is not followed by malaise.

Neuroanatomy of the Essential Temporal Cortex

Four regions of the temporal lobes have been implicated in object-recognition memory, as defined by the tasks defined in the section entitled ‘Measurements of object recognition.’ These include the IT, PR, HC, and IC. The entorhinal cortex (EC) is also included below because it furnishes essential input to and output from HC. **Figures 1** and **2**, respectively, illustrate the relevant neuroanatomy in the rat and primate cortex.

Inferotemporal Cortex

The IT is a high-level visual-information-processing area. In the human, IT occupies the lingual and fusiform gyri. In nonhuman primates, it occupies the ventral temporal gyrus (**Figure 2**). There is no apparent IT homolog in rodents.

IT is classified as a neocortex because it is organized into six layers. Layer I – the molecular layer – mainly contains the dendrites of pyramidal neurons from other layers. Layer II – the external granule cell layer – contains densely packed small-granule and pyramidal cells. Layer III – the external pyramidal cell layer – contains medium-sized pyramidal neurons. Layer IV – the internal granule cell layer – contains closely packed spiny-stellate and granule cells as well as myelinated fibers. Layer V – the internal pyramidal cell layer – contains medium- and

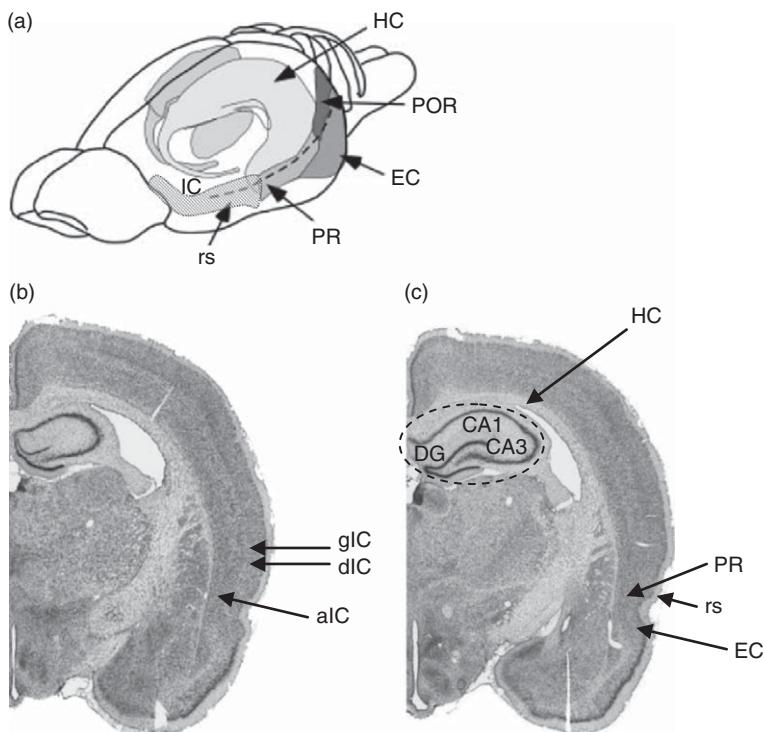


Figure 1 Rat temporal cortex. (a) Location of the temporal lobe cortex in the rat brain. Shading indicates location of the hippocampus (HC), postrhinal (POR), entorhinal (EC), perirhinal (PR), and insular (IC) cortices. POR, EC, PR, and IC are situated along the rhinal sulcus (rs). POR is termed as the parahippocampal cortex in primates. (b) Coronal section of rat (*rattus norvegicus*) brain showing granular, dysgranular and agranular insular cortex (gIC, dIC, aIC, respectively). (c) A more posterior coronal section showing EC, rs, PR, and the hippocampal formation (dentate gyrus and CA fields). (a) From Furtak SC, Wei S, Agster KL, and Burwell RD (2007) Functional neuroanatomy of the parahippocampal region in the rat: The perirhinal and postrhinal cortices. *Hippocampus* 17: 709–722, © 2007 Wiley-Liss, Inc. Reprinted with Permission from John Wiley & Sons, Inc. (b, c) Reprinted with permission from Dr. Edward G. Jones and brainmaps.org, University of California Davis.

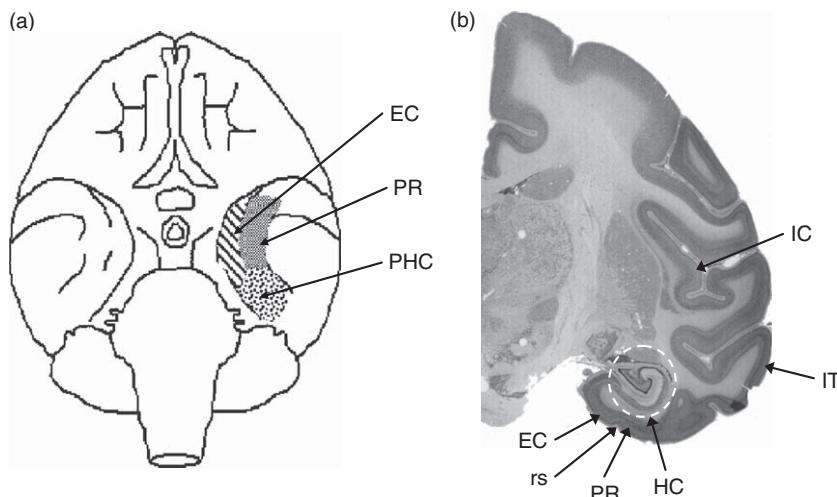


Figure 2 Monkey temporal cortex. (a) Ventral view of a monkey brain. The entorhinal (EC; diagonal stripes), perirhinal (PR; gray), and parahippocampal (PHC; mottled shading) cortices are visible on the exterior surface. (b) Coronal monkey (*macaca mulatta*) brain section showing the relevant temporal cortex structures. EC and PR are situated along the two banks of the rhinal sulcus (rs). Also shown are the hippocampal formation (dentate gyrus and CA fields), inferotemporal cortex (IT), and insular cortex (IC). (a) Reprinted with permission from Squire LR, Stark CEL, Clark RE (2004) The medial temporal lobe. *Annual Review of Neurosciences* 27: 279–306. (b) Reprinted with permission from Dr. Edward G. Jones and brainmaps.org, University of California Davis.

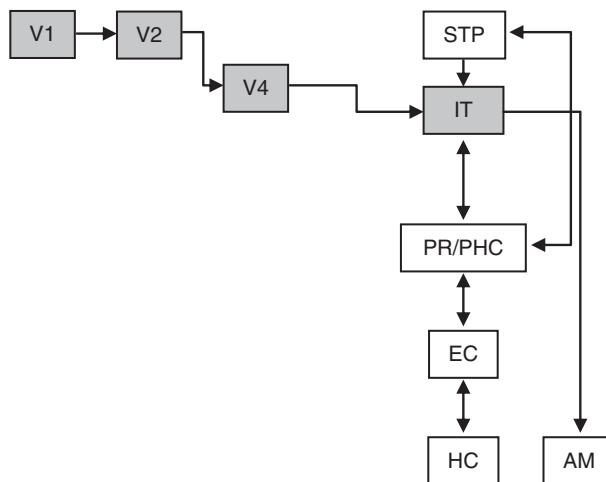


Figure 3 Ventral visual pathway. The structures within the ventral visual stream are shown in gray. Visual information from the primary visual cortex (V1) reaches the inferotemporal cortex (IT) via V2 and V4. The perirhinal (PR) and entorhinal cortices (EC) are reciprocally connected to the IT. The amygdala (AM) receives input from the IT. The hippocampus (HC) is indirectly connected to the IT via the EC. The PR is also reciprocally connected to the AM.

large-pyramidal cells as well as intrinsic granule cells. Layer VI – the multiform or fusiform layer – contains small-pyramidal neurons, spindle-shaped cells, granule cells, and a variety of stellate neurons.

Visual information from the primary visual cortex (V1) reaches the IT via its projections through V2 and V4. This network – referred to as the ventral visual stream – is illustrated in **Figure 3**. The ventral stream is sometimes termed the ‘what’ pathway. By contrast, the dorsal visual stream, not shown in **Figure 3**, is sometimes termed the ‘where’ pathway. IT is reciprocally connected to the PR and EC and also projects to the prefrontal cortex, the amygdala, and the basal ganglia.

Perirhinal Cortex

The PR is a high-level, polymodal, association cortex. In rodents, it consists of a strip that occupies the dorsal and ventral banks of the rhinal sulcus (**Figure 1**). The PR is distinguishable from neighboring areas based on its extraordinarily low level of myelin and laminar distribution of neuronal cell types. At least 15 neuroanatomical categories of neurons have been discovered in the rat PR using the Golgi-staining method. One striking characteristic of the PR is a much larger proportion of cell types that are less common in other cortices – a fact that creates the visual impression of enormous morphological diversity.

The PR is classified as the periallocortex because it is organized into five layers. Layer I – the most superficial layer – contains axons and dendrites, but no cell bodies. Layers II and III are counted as two layers even though they are difficult or impossible to distinguish from each other in the rat PR. Layers II/III and V contain many

upright pyramidal cells, large spiny stellates, and numerous smaller cell types. Layer VI is uniquely characterized by the fact that the dendrites are aligned parallel to the external capsule and pial surface.

The neuroanatomical connections to and from the PR can be subdivided into three broad categories: cortical, hippocampal, and subcortical connections. The PR receives massive input from all unimodal sensory and polymodal association cortical areas, and is reciprocally connected to the IC and IT. The PR also receives afferents from the piriform cortex and is strongly connected to the hippocampal formation through its reciprocal connection with the EC. Furthermore, the PR is reciprocally connected to the lateral and basal nuclei of the amygdala (LA and BA, respectively) and also receives afferents from the thalamus (dorsal nucleus and medial geniculate body).

Hippocampal Formation

The HC proper is an elongated structure within the medial temporal lobes (**Figure 1**). Throughout its length, HC consists of three subfields, designated CA1, CA2, and CA3. The hippocampal formation includes, in addition to the HC, the dentate gyrus (DG) and the subiculum complex (subiculum, presubiculum, and parasubiculum).

The HC is typically classified as archicortex or allocortex because the CA fields are organized into three prominent layers: the stratum oriens, the stratum pyramidale, and stratum radiatum/stratum lacunosum-moleculare. Stratum oriens contains the basal dendrites of pyramidal cells. Stratum pyramidale contains the cell bodies of pyramidal neurons. Stratum radiatum and stratum lacunosum-moleculare contain the apical dendrites of pyramidal cells. DG also has a trilaminar organization,

with the molecular layer being the most superficial, followed by the granule cell body layer, and finally, the polymorphic layer.

Entorhinal Cortex

The EC furnishes an essential part of the input to and output from HC. Much of the higher-level sensory input to EC originates in the PR and IC. The medial and lateral parts of the EC have different types of connections. The medial EC receives afferents from the postrhinal cortex, whereas the lateral EC receives inputs from the PR and the IC. These cortical afferents (among others) reach layers II–III of the EC. From there, activity traverses the internal circuitry of HC. Information from the HC is projected back to layers IV–VI of the EC, which projects to the PR and the IC. In summary, the HC is extensively connected to the PR and IC via the EC. The HC is also reciprocally connected to the IT via the EC and PR (**Figure 3**).

Insular Cortex

The IC is important for integrating high-level gustatory, olfactory, and visceral information. In rodents, the IC is a broad strip, situated anterior to the PR along the rhinal sulcus (**Figure 1**). In primates, the IC is situated deep within the lateral sulcus of the brain (**Figure 2**). The IC is reciprocally connected to both the PR and the EC. Based on cytoarchitecture, one can distinguish three subdivisions of the primate IC: agranular IC (aIC), dysgranular IC (dIC), and granular IC (gIC). The aIC occupies the anterior portion, dIC occupies the middle portion, and gIC occupies the posterior portion of the IC. The aIC is further divided into dorsal aIC and ventral aIC. The ventral aIC is classified as allocortex because it has three layers: II/III, V, and VI. The three layers are easily distinguishable because they are segregated by cell-free zones called the *lamina dissecans*. The dorsal aIC, classified as proisocortex, has only two layers: II/III and V/VI. Layers V and VI are not distinguishable because the dorsal aIC region lacks the *lamina dissecans*. Layer II/III contains pyramidal cell bodies. Layers V and VI contain pyramidal cell axons that project to regions near the rhinal sulcus.

The IC is connected to several structures in a topographically specific fashion: only aIC projects to the mediodorsal thalamic nucleus; only dIC projects to the presupplementary motor cortex; and only gIC is reciprocally connected to the somatosensory cortex. The differences in connectivity suggest that the three divisions of IC may be functionally dissociable. All three divisions are connected to the PR, EC, and the amygdala. PR is reciprocally connected to the aIC and receives input from the dIC. The EC is reciprocally connected to aIC and dIC

and projects to the gIC. The lateral (LA), basolateral (BLA), and central (Ce) nuclei of the amygdala receive input from the aIC and dIC. The LA and BLA project to the gIC. Finally, the gustatory thalamic nucleus is reciprocally connected to the gIC and receives input from the dIC. In summary, the IC is well connected to the PR, EC, the gustatory thalamus, and the amygdala.

Temporal Cortex and Object Recognition

Each of the four regions of temporal cortex described in the section titled ‘Neuroanatomy of the essential temporal cortex’ has been linked to one or more of the object-recognition tasks described in the section titled ‘Measurements of object recognition.’

Inferotemporal Cortex

The IT is implicated in face recognition and, more generally, in recognition of complex stimulus patterns. In the 1970s, Pribram and Mishkin discovered that removal of IT in monkeys severely impaired visual-object recognition. In the human, damage to the IT leads to prosopagnosia – a condition in which a person is unable to recognize faces. Damage to the IT can also impair discrimination among colors (achromatopsia), animal species, and categories of manmade objects.

Human fMRI studies suggest that the fusiform gyrus (FFA), a part of the IT, is particularly responsive to faces. The FFA is also activated when participants are asked to discriminate among bird species, types of cars, or complex nonsense shapes called Greebles. The evidence to date suggests that the FFA is involved in face recognition, and also plays a role in discriminating complex nonface visual objects.

The involvement of the IT in face recognition has been investigated in large part via electrophysiological studies in monkeys. Some neurons in the IT seem to be selectively tuned to faces. These cells are responsive irrespective of whether the faces are real or plastic, human, or monkey. They are also responsive irrespective of the size or orientation of the faces. Importantly, these cells are not responsive if the components of the face are rearranged. They are also relatively unresponsive to simple stimuli such as gratings, or complex stimuli such as food or snakes.

Some cells within the IT are highly responsive to components of the face, such as the eyes, mouth, or hair. One implication of this fact is that the IT may support face recognition based on a distributed network of cells that individually encode particular aspects of faces. This possibility stands in contrast to the alternative that so-called gnostic or dedicated face cells are responsible for representing different faces. A distributed encoding

mechanism may be more economical. The modern consensus is that the IT supports face recognition through distributed networks of neurons that are individually tuned to simpler shapes, colors, and textures. Face-responsive cells have also been reported in the amygdala. This is not surprising given that the IT projects directly to the amygdala (**Figure 3**). However, amygdalar cells appear to be selective for certain facial expressions, rather than face recognition. It is unclear whether the PR, HC, or IC play important roles in face recognition.

Perirhinal Cortex

The PR is essential for the performance of four different categories of object-recognition tasks. No other temporal-lobe region has been demonstrated to have this task- and modality-independent relationship to object recognition. The four general categories of tasks are discussed under separate headings below.

Delayed (non)-matching-to-sample

Abundant evidence demonstrates that damage to the PR produces the largest deficits in visual object-recognition memory. Seminal studies in the late 1980s compared the effects of the PR and parahippocampal (PHC) cortical lesions with lesions of the HC and amygdala. The PR-PHC lesions severely impaired DMS performance in monkeys even when the HC and amygdala were intact. Studies by Elizabeth Murray and colleagues show that damage restricted to the PR is necessary and sufficient for severely impairing DMS performance in monkeys. Combined PR and EC lesions drastically impair DMS and DMS performance, but lesions limited to the EC produce only a mild and transient deficit. Thus, the PR is strongly implicated as being the most critical structure in the medial temporal lobe (MTL) for visual object recognition.

Decreased neuronal responding to familiar stimuli may be one mechanism by which the PR supports familiarity-based object recognition. Approximately 25% of the PR neurons show attenuated responses to familiar stimuli. Importantly, neuronal responses in the PR have been shown to attenuate in as little as one stimulus repetition, a finding that is consistent with object-recognition memory, which can form in a single trial. Attenuated responding in the PR occurs even when several objects are presented simultaneously or serially, suggesting that this mechanism has a large capacity. Given that unique stimuli are presented on each trial in DMS, it is possible that object recognition of multiple familiar stimuli is supported by attenuated neuronal responding in the PR. In this way, attenuated PR responding may encode stimulus familiarity.

Performance on DMS and DMS tasks depends on muscarinic receptor activation. Systemic injections of

scopolamine or atropine, which are cholinergic muscarinic receptor antagonists, impair performance in the human and rats. Intra-PR infusion with scopolamine disrupts DMS performance in monkeys. Interestingly, performance on the DMS task has been reported to enhance the release of acetylcholine in the PR. Systemic injections of the acetylcholinesterase inhibitor, physostigmine, enhances recognition memory in monkeys and the human.

Spontaneous object recognition

The PR plays a prominent role in SOR. Inactivation of the PR via lidocaine infusion impairs SOR performance across a wide spectrum of time points, which suggests that the PR may be necessary for the encoding, maintenance, and retrieval of object-recognition memory. The cholinergic system in the PR is implicated in SOR because intra-PR infusion of scopolamine impairs SOR. Finally, single-unit recordings in the PR reveal attenuated neuronal responses during presentations of familiar objects in SOR. Both SOR and DMS may be tapping a common PR function.

Coolidge Effect and conspecific recognition

The PR also plays a critical role in the Coolidge effect. Ibotenic acid lesions of the PR and the EC (but not of the HC) abolish the Coolidge effect. In one study, male golden hamsters were mated with a female until satiation. The males were then presented with both the familiar female and a novel one. Both females were anesthetized. Investigatory and copulatory behavior was measured. A control test confirmed that lesioned males do not differ from control animals in terms of general investigatory behavior. PR-EC-lesioned males (but not HC-lesioned males) failed to demonstrate the Coolidge effect. Other studies have similarly reported that combined PR-EC lesions (but not fimbria-fornix lesions) impair discrimination between novel and familiar conspecific odors. Similarly, PR-EC lesions (but not HC lesions) impair recognition of juvenile conspecifics. Both PR and EC appear to play fundamental roles in the recognition of conspecifics.

Taste recognition

The PR is strongly implicated in taste-aversion tasks. The disruption of the cholinergic system in the PR impairs taste recognition as assessed by AN and CTA. More specifically, infusion of scopolamine into the PR impairs AN and CTA. Pretraining, but not pretesting, inactivation of the PR blocks CTA, implicating the PR in CTA learning but not retrieval.

Hippocampus

The role of the HC in object recognition has been a subject of intense debate since the 1970s. Early studies suggested that the HC and the amygdala were critical for object-recognition memory. Specifically, combined lesions of the HC and the amygdala impaired visual- and tactile-recognition memory, but lesions restricted to either structure did not. Combined HC and amygdalar lesions also impaired DMS performance, retention of object discrimination at long delays, and concurrent object discrimination. In some or all of these studies, the PR may also have been damaged. Indeed, subsequent research found that attempts to ablate the HC and the amygdala only resulted in impaired DMS performance when the surrounding PR and PHC were also damaged. Furthermore, lesions that were restricted to the PR and PHC produced an equally severe impairment. These studies collectively suggest that the PR plays a greater role in DMS than does the HC.

In the 1990s, the DMS task was adapted for use in rats. Although some studies reported that damage to the HC or to the fornix impairs DMS performance, an equal number of studies failed to find this impairment. It is thought that HC lesions only impair DMS performance when the delay period between the sample and choice phase is sufficiently long. In fact, HC-lesion deficits in DMS performance are evident at 10 min, 1 h, and 24 h, but not at 10 s and 1 min.

Physiological studies reveal that attenuated neuronal responding to familiar stimuli is not common in the HC. Although one study reported attenuated responding in the HC, this occurred in only 2% of HC neurons, as opposed to 25% of PR neurons. Enhanced neuronal responding has also been observed in the HC, but this is less common. Relative to the PR, it appears that the HC plays a much more limited role in object-recognition memory.

Insular Cortex

The IC is implicated in CTA and AN. Early studies reported that electrolytic lesions of the amygdala impair CTA and AN. However, it was unclear whether the impairment was due to damage to amygdalar neurons, or to fibers of passage within the amygdala. A double-dissociation study tested this confound by comparing the effects of ibotenic and electrolytic lesions of the amygdala and IC on CTA and AN. Electrolytic, but not excitotoxic, amygdalar lesions impaired CTA and AN. Excitotoxic IC lesions severely impaired CTA and AN. Given that axons to and from the IC pass through the amygdala, the results suggest that the IC is more critical than the amygdala for taste-aversion tasks. The physiological and chemical mechanisms underlying the function of the IC in CTA remain to be unraveled. One might expect the IC to also

be critical for the Coolidge effect and conspecific recognition, but we found no evidence to support this possibility.

Synthesis

The concept of an object commonly conjures up visually identified features that have essential spatial relationships to each other. Recently, however, the concept of object-hood has been extended to include auditory, olfactory, or gustatory, and multimodal objects. Here we consider a wide range of object-recognition tasks, some of which do not depend on visual or spatial stimulus attributes. Evidence to date suggests that the PR is critical for object recognition, irrespective of the stimulus modality. We anticipate that the PR will also play a critical role in multimodal object-recognition memory.

See also: Agnosia; Amnesia; Animal Models of Learning and Memory; Brain Aging: Structural Imaging Biomarkers of Risk of Alzheimer's Disease; Cognition: Learning and Memory: Pavlovian; Communication of Emotions in Animals; Declarative Memory; Disorders of Face Processing; Emotion–Cognition Interactions; Language and Communication – Brain Substrate; Male Sexual Behavior; Mating Behavior; Neural Basis of Recognition Memory in Nonhuman Primates; Neural Basis of Working Memory; Novelty; Social Bonding and Attachment; Social Communication; Social Learning and Behavior Transmission; Social Relationships and Social Knowledge; Taste Perception and Behavior in Rodents and Flies.

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Thermoregulation

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Glossary

Ablation – Surgical removal.

Adipose – Of, or relating to fat.

Caudal – Situated in, or toward the hind end of the body.

Comorbid – Pertaining to two diseases occurring together.

Countercurrent – Materials flowing in opposite directions.

Cytokine – One of several intercellular proteins that mediate an immune response.

Dorsomedial – Located toward the back and near the midline.

Pyrogen – A fever-producing substance.

Rostral – Situated in, or toward the front end of the body.

Sepsis – A toxic condition resulting from the spread of bacteria from a site of infection.

Vaso- – Of, or relating to blood vessels.

Ventromedial – Located abdominally and near the midline.

systems mitigate heat escaping through the extremities, by transferring heat from outgoing arterial to incoming venous blood. Metabolic adaptation includes the ability to store metabolic energy in the form of fat. Through thermogenesis, energy can be released rapidly (e.g., shivering) or gradually (e.g., liver). Furthermore, evolutionary pressures also select for advantageous anatomical features, such as shortened extremities, or increased body size to more easily facilitate a steady core body temperature.

As thermoregulators, humans have successfully adapted to a diverse set of climates. In cooler conditions, the human body engages in thermogenesis (e.g., liver processes and muscle contractions) to produce heat, and vasoconstriction to retain heat. In warmer environments, humans engage in vasodilation and sweating to transfer excess heat through evaporation. In addition to physiological mechanisms, humans also employ other social (e.g., huddling), cultural (e.g., clothing), and technological adaptations to produce or retain heat.

Thermometers provided the means to accurately measure temperature in animals. Because rates of heat loss and heat production vary in different parts of the body, local temperature readings may differ considerably. Although circulating blood tends to standardize body temperature, the identification of body parts that closely match those of the internal organs have proven useful in ascertaining core temperature. These body parts include the rectum, uterus, bladder, mouth, ear, and groin.

Behavioral Physiology

Homeostasis involves the dynamic interaction between an organism's internal processes and its external environment to maintain physiological stability. One such homeostatic process is thermoregulation. Among animals, there are thermoconformers and thermoregulators. A thermoconformer's body temperature changes based on external temperature. A thermoregulator, however, maintains its body temperature within a particular range, while still being responsive to various external, environmental stimuli. Among thermoregulators, failure to achieve thermoregulatory balance may result in physiological damage, metabolic irregularities, or even death.

In order to maximize heat loss in warm environments, mammals typically employ a number of adaptations. These include physiological strategies such as evaporative cooling (i.e., panting and perspiration), highly vascularized extremities, and compartmentalized fat storage to reduce insulation (e.g., camels). Other strategies involve behavioral adaptations such as daytime burrowing and increased nocturnal activity. Conversely, mammals utilize a variety of strategies to retain heat in cold environments. Physiologically, vascular countercurrent exchange

Heat Loss

When environmental temperature is above core body temperature, humans lose heat through evaporation by secreting sweat. Furthermore, dermal hairs lie flat to prevent air retention between them, and thus complement convective heat loss. Vasodilation is another mechanism by which humans lose heat. Relaxation of smooth muscle in arteriole walls increases arterial blood flow. By redirecting blood to distal parts of the body, such as the skin, heat is lost through radiation and convection. In contrast to humans, only few other mammals lose heat primarily through sweating (e.g., horses). Rats, mice, cats, and dogs possess sweat glands exclusively on their foot pads. Instead, many mammals (e.g., dogs) pant to cool their bodies. Highly vascularized lungs, with a large surface area, facilitate heat exchange between the air and their bodies.

Heat Retention/Production

In contrast to heat loss, humans apply the opposite mechanisms to retain heat whenever environmental temperature dips below core body temperature. Sweat pathways are deactivated. Vasoconstriction is achieved by constricting subdermal capillaries and thus, shunting blood away from the skin and the body's periphery. In extremely cold conditions, prolonged rerouting of blood away from the extremities causes numbness and cellular damage (e.g., frostbite). To augment heat retention, humans contract minuscule subdermal muscles (erector pili) to erect dermal hair follicles. These erect hairs form an insulating layer capable of trapping heat. Furthermore, humans and some animals produce heat in an exothermic process called thermogenesis. Effector signals from the hypothalamus reach peripheral muscles to cause shivering. Humans engage in short-term shivering, where heat is produced through high-intensity glucose metabolism in a relatively short period of time. Other animals, such as hibernating bears, are capable of long-term shivering, often for months, by gradually and steadily metabolizing fat stores.

Circadian Rhythm

Daily variations in body temperature have been observed in mammals, particularly humans and rats. The daily peak of body temperature rhythm in rats was found to consistently follow the daily peak of heat production rhythm and to precede the daily peak of heat loss rhythm. Furthermore, the daily rhythm of heat balance is 180 degrees out of phase with the rhythm of body temperature. Animals with lesions in the dorsomedial nucleus of the hypothalamus (DMH) maintained a normal circadian variation, though body temperature remained 0.5°C lower. Rat studies have shown that the circadian rhythm may be inverted by restricting food access to daylight hours. When only permitted to eat during the latter half of the light period, these animals awaken and increase body temperature a few hours before food administration. Lesion studies have determined that, the ability to alter rhythms in body temperature, as well as the wake–sleep cycle, feeding, activity, and corticosteroid rhythms, correlate with DMH activity.

Sleep

Mammals sleep to conserve energy, during most of which, decreased body and brain temperatures are observed (exception: rapid eye movement (REM) cycle). Small mammals tend to sleep the most, since they also have higher energy demands for thermoregulation and locomotion, while possessing relatively lower energy reserves. Disruption of normal sleep cycles has been associated

with thermoregulatory irregularities. For example, chronically sleep-deprived rats show increased preference for cooler ambient temperatures ($\sim 10^{\circ}\text{C}$), which suggests that sleep has cooling function. Conversely, rats sleep-deprived for 2 weeks show a significant decrease in body temperature despite a doubling in metabolic rate, suggesting sleep's role in heat retention. In humans, increased hypothalamic temperature induces sleep, and body heating prior to sleep increases subsequent slow wave sleep.

Mechanism

Hypothalamus

Found in all mammalian brains, the hypothalamus is located ventral to the thalamus, where it coordinates certain metabolic processes and various autonomic nervous system activities, as well as synthesizes and secretes particular hormones. Stimulation and ablation studies have determined that the preoptic anterior part of the hypothalamus (PO/AH) is crucial for thermoregulation. Approximately, 40% of PO/AH neurons are temperature sensitive, while the rest are insensitive. Local warming of PO/AH neurons evoked heat loss responses, whereas local cooling induced heat production. This observation led to the idea that the PO/AH is an area responsible for thermoregulatory control, that is, the body's central thermostat.

Neuroanatomical Correlates

Observed heat loss behavior includes vasodilation, panting, and sweating, while heat production behavior involves vasoconstriction, shivering, breakdown of brown adipose tissue, and huddling. Further studies have determined that heat loss and production are triggered by the actual ambient temperature, and not by the rate of change in temperature, since no burst of neuronal firing rates was detected during rapid temperature change. Hypothalamic temperature does not vary considerably (range: $\pm 1^{\circ}\text{C}$) even when ambient temperature is changed drastically. However, drops in hypothalamic temperature below this range often induce a coma and other neuroprotective mechanisms.

Changes in the activity of neurons in the PO/AH have been correlated with initiation of thermoregulatory mechanisms. Nearby areas, such as the DMH, may include thermoeffector neurons. Electrical stimulation of the DMH induced an increase in heat production behavior. Retrograde labeling shows dye beads transported to the lateral hypothalamus (LH), PO/AH, ventromedial preoptic (VMPO), and paraventricular area (PVN). It is thought that the PO/AH may have direct control of the DMH.

Neuronal Thermosensitivity

Various *in vivo* and *in vitro* electrophysiological studies have identified four types of thermoregulatory neurons: warm-sensitive, cold-sensitive, temperature-insensitive, and silent. Among these types, the vast majority of the PO/AH neuronal population comprise warm-sensitive (~30%) and temperature-insensitive (~60%) neurons. One main difference between these two types is that warm-sensitive neurons not only inherently respond to local thermal input, but also to hypothalamic, skin, and/or spinal temperature. Conversely, temperature-insensitive neurons do not exhibit temperature-related changes in activity.

Thermal Classification

Historically, temperature sensitivity has been ascertained in one of two ways: either through the determination of a Q_{10} thermal coefficient, or through calculation of the neuronal firing rate regression line slope. The slope reflects the number of action potentials (APs) as a function of hypothalamic temperature.

Using the latter criteria, temperature-sensitive neurons can be divided into two subgroups: warm- (35% PO/AH) and cold-sensitive (5%). Warm-sensitive neurons fire at ≥ 0.8 impulses/s/ $^{\circ}\text{C}$, when the PO/AH temperature is changed by $\pm 2\text{--}3$ $^{\circ}\text{C}$. These neurons respond to both direct changes in hypothalamic temperature and peripheral thermal stimulation. They also have a distinct lateral/medial orientation in dendritic morphology, which is consistent with their roles as integrators of thermal information. Cold-sensitive neurons, on the other hand, fire at ≤ -0.6 impulses/s/ $^{\circ}\text{C}$ when PO/AH temperature is changed by $\pm 2\text{--}3$ $^{\circ}\text{C}$. Unlike warm sensitivity, cold sensitivity is not an inherent property, and may be a result of synaptic input from nearby warm-sensitive and -insensitive neurons.

In contrast, temperature-insensitive neurons show little change in firing rate when temperature deviates from the normal physiological condition (~ 37 $^{\circ}\text{C}$). Temperature-insensitive neurons fire at ≤ 0.79 impulses/s/ $^{\circ}\text{C}$ and ≥ -0.6 impulses/s/ $^{\circ}\text{C}$ when the PO/AH temperature is changed by $\pm 2\text{--}3$ $^{\circ}\text{C}$. Essentially, this range is between the firing rates of warm- and cold-sensitive neurons. Unlike their warm-sensitive counterparts, temperature-insensitive neurons extend their dendrites in rostral/caudal directions, consistent with their roles as indicators of baseline of hypothalamic activity.

The characterization of physical differences between temperature-sensitive and -insensitive neurons is an active area of research. These differences in firing rates may be determined by various ionic conductances, such as tandem-pore K⁺ leak channels (TWIK-related acid-sensitive K⁺ (TASK), TWIK-related K⁺ (TREK), and TWIK-related arachidonic acid-stimulated K⁺ (TRAAK), transient K⁺ A-current channels, hyperpolarization-activated cyclic

nucleotide-gated channels (HCNs), and vanilloid transient receptor potential (TRP) channels. The proportion of these channels within the hypothalamus may explain the difference among warm-, cold-sensitive, and temperature-insensitive neurons. It has been observed that increased temperature positively correlated with both firing rate thermosensitivity and A-current inactivation, resulting in a higher probability of reaching firing threshold.

Hammel's Model

In the 1960s, the six-neuron Hammel's model (see Figure 1), named after its developer, proposed that set-point temperature was determined by the integration of afferent signals with neuronal interactions within PO/AH. The model proposed that warm-sensitive neurons positively synapse on warm effector neurons, whereas temperature-insensitive neurons inhibit them. The temperature where excitation equals inhibition determines the set point. Above the set-point temperature, warm effector neurons receive more stimulation than inhibition, thus leading to heat loss behavior. Conversely, temperature-insensitive neurons positively synapse on cold effector neurons, whereas warm-sensitive neurons inhibit them. Below the set-point temperature, cold effector neurons receive more stimulation than inhibition, thus initiating heat retention/production behavior.

Medical Implications

Fever, General Pathology

Several studies estimate that the average core temperature for healthy adults is approximately 98.2 $^{\circ}\text{F}$ or 36.8 $^{\circ}\text{C}$. When presented with an immunological challenge, the human body undergoes a cascade of factors that signal the brain. The resulting rise in body temperature of 1–4 $^{\circ}\text{C}$ is defined as a fever. Metabolically, specific cytokines cause production of prostaglandin (PGE₂) in the brain, which in turn affects neuronal activity in the ventromedial preoptic (VMPO). Introduction of PGE₂ to this region or stimulation of afferent pathways causes significant rise in body temperature (>1 $^{\circ}\text{C}$). According to Hammel's model, an increase in the firing rates of temperature-insensitive neurons and/or a decrease in the firing rates of warm-sensitive neurons result in a new, higher set point. The presence of PGE₂ causes warm-sensitive neurons to decrease their firing rates and temperature-insensitive neurons to be unaffected or increase their firing rates.

Fever, Evolution of

Endotherms and ectotherms (both vertebrates and invertebrates) initiate a febrile response when challenged with endotoxins or pyrogens. This widespread occurrence

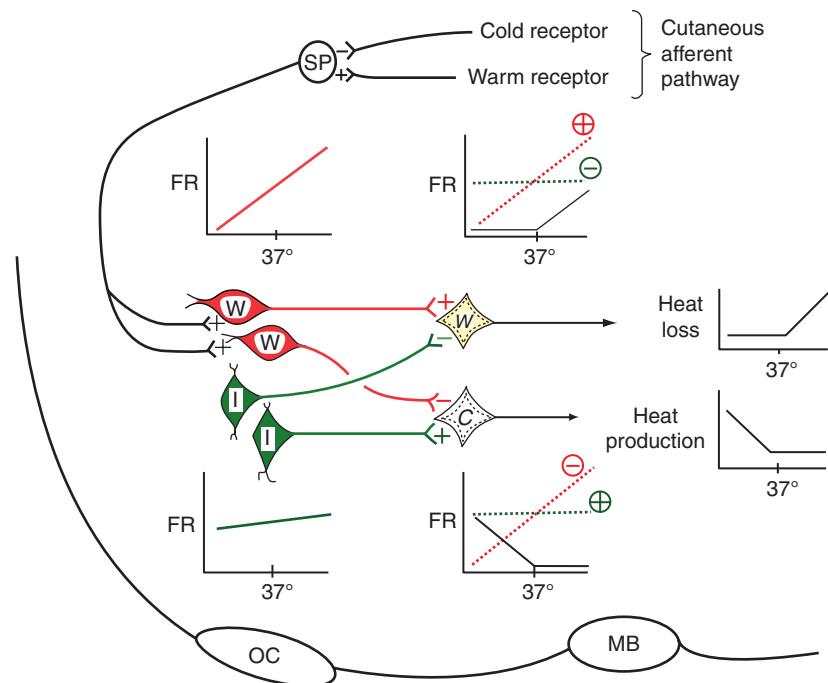


Figure 1 Hammel's model. A modified six-neuron model explaining the hypothalamic determination of set-point temperature. Key: FR, firing rate; W, warm-sensitive neuron; I, temperature-insensitive neuron; w, heat loss effector neuron having synaptically derived-warm sensitivity; c, heat production effector neuron having synaptically-derived cold sensitivity; SP, dorsal horn spinal neuron; OC, optic chiasm; MB, mammillary body. Adapted with permission from Boulant JA (2006) Neuronal basis of Hammel's model for set-point thermoregulation. *Journal of Applied Physiology* 100(4): 1347–1354.

suggests that, fever most likely evolved hundreds of millions of years ago.

There has been some debate as to whether the febrile response is evolutionarily beneficial or maladaptive. For example, in endotherms, such as birds and mammals, maintenance of body temperature at 2 or 3°C above set-point levels often results in an increase in energy consumption by at least 20%. Despite this metabolic expenditure, several studies have shown that suppression of the febrile response in many species resulted in increased mortality, while artificial inducing fever increased resistance in others. However, adverse effects through pyrogenic cytokines (such as interleukin-1, tumor necrosis factor, interleukin-6, and interferons) have been shown to add to an infection's pathophysiological burden on the host, and, in some instances, even induce sepsis.

Despite the risk, it is unlikely that such an energetically expensive process would have persisted for millions of years, among diverse groups of organisms, if fever did not have an adaptive function. One evolutionary explanation proposes that, fever may lead to a sacrifice of the host, if that individual poses a threat to the species as a whole. From this perspective, the febrile response might have evolved as a mechanism for facilitating the recovery of infected individuals with localized, or mild to moderate systemic infections, while simultaneously accelerating the

demise of hopelessly infected individuals, who pose an epidemic threat to the species.

Thermal Dysregulation

Because of the hypothalamus' importance in thermoregulation, injuries, tumors, genetic abnormalities, and its exposure to pyrogenic and other exogenous compounds may cause thermal dysregulation. Because the PO/AH can be influenced by factors besides temperature (e.g., glucose levels, testosterone, estrogen, and osmotic balance), dysfunctions in maintenance of these factors may be comorbid with thermal dysregulation. Furthermore, metabolic and physiologic conditions, such as hormonal imbalance and poor blood circulation, may contribute to abnormal body temperature.

Conclusion

To summarize, thermoregulation is a dynamic, homeostatic interaction between an organism's internal processes and its thermal environment. To maintain physiological stability, many organisms employ processes such as heat loss, retention, and production. These thermoregulatory mechanisms influence other aspects of life, such as sleep and circadian rhythms. Among mammals,

the hypothalamus (located deep within the brain) coordinates certain metabolic processes, regulates various autonomic nervous system activities, and synthesizes/secretes particular hormones critical to thermoregulation. Through thermal dysregulation and protective mechanisms like fever, we are reminded of thermoregulation's importance in maintaining life.

See also: Cytokine Effects on Neuronal Processes and on Behavior; Motivation; Thermo- and Mechanosensation via Transient Receptor Potential Ion Channels.

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Transient Global Amnesia: Neuropsychology, Psychopathology, and Neuroimaging

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Glossary

Diffusion-weighted (magnetic resonance) imaging (DWI) – A technique that measures the diffusive motion of water molecules in biologic tissue. It is commonly used in clinical practice for early detection of cytotoxic edema due to ischemia (water moves from the less restricted extracellular environment to the more restricted intracellular one). This phenomenon results in reduced extracellular water motion (random displacement of the molecules), leading to an attenuation of analog-to-digital converter (ADC) signal and a hyperintensity on DWI sequences.

Episodic memory – A term introduced by Endel Tulving within the context of the fractionation of memory. It is the memory of personal events, as opposed to the memory of knowledge (semantic memory). Episodic memory makes mental time travel possible in the sense that it allows the reexperiencing of a situation. It is defined through three important clues: the sense of subjective time, autonoetic awareness, and self.

Lexical–semantic priming – A semantic paradigm allowing the assessment of semantic memory implicitly. The lexical–semantic priming effect is attested by a faster reaction time and/or greater accuracy when a target item (giraffe) is preceded by a semantically associated prime (zebra) rather than an unrelated one (table).

Wechsler Adult Intelligence Scale – An individually administered adult scale measuring general intelligence through 15 subtests dealing with both verbal and performance abilities.

Working memory – A limited-capacity and short-term memory system that temporarily stores information in view of its manipulation. According to Baddeley and Hitch's model, it is a multicomponent system comprising two slave systems (the phonological loop and the visuospatial sketchpad) devoted to the maintenance of verbal and visuospatial information, respectively, which are supervised by a coordinating system called the central executive.

Introduction

Among the variety of amnestic disturbances, transient global amnesia (TGA) remains one of the most enigmatic syndromes. Reasons for this may lie in the fact that TGA has a relatively low incidence (3–10 per 100 000 per year) and that it may have long been immersed in the psychiatric literature and once viewed as an atypical case of psychogenic/hysterical amnesia. In fact, its definition as a distinct entity is relatively recent, with its first descriptions appearing in 1956: Guyotat and Courjon reported 12 cases of *ictus amnésique idiopathique* in France, while Bender described the “syndrome of isolated episode of confusion with amnesia” in the US. Fisher and Adams subsequently published an account of 17 cases and discussed the possible etiologies. Although these authors were specific in their description, the diagnosis of TGA was still applied to a wide variety of clinical situations that only shared with TGA an acute and dense memory loss as their core feature, which resulted in persistent heterogeneity in the subsequent publications. It was not till 1990 that Hodges and Warlow established the following criteria, which are still unanimously accepted for the diagnosis of TGA:

1. attacks must be witnessed and information available from a capable observer who was present for the most part of the attack;
2. there must be clear-cut anterograde amnesia during the attack;
3. clouding of consciousness and loss of personal identity must be absent, and the cognitive impairment limited to amnesia (i.e., no aphasia, apraxia, etc.);
4. there should be no accompanying focal neurological symptoms or functionally relevant focal signs;
5. epileptic features must be absent;
6. attacks must resolve within 24 h; and
7. patients with recent head injury or known active epilepsy (i.e., remaining on medication or one seizure in past 2 years) are excluded.

From a group of 114 patients diagnosed with these criteria, Hodges and Warlow found that TGA most commonly occurs in middle-aged or elderly people (mean age 62.3, most patients between 50 and 80 years) and that amnesia lasts 4.2 h on average. Regarding the behavioral manifestations during the attack, Hodges and Warlow observed repetitive questioning, disorientation in time; they also noticed that a number of subjects were perplexed, anxious, or agitated. None had neurological symptoms, such as ataxia, limb weakness, or sensory disturbance, and all were able to perform complex tasks (e.g., driving a car). The only commonly noted nonfocal symptoms were headache, nausea/vomiting, and dizziness that might occur at any time from the onset of the attack. One conspicuous feature noted in their report is the frequent presence of precipitants of the attacks. In the previous 24 h, a number of subjects had faced an emotionally stressful event (e.g., learning that a close relative had cancer, witnessing a severe car accident, being involved in a gas cylinder fire, being told that a close friend had had a heart attack, attending the grave of a deceased spouse, or having a violent family argument), or experienced physical pain, or performed some physically straining action (e.g., having sexual intercourse, swimming in cold water, undergoing a medical procedure, or doing vigorous exercise). Hodges and Warlow concluded that TGA is a heterogeneous clinical syndrome and considered that there is little support for a cerebrovascular etiology in pure TGA defined according to their criteria. They also raised the role of physical and emotional stress in the etiology of TGA. Once Hodges and Warlow's criteria were fully accepted, TGA reports became more frequent with time and the nosological concept of TGA was better and better delineated. However, as the knowledge of the syndrome spread, questions initially tackled by Hodges and Warlow emerged. First, even though neuropsychological assessment of patients in the acute phase is difficult to carry out because of the suddenness and the short duration of the attack, TGA is an interesting model for testing the memory organization. This syndrome represents an interesting approach to understand the episodic memory disorders, especially in the light of the recent theoretical development of this concept. Second, although the psychopathological aspects have been reported in the first cases, they remain almost unexplored, highlighting the need for a comprehensive investigation. Third, the etiology of TGA is still unknown. These issues are successively discussed in the subsequent sections of this article.

Neuropsychology of TGA

TGA comprises, on the one hand, a massive anterograde amnesia that expresses itself chiefly by repetitive questioning, and, on the other hand, a variable retrograde amnesia that may extend back from a few hours to several months prior to the onset of the attack. Only a few group studies of patients examined during TGA have been published because TGA patients often access medical care only when the attack is already over. This fact may contribute to the heterogeneity of the findings. However, a general survey of the publications suggests that during a TGA attack some memory functions appear to be completely lost, while others are only partially lost or impaired and some others are preserved.

It must be stressed, in the first place, that TGA patients have no impairment of intelligence or of cognitive functions other than memory, such as attention, language, praxia, or visuoconstructive abilities. Speech is spontaneous, coherent, and fluent. Patients also correctly perform the copy of complex geometrical figures and score within the normal range at general intelligence tests such as the Wechsler Adult Intelligence Scale.

The leading feature of TGA is total anterograde amnesia and all observers agree that learning into episodic memory is completely suppressed, whatever the type of information (numbers, letters, faces, figures, objects, stories, paired associated lists, or spatial locations) format (verbal, visual, or tactile) or testing procedure (recall or recognition). The issue of whether anterograde amnesia in TGA is caused by encoding or storage impairment has stirred considerable interest and it seems that both patients with preferential storage disorders and those with preferential encoding deficits may be observed. This distinction may be closely related to the time of testing, that is, impaired encoding is more likely to be found in the early phase of the attack.

The next most important aspect of memory impairment during TGA is retrograde amnesia, which affects past memories to a variable extent, from days to months, sometimes even more. There is a gradient between recent and remote memories, with recent memories being more affected than remote ones. However, not only is there a gradient, but retrograde amnesia during TGA is also patchy in nature, depending on the type of memory involved. This is because, of all memories we have of the past, some pertain to the semantic system, while others still remain episodic in nature, being closely linked to episodes of our personal life. This may be sorted out through neuropsychological testing paradigms specifically designed to evaluate the episodic load of one particular remote memory. It has thus been demonstrated that the more episodic an ancient memory is, the higher the likelihood for it to

be missing or altered during TGA, while the semantic components are spared, so that in its retrograde as well as in its anterograde component, the amnesia of TGA mainly affects the episodic memory system. Retrograde amnesia completely recovers after the attack, and the patient is only left with a lacunar amnesia that covers most of the time of the episode.

Semantic memory includes conceptual knowledge (knowledge about common objects), knowledge of famous people and public events, and personal autobiographical knowledge. It has been mostly investigated during the acute phase of TGA through explicit tasks such as giving an object's name, definition, or category but seldom through implicit tasks assessing the lexical–semantic priming effects. Reduced verbal fluency has sometimes been observed, and ascribed to impaired retrieval strategies, but conceptual knowledge is spared. Regarding lexical–semantic priming, it has been shown that despite their absolute anterograde episodic amnesia, patients are still able to acquire new conceptual semantic information such as learning how to solve sentence puzzles during the very acute phase of TGA attack. The dissociation between sparing of semantic memory and episodic amnesia impairment is such that patients who have just learned how to solve sentences puzzles have no explicit memory of the learning phase that took place a few minutes before. Performances at tests assessing the knowledge of famous people and public events fall below the normal range. Errors affect exclusively the most recent knowledge and suggest difficulties in the retrieval process, as further supported by the better scores in recognition than in free recall tasks. Impairment in personal autobiographical knowledge also involves the most recent period of time rather than earlier memories, extending at most back to 1 year before the attack.

Procedural memory has been explored in two ways showing that, during the attack, patients were able (1) to remember already-learned procedures as complex as driving a car, conducting an orchestra, or even taxiing an airplane for takeoff, and (2) to learn new cognitive procedures, such as perceptual-verbal and perceptual-motor skills (e.g., Tower of Toronto, mirror reading, or copy of complex geometric figures). Similarly, long-term perceptual priming (as studied with word completion or fragmented-picture identification tasks) can occur during TGA.

Working memory consists of two slave systems, the phonological loop and the visuospatial sketchpad, and of a supervisory system called the 'central executive.' The slave systems function normally during the acute phase of TGA. The integrity of the central executive is still a matter of debate, but its impairment, if any, is probably limited.

Psychopathology of TGA

That TGA may be triggered by emotional stress and that patients display emotional changes during the attack has prompted the hypothesis that TGA could have some relationship to psychogenic amnesia. Regarding emotional triggers, however, they do not appear to bear any specificity, and they only stand among a number of other possible events that have long been known to be classical TGA triggers for TGA as well. Similarly, there is no evidence so far that emotionally triggered TGA attacks have a specific clinical profile as compared with the remaining cases. It seems clear, however, from group studies, that TGA patients who suffered the attack after an emotional precipitant have, more often than not, a personal history of anxiety. This may mean that, in such people, a stressful event is more likely to have physical consequences than in others, as is true for various psychosomatic diseases. As far as TGA is concerned, it is possible that the physiological disturbances resulting from the stress reaction impinge on the brain chemistry in such a way as to modify the concentration of one or another of the molecules involved in the metabolism of the limbic system. To date, however, the mechanism by which such interference might occur is still a matter of pure speculation.

The emotional changes that occur during the acute phase of TGA may be more relevant to the present discussion, because triggers, which may or may not exist, and which vary from one patient to another, occur in all patients. Again, anxiety is the core feature of these changes, with associated symptoms of depression sometimes, as demonstrated by examinations carried out during attacks by means of specific scales. The emotional state interferes with memory processes, and it is common knowledge that, for example, depression impairs episodic memory, or that emotional items are processed differently than neutral ones. The influence of the emotional state of patients upon memory scores during the acute phase of TGA has been demonstrated, and so has been the relationship between emotional changes during the attack and persistent subtle memory deficits that can sometimes still be observed the day after or even later. The amnesic syndrome of TGA, however, is nowhere near to those of psychogenic amnesia. Hysterical amnesia, for example, is primarily retrograde in nature, predominantly affects personal memories such as identity or significant autobiographical events of the past, and usually lasts from days to months; however, no such clinical profile has been shown so far to result from a demonstrable brain lesion or dysfunction. While hysterical conversion does occur in anxious and depressed subjects, the significance of behavioral and emotional changes in this setting is thus completely different from that of TGA patients.

Episodic memory is primarily dependent on the integrity of well-identified cerebral structures, located within the limbic system, which also supports the expression and experience of emotions. It is thus tempting to speculate that any dysfunction of this system severe enough to cause a total episodic amnesia could have some impact on the emotional state as well. Further, the repetitive and anxious questioning of patients about time during the acute phase, as well as the questions invariably asked the day after about what they may have done during the period of which they have no memory, may somehow reflect the feeling of losing their personal identity, insofar as it is episodic memory that supports the perception of someone's life continuity.

Pathophysiology of TGA

TGA is a completely transient disorder that leaves no trace except for a memory gap. To find (1) the nervous structure the dysfunction of which causes TGA, and (2) the cause and mechanism of this dysfunction, are thus particularly demanding tasks.

As far as the location of the brain dysfunction is concerned, there is growing evidence that this could be the hippocampal cortex. The hippocampus is located on the medial aspect of the temporal lobe, within the fifth temporal gyrus, and is a key element of the brain memory circuitry. Other parts of this circuitry (the so-called Papez loop) are equally important, but the clinical profile of TGA episodic amnesia is closer to that of amnesic syndromes resulting from hippocampal damage than to any other type of amnesia. Unfortunately, material evidence is still lacking. Conventional morphological brain imaging (computed tomography (CT) and 1.5-T magnetic resonance imaging (MRI)) are always normal in TGA, and the results of the few studies using functional imaging such as positron emission tomography (PET) or single-photon emission tomography (SPECT) have been too inconsistent to allow any definitive conclusion. Recent studies with 3T-MRI seem more promising in that small hippocampal hyperintensities on diffusion-weighted sequences have been found in some patients but their meaning is still uncertain.

When faced with a transient dysfunction of the central nervous system, the neurologist always thinks of either epilepsy or transient ischemia. None of these mechanisms is at work in TGA. Transient amnestic epilepsy does exist but its duration is shorter than that of TGA, and epilepsy is generally a chronic disease such that seizures recur frequently, which is not the case of TGA, although as many as 20% recurrence may exist.

Electroencephalographic tracings done during TGA attacks are usually normal. Regarding transient ischemia, it is more a matter of differential diagnosis. A few cases of

acute and transitory amnesia associated with stroke (mainly anterior thalamic ischemia or hemorrhage) but then the lesion is demonstrated by the CT or MRI examination. On epidemiological grounds, the only common factor between transient ischemic accidents (TIAs) and TGA is the age range of the patients. Otherwise, TGA patients share neither the vascular risk factors nor the poor vascular prognosis of TIA patients. Finally, unlike in TIAs, there is no such permanent purely episodic and isolated cerebral vascular syndrome that would be the stroke equivalent of TGA. Thus, while, on an individual basis, an episode of TGA may lead to the disclosure of hypertension or diabetes, there are no firm grounds to state that TGA is a transient ischemic cerebral syndrome.

The viewpoint that TGA may be triggered by vigorous exercise has led some authors to search for anatomical abnormalities that could predispose to TGA in case of increased intrathoracic pressure. Abnormally high incidences of patent foramen ovale and venous jugular incontinence were found in TGA patients versus control subjects, which might carry an increased risk of paradoxical cerebral embolism and venous cerebral ischemia, respectively. No evidence for either of these phenomena has been gathered to date and the significance of these findings should probably be reassessed. The time course, duration, reversibility, and the frequent occurrence of headache at sometime during or immediately after TGA have suggested that there could be some pathophysiological connection with migraine. A number of experts think that the migraine aura, which consists of reversible cerebral symptoms (paresthesiae, visual disturbances, and/or aphasia) that usually last from 15 to 60 min occurring before headache, is associated with cortical spreading depression (SD). This phenomenon has been described experimentally in animals and can be triggered by a variety of stimuli directly applied on the brain cortex (electrical, chemical, mechanical, etc.). It consists of a depolarizing wave that extends on the brain cortex and induces local electrical changes. While its relevance to human pathology is unknown, SD could account for acute, relatively lasting, and reversible phenomena such as those seen in the migraine aura or in TGA. To explain the clinical homogeneity of the TGA syndrome, it can be hypothesized that the same neuronal mechanism (SD) is at work in all cases during the attack. This, on the other hand, could be triggered by a variety of causes, either external such as physical or emotional stress, or individual such as cerebral circulatory disturbances, in combination with any possible predisposing physical or psychological factor. This would account for the apparent heterogeneity found in all studies of TGA patients, with such different subgroups as anxious people, migrainous individuals, and subjects with vascular risk factors of any type, as demonstrated recently in a large group study.

See also: Amnesia.

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Cognitive Control in the Service of Self-Regulation

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Glossary

- Cognitive control** – The organization of thoughts, behaviors, and emotions to achieve selected goals.
- Conflict** – The cognitive state that arises from competition between different behavioral options when only one option may be selected.
- Dorsolateral prefrontal cortex** – An area of the prefrontal cortex that comprises Brodmann's areas 9 and 46 and that subserves working memory and top-down attention.
- Response inhibition** – Inhibition of behavioral responses that are goal or task irrelevant.
- Self-regulation** – It encompasses cognitive control as well as the selection of goals and their interactions with current and learned parental, social, and cultural influences.
- Striatum** – The major input structure of the basal ganglia, consisting of the caudate nucleus and putamen. Portions of the striatum are important for the encoding of well-learned motor and cognitive tasks.
- Stroop Task** – Cognitive task in which each trial consists of a colored word. In congruent trials, the ink color is the same as the color that the word denotes. In incongruent trials, the ink color differs from the color that the word denotes. The subject's task is to name the ink color. The conflict inherent in incongruent trials causes participants to take longer to respond and make more errors on those trials than on congruent ones.
- Top-down attention** – Goal-driven selection of task-relevant stimuli or features that may not be the most salient and might, therefore, not be attended otherwise.
- Ventrolateral prefrontal cortex** – An area of the prefrontal cortex that comprises Brodmann's area 47 and that is important for response inhibition.

Introduction

Conflict arises from competition between different behavioral options when only one may be selected. Conflict is ubiquitous in everyday life, in that we are constantly pulled in multiple directions. On a given day, we may need to accomplish a task that others are depending on us to complete while we are simultaneously tempted by

more pleasing activities. We may have to finish a review for an employer or pay the bills when a friend calls with an invitation to watch a sporting event. Even choosing not to act may produce conflict, since it involves an assessment of the risks and benefits of not undertaking possible actions.

Appropriate resolution of conflict occurs when the choice among competing behavioral options offers the greatest overall benefit to the individual. Often, this requires the inhibition of automatic or immediately rewarding options in favor of nonautomatic, effortful options that are better aligned with the organism's long-range goals. This requires cognitive control – the goal-oriented organization of thoughts, behaviors, and emotions.

The concept of cognitive control poses the difficult questions of who is doing the controlling and why? Who is controlling the controller? These questions raise the age-old paradox of the homunculus pulling at the strings of thought and action. The paradox boils down to the problem of the origins of goal selection. Why do we choose to pursue certain goals rather than others? These choices are the products of the complex influences and interactions of individual, familial, societal, and cultural values, reinforcements, and other determinants. Cognitive control, in contrast, refers to the mechanisms supporting the coordination of thought and action to achieve given goals; the origin of the goals themselves is beyond the scope of cognitive control.

The term 'cognitive control' comes from cognitive psychology and cognitive neuroscience; a related term that is used in these and a variety of other disciplines is 'self-regulation'. The latter term is, sometimes, used with slightly different meanings across disciplines. For our purposes, the concept of self-regulation is related to, but broader than, that of cognitive control. Whereas cognitive control refers to a set of cognitive mechanisms that allow action to be aligned with internal goals, the concept of self-regulation also includes the origin of those goals and their interaction with current and learned parental, social, and cultural influences. Self-regulation often engages cognitive control mechanisms to achieve goals, but it also includes the broader context and choice of those goals. For example, self-regulation may include a set of internalized moral imperatives according to which an individual seeks to behave, and it may establish goals consistent with those moral imperatives. Cognitive

control can then be engaged to achieve those goals (e.g., a person may inhibit the impulse to buy an expensive material object so as to use the money instead to help a friend in need).

Self-regulation and cognitive control play a central role in normal mental and behavioral functioning, and its impairment is a central sign in many neuropsychiatric and behavioral disorders. We can consider the example of people who suffer from bipolar disorder (BD), who have great difficulty inhibiting their responses to behavioral options that offer immediate rewards, such as sex or spending sprees. At the same time, they may neglect longer-term goals that would produce more rewarding long-term gains, while they simultaneously incur many painful consequences of their impulsive behavior.

Attention in the Service of Cognitive Control

Attention allows an individual to resolve conflict by selecting task-relevant information and ignoring task-irrelevant information. This is best illustrated by a classic example from experimental psychology, the Stroop interference effect, named after its discoverer. Participants are asked to identify the ink color in which words are written. Conflict arises because the words also spell names of colors, which may or may not be the same as the ink color. Each trial consists of a single colored word. In congruent trials, the ink color is the same as the color that the word denotes. In incongruent (conflict) trials, the ink color differs from the color that the written word denotes. Participants take longer to respond and they make more errors on incongruent trials than on congruent

ones. This is one of the most robust and best-replicated findings in all of behavioral neuroscience.

Responding in incongruent trials is slow and prone to error because reading is a highly practiced, automatic task, whereas color naming is not. To name the ink color rather than read the word, participants must, therefore, inhibit the more automatic task in order to correctly perform the less automatic one. This requires the allocation of attentional resources and the resolution of the two competing behavioral tendencies, the naming of colors and word reading. Resolving these two tendencies requires more time to complete the task. Failure to boost activity appropriately in the neural pathways associated with color naming or to suppress activity in the neural pathways associated with word reading produces error, which nearly always is responding with the color that the conflicting word denotes. In contrast, when the ink color is the same as the color that the word denotes, the response tendencies for the two tasks are identical, and the task is much easier and less prone to error. In fact, the presence of a congruent word speeds the naming of the ink color, compared to the naming of the ink color of, for example, a colored patch.

One model of the Stroop task helps to explain how the human uses attention to boost activity in task-relevant neural pathways when faced with competing activation in task-irrelevant pathways. Several versions of this model have been developed over the years, but the basic mechanisms underlying all of them are similar. A typical version of the model is depicted in **Figure 1**. It includes a sensory input layer, an association layer, and a response layer – all of which are connected via feedforward connections. Separate units in the input layer represent the written text and the ink's color of a colored word. These

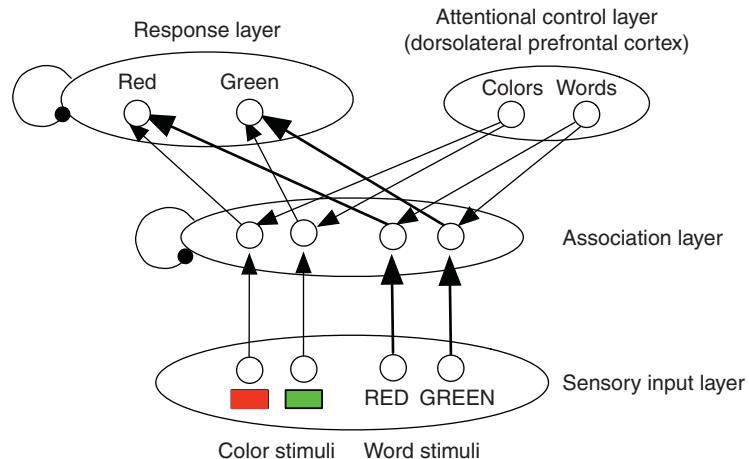


Figure 1 Connectionist model of the Stroop task. The model consists of three layers – sensory input, association, and response layers – that communicate through feedforward connections. Additionally, an attentional control layer provides top-down control to bias either the word-reading or the color-naming pathways. Layers are indicated by ovals; processing units within layers are indicated by circles. Connections between units are indicated by arrows. The width of the arrows represents the strength of the connections.

units project to corresponding units in the association layer, where lateral inhibition introduces competition between the units. Further competition occurs at the response level via lateral inhibition between the units that represent the possible responses. An attentional control layer, representing the dorsolateral prefrontal cortex (DLPFC), projects to the association layer. The role of the attentional control layer is to bias activation in the association layer in a task-relevant way. Such biases are often called ‘top-down’ influences on activity in the association layer, reflecting the common notion that the brain is organized hierarchically, with the DLPFC at the top of the hierarchy controlling activity in areas lower in the hierarchy, such as the posterior cortex and subcortex. We note that the validity of this hierarchical view of the brain is not essential to the validity of the connectionist model. All that is essential to the latter is the capacity to flexibly, but selectively, alter the strength of activity in one pathway compared with activity in another pathway according to task demands.

The strength of connections from one layer to another, indicated by the width of the arrows in **Figure 1**, reflects the degree of automaticity of the competing behavioral tendencies of word reading and color naming. Thus, connections are stronger for the word-reading than for the color-naming pathway. Incongruent stimuli will, therefore, activate the units in the word-reading pathway more strongly and, without top-down attentional control, will produce a response that denotes the written word. The attentional control layer, however, contains units that can provide top-down activation that can differentially boost activity in either the color-naming or the word-reading pathway. Appropriately activating the ‘colors’ unit in the attentional control layer increases responsivity of the color-naming pathway to support color naming, even for incongruent stimuli. Nevertheless, color naming is slower and more prone to errors when responding to incongruent than to congruent stimuli, because incongruent words interfere with the processing and naming of colors via the competitive dynamics present in the association and response layers.

A key question not addressed by the model is how the instructions to name the colors or read the words are translated into appropriate activations in the attentional control layer. This question is even more complicated if one generalizes the model to address the allocation of attention that is required to pursue dynamically changing goals as encountered in everyday life. How are goals translated into the appropriate patterns of activation in the attentional control layer? How are the appropriate connections from that layer to the associative layer formed?

Unfortunately, the model is not yet designed to address fully questions that extend beyond cognitive control and into the broader domain of self-regulation. To

perform appropriately in the Stroop task, for example, participants must have the goal of complying with the task instructions. But what is the origin of that goal? It likely is a product of complex interactions among the participant’s understanding of the desirability of certain types of behavior in given social or academic situations, the desire to perform well, or the motivation provided by external rewards, if available. While these processes of goal selection are taken for granted in most experimental situations, their influence is likely to be even greater, and their interactions more complex in real life. Undoubtedly, the appropriate allocation of attention or inhibition of task-irrelevant responses requires clear goals. In experimental settings, these goals often are clearly defined by the experimenter (but assuming that the participant wants to comply with task instructions). However, in real life, goals themselves are dynamic and often underdetermined. A key component of self-regulation is the ability to select goals consistent with one’s values. Future research on self-regulation should emphasize the process of goal selection, the relation between goals and learned values, and the origin of values themselves.

The degree to which a given task is automatic or requires effort is not fixed but, instead, can be changed by practice. The effects of practice and prior experience on the degree of automaticity of behavioral tendencies may reflect the strengthening of synapses from the input layer to the association layer, and from the association layer to the response layer for the relevant task. As the pathway corresponding to a given behavioral tendency is strengthened, execution of tasks that engage that behavior relies less on top-down control by the DLPFC. Evidence for this claim comes from the finding that activation of the DLPFC decreases with additional training in given tasks.

The prefrontal cortex and basal ganglia are intimately interconnected in cortico-striato-thalamo-cortical (CSTC) loops. At least five such loops have been identified, each involving projections to and from different cortical areas. With extensive training, activity during performance of motor-planning tasks shifts from the CSTC loop that involves the DLPFC to the CSTC loop that involves sensory and motor cortices. Behavioral tendencies that are more controlled and effortful on the one extreme, therefore, lie along a continuum with more automatic behavioral tendencies on the other extreme, with the degree of control and automaticity changing along this continuum as a consequence of prior experience and practice.

Neural Monitoring of Conflict

Discerning the nature of conflicting behavioral tendencies and detecting when they occur is essential to the appropriate resolution of conflict. Conflict can occur when the

processing of two or more stimuli competes or when a response must be selected from among two or more competing behavioral responses. Considerable experimental evidence suggests that the ACC (Brodmann's Area 24) is the neural locus for detecting conflict both at the level of stimulus processing and at the level of response.

The ACC can help to resolve detected conflict only if it signals the presence of the conflict to a neural system, such as the DLPFC, that can then appropriately deploy the attentional resources and control required to resolve the conflict that accompanies the simultaneous presence of conflicting stimulus processing or response tendencies, as occurs in the Stroop effect. The ACC and DLPFC, therefore, cooperate by detecting conflict within the ACC and then deploying the cognitive control systems within the DLPFC that are required for correct, goal-directed responding.

The ACC also seems to participate in detecting the occurrence of errors in responding. Studies using event-related potentials (ERPs), for example, have established firmly that the so-called 'Error Related Negativity,' a peak deflection over the ACC, occurs approximately 100 ms following an erroneous response. Multiple functional magnetic resonance imaging (fMRI) studies also report activity in the ACC following errors. These findings are consistent with those from studies using single-unit recordings in monkeys, which show that increases in activity of ACC neurons signal error when monkeys fail to inhibit a planned, task-guided saccade. The seemingly different conflict-detecting and error-detecting activities of the ACC can potentially be reconciled by considering the detection of errors as a form of conflict, one in which a person recognizes that the actual, erroneous response differs from (and, therefore, conflicts with) the correct response.

Similar to conflict in the Stroop task, which we might designate 'cognitive conflict,' 'emotional conflict' arises when an emotionally salient stimulus competes with task demands. Conflict in the classical color-naming Stroop task activates the dorsal division of the ACC. In the emotional Stroop task – in which conflict is produced by the presentation of words with emotional valence (e.g., 'murder') – conflict, instead, activates the ventral, or affective, division of the ACC. The dorsal and ventral ACC exhibit opposite patterns of activation, with the ventral ACC decreasing and the dorsal ACC increasing activity during cognitive conflict, whereas the dorsal ACC decreases and the ventral ACC increases activity during emotional conflict.

Neural Mechanisms of Control

The DLPFC uses top-down attention to enhance processing along task-relevant neural pathways. Top-down attentional processes should be distinguished from

bottom-up ones. Bottom-up attention is a consequence of the salience of stimulus features (e.g., loud noises) that automatically attract attention. Top-down attention, in contrast, involves the goal-driven selection of task-relevant stimuli or features that may not be the most salient and, thus, would not necessarily be attended using only the bottom-up mechanism. In the connectionist model of the Stroop Task, bottom-up processing is reflected in the strength of connections between the input, association, and response layers. Top-down processing is modeled by the top-down influences of the attentional control layer onto the association layer. In the Stroop task, when naming colors, participants use top-down processing to attend to colors and to override the bottom-up tendency to attend to and read words.

Top-down attention functions similarly irrespective of whether salience is determined by static stimulus features or by the frequency of stimulus presentation. Stimuli that are presented in an unpredictable, oddball fashion have greater salience and are thus more prone to capture attention. If such oddball stimuli are behaviorally irrelevant, greater top-down attentional control must be used to inhibit their processing.

The top-down attentional control exercised by the DLPFC is flexible and can change in the context of changing task goals, task demands, or motivational states. This contrasts with a habit-learning system that is based within the dorsal striatum and that guides action selection via previously learned stimulus-response (S-R) associations. One theory suggests that the prefrontal and dorsal striatal systems balance their control over behavior according to Bayesian principles, such that each system guides behavior when it is likely to be the most accurate of the two (i.e., when the uncertainty associated with its predictions is smaller than that associated with the other system's predictions).

Response Inhibition

In addition to the deployment of attention for selective stimulus processing, cognitive control depends on the inhibition of goal- or task-irrelevant responses. Appropriate development of response inhibition is also critically important for normal development, and impaired maturation of this cognitive ability may produce or predispose to disorders of impulse control. The prefrontal cortex is fundamental for response inhibition, just as it is for attention, consistent with the idea that the prefrontal cortex exerts top-down control over other brain regions and circuits. Top-down control is precisely the type of mechanism necessary to inhibit prepotent responses and responses that have already been initiated. The specific portions of the prefrontal cortex that are involved in response inhibition differ, however, from

those that have been implicated in attentional facilitation. Whereas attention relies largely on the DLPFC (and related, interconnected portions of parietal cortex), response inhibition appears to rely instead on the ventrolateral prefrontal cortex (VLPFC). Different areas of prefrontal cortex seem, therefore, to be specialized for different cognitive control functions.

Response inhibition is often studied using the Go/No-Go task. The participant must perform an action, such as pressing a button, when a given stimulus is presented ('Go' trials), and refrain from performing the action when a different stimulus is presented ('No-Go' trials). If Go trials are much more frequent than No-Go trials, the participant has difficulty inhibiting the response on No-Go trials, producing a higher error rate on the No-Go trials.

Another task commonly used to study response inhibition is the Stop-Signal Reaction Time (SSRT) task. In this task, participants perform a speeded response to a stimulus. On some trials, however, that stimulus is followed after a brief delay by a stop signal indicating that the response should not be performed. This task permits estimation of the SSRT, a measure of the time required to suppress a response. The greater this measure, the greater the difficulty in inhibiting a behavioral response. Many functional imaging studies show activation of the VLPFC in Go/No-Go and SSRT tasks, and lesion studies have shown that the extent of damage in the right VLPFC correlates with the SSRT, providing direct evidence for a causal role of the VLPFC in inhibiting the behavioral response during the SSRT task. Damage in other areas of the prefrontal cortex does not correlate with the SSRT, suggesting that response inhibition may depend primarily on VLPFC and not on other areas of prefrontal cortex. The prefrontal cortex and basal ganglia, moreover, seem to subserve distinct aspects of response selection and inhibition, with the striatum subserving the lower-order translation of stimuli to behavioral responses and the VLPFC and DLPFC subserving adaptation to changing task rules. Finally, backward-masking procedures used in conjunction with electrophysiological recordings have shown that inhibitory cues can be processed similarly with or without conscious awareness, and therefore the 'higher' cognitive process of behavioral inhibition can be influenced by stimuli that are outside of awareness.

Response inhibition seems to mature to a greater degree (i.e., it changes over a larger range of values) than does selective attention from childhood to adulthood. Performance differences on a Go/No-Go task are greater than performance differences on tasks of attentional control when measures of performance in young children are compared with performance measures on the same tasks in adults. Moreover, the difference in frontostriatal blood-oxygenation-level-dependent (BOLD) activation between young children and adults is greater for tasks requiring response inhibition than for tasks requiring selective

attention. The development of response inhibition may, therefore, be even more important than that of selective attention for behavioral control and the successful transition from childhood and adolescence into adulthood.

Impaired Control and Psychopathology

Impairments in attention or inhibitory control have been implicated in a wide variety of disorders, including attention-deficit/hyperactivity disorder, anorexia nervosa, bulimia nervosa, obsessive-compulsive disorder, and schizophrenia. In this article, we focus on the evidence for impaired cognitive control in BD as an example of the relevance of control to the understanding of psychopathological processes. A similar approach has been used for several other disorders.

BD is a chronic, but treatable, disorder of affect, thought, and behavior that produces long-term interpersonal and functional disability. *The Diagnostic and Statistical Manual*, Version IV (DSM-IV) defines BD by the history of at least one manic episode, defined as elevated mood and energy that lasts for at least a 2-week period. In addition, patients with BD often have periods of depression, with low mood, low energy, and loss of interest in people and activities that were previously enjoyable. Sufferers of BD have difficulty controlling their behaviors, thoughts, and emotions. They engage in impulsive behavior (e.g., spending sprees); they often have racing thoughts when in a manic state and difficulty controlling ruminations about negative experiences when in a depressed state; and they are emotionally labile, unable to control their emotions, which can change quickly over a large range, from profound sadness to excited elation. These symptoms suggest an impaired ability to exercise cognitive control of behaviors and thoughts as traditionally defined, as well as difficulty with the cognitive control of emotion, which sometimes is termed 'emotional regulation.'

Consistent with a putative deficit in cognitive control, patients with BD exhibit anatomical and functional abnormalities in brain regions and circuits that subserve cognitive control, including the ACC, DLPFC, and VLPFC. Persons with BD, for example, have reduced ACC and DLPFC activity compared with healthy controls while performing the Stroop task. Activity of the VLPFC is also lower in patients with BD who are in the euthymic state as they make erroneous, impulsive responses on the Go/No-Go task, suggesting that a trait deficit in top-down prefrontal control may underlie a behavioral tendency toward impulsivity in these people.

In addition to these deficits in cognitive control as traditionally defined, persons with BD are also reported to have anatomical and functional abnormalities in brain regions such as the orbitofrontal cortex and the ventral

portion of the medial wall, that have been implicated in emotional regulation. In addition, prefrontal control of affective state seems to be impaired in persons with BD, reflected in hypoactivation of the subgenual prefrontal cortex (Brodmann's Area 25), during depressive episodes. The abnormalities in prefrontal BOLD activation seen during depressed and manic episodes are generally hemisphere- and state-specific, with left prefrontal reductions reported during depressive states and right prefrontal reductions reported during mania.

Conclusion

The constructs of self-regulation and cognitive control that we have reviewed are useful in understanding both normal function and psychopathological processes. We have outlined self-regulation at several levels. First, we illustrated the differences between behaviors that are automatic and those that require effortful control. Second, we defined the central concept of conflict as it relates to the need for self-regulation and control, and we reviewed how the brain is thought to detect conflict and resolve it by appropriately deploying cognitive control. Third, we reviewed the role of response inhibition in the control of behavior. Finally, we have exemplified the usefulness of these concepts to understanding psychopathological processes through discussion of what are believed to be core deficits in self-regulatory control in persons with BD.

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See also: Animal Models of Bipolar Disorder; Attention and Speed of Information Processing; Brain Imaging; Cognition: Attention and Impulsivity; Emotion–Cognition Interactions; Evolutionary and Developmental Issues in Cognitive Neuroscience; Motivation; Neural Basis of Attention-Deficit/Hyperactivity Disorder.

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Conscious and the Unconscious

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Glossary

Continuous flash suppression – A method discovered by Tsuchiya and Koch for making visual stimuli subliminal. When a static stimulus is presented to one eye and a stream of rapidly changing images to the other eye, the static stimulus is consciously suppressed but still processed. Unlike pattern masking, continuous flash suppression allows conscious and unconscious stimuli to be presented for the same period of time (e.g., >1 s).

Event-related cortical potential (ERP) – An electric potential recorded from the scalp by an electroencephalogram in which the trace has been time-locked to the occurrence of a stimulus and noise-reduced by averaging over many trials. Characteristic negative-going and positive-going changes in voltage occur over the several hundred milliseconds following a stimulus onset that can, in principle, indicate the nature of the processing at different times. For example, it has been shown that a positive change in voltage at around 300 ms (the P300), associated with attention and memory processing, can be elicited by stimuli the person is not conscious of.

Implicit learning – Ability to acquire knowledge that is unconscious. For example, we can use the rules of grammar to comprehend and produce grammatical utterances within a fraction of a second, yet we cannot describe more than a few rules of grammar. We can also learn to appreciate certain styles of music, to obey cultural rules, or to gain perceptual motor mastery of a domain without consciously knowing the underlying regularities.

Implicit memory – The influence of a past event, on current behavior and judgments without being able to recollect the prior event. For example, people with anterograde amnesia, due to damage to the hippocampus and temporal lobes, are unable to form new biographical memories yet can be influenced by new events. If shown a list of words, they may not recall seeing the words, yet when given the starting letters of the words they will be more likely to complete the letter stems as the words because of having seen the list of words. Implicit memory is different from implicit learning in that implicit memory need not involve acquiring any unconscious knowledge: completing letter stems as a word involves only knowledge of which the person is conscious (*viz.*, the spelling of the word). In this case,

there are no unconscious contents, but implicit memory is often called unconscious memory because it is a form of memory – broadly construed – that the person is not conscious of as memory.

Pattern masking – One of the most common laboratory techniques for producing subliminal perception. The mask consists of jumbled parts of the target stimulus. If the mask occurs just before (forward masking) or just after (backward masking) the target, with the difference in stimulus onsets being on the order of 10–100 ms, an otherwise consciously reportable briefly flashed target is rendered subliminal. The mask is just as effective if presented to a different eye from the target, indicating the mask interrupts processing at a central level.

Signal detection theory (SDT) – It allows the experimenter to distinguish ‘discriminability’ and ‘bias’: discriminability is the ability of a subject to discriminate the presence versus the absence of a stimulus, and bias is the tendency of the subject to say ‘present’ rather than ‘absent.’ Imagine a light is shown very briefly on half of trials and the subject has to say ‘yes’ or ‘no’ in each trial. Subjects may be biased to say ‘no’ a large or small proportion of the time without their underlying perceptual ability to discriminate changing. In SDT, discriminability depends on the distance apart from the distribution of neural activation for when the stimulus is present and for when it is absent; the bias to say ‘yes’ depends on whether the criterion amount of neural activation above which ‘yes’ is said is low or high. With a strict criterion, a person may say ‘no’ almost all the time when a stimulus is presented and still have a high ability to discriminate.

Subliminal perception – The influences on thoughts, feelings, or actions of stimuli that affect neural activity but are not consciously reported. Unlike implicit learning, subliminal perception need not involve changes in the pattern of synaptic connections.

One of the most fundamental problems facing psychology and neuroscience is the nature of consciousness. In this article, we sketch a variety of broad theoretical positions, describe a range of behavioral and neurophysiological measures of consciousness in the context of these theories, and review key findings concerning the existence and nature of unconscious perception and

learning. Finally, we consider possible functions of consciousness.

Theories of Consciousness

Worldly Discrimination Theory

According to worldly discrimination theory (WDT), the content directly expressed in any behavior (e.g., pointing to where a dog is) is the content of a conscious mental state (e.g., consciously knowing “There is the dog”). Thus, a person shows that s/he is consciously aware of a feature in the world when s/he can discriminate it with choice behavior. This theory often makes use of signal detection theory (SDT), a statistical framework for quantifying the discriminability of a stimulus. SDT itself is mute on the subject of consciousness, and can thus be combined with different theories. The combination of SDT with WDT asserts that continuous information available for discriminations is necessarily the content of conscious mental states. That is, if over many trials a person can reliably indicate that a certain stimulus was present or not (to any degree above chance), then the information allowing that discrimination must be conscious, on WDT. This theory captures one property of conscious knowledge, namely that it allows choice behavior. However, rightly or wrongly, it does not respect other properties associated with consciousness. Consider blindsight patients, who have damage to the primary visual area in the cortex, V1. In the ‘blind’ part of their visual field they can make some discriminations very accurately; for example, whether an object is moving up or down. According to WDT, blindsight patients see movement consciously because forced-choice discrimination is the result by which we infer they see at all. However, two properties of blindsight suggest intuitively that the seeing is not conscious, and indeed are the reason why their ‘sight’ is called ‘blind.’ First, blindsight patients do not spontaneously attempt to use the information practically or inferentially. If they are thirsty, a glass of water in their blind field is not drunk. Second, blindsight patients themselves think they do not see. Indeed, they may insist that the attempt to discriminate shapes, motions, or sizes of objects in their blind field is nonsensical and they can offer only pure guesses. Other theories have tried to locate a divide between conscious and unconscious processes that respect one or both of these intuitions.

Integration Theories

According to integration theories, conscious contents are widely available to many cognitive and/or neural processes. This core idea has been variously expressed as, for example, fame in the brain, broadcast within a

global workspace (an idea made popular by Bernard Baars), activity within a ‘dynamic core,’ or integrated information (as elaborated respectively by Gerald Edelman and Giulio Tononi). The intuition offered by Edelman and Tononi is that, on the one hand, consciousness cannot arise in a system only making a simple discrimination similar to a thermostat: our conscious experience seems to always involve making many discriminations at once. On the other hand, consciousness cannot arise in a system merely making a set of independent discriminations similar to a photographic plate: our conscious experience seems interconnected, integrated, and unified. According to dynamic core and integrated information theories, a mental state is conscious if it provides a sufficiently informative discrimination among a large repertoire of possible states, where successful discrimination requires both differentiation and integration.

Higher-Order Thought Theories

According to higher-order thought (HOT) theories, a mental state is conscious when we are aware of being in that state, a view pursued by David Rosenthal and Peter Carruthers among others. Accordingly, it would be odd to say that a mental state is conscious yet, the person is not aware of being in that state. HOT theories differ from WDT in that it is the ability to tell the mental state one is in, rather than what state the world is in, that determines whether a mental state is conscious. In the context of SDT, HOT theory is associated either with the criterion of standard SDT, or with the second level of discrimination: discriminating not the world (as in WDT), but the accuracy of one’s responses (i.e., whether responses reflect knowledge or guessing).

The above theories, excepting some neural integration theories, describe conditions for asserting whether or not a particular mental state is conscious. They do not generally pertain to whether an organism is conscious or unconscious. These are scientifically different problems as, for example, a person who is unconscious (e.g., in a coma or dreaming) can have conscious mental states (as suggested by functional magnetic resonance imaging (fMRI) scans of some coma patients asked to imagine different activities: relevantly different parts of the brain become active). As we will see, measures of consciousness can and do address the conscious status of both mental states and individuals.

There is no consensus about which of the above theories best captures intuitions concerning consciousness, which is best supported by evidence, or which should be exclusively adopted in scientific research. Consequently, for current purposes we will treat the measures motivated by each theory as picking out a separate aspect of consciousness or mental life, of interest in its own right. Thus,

the behavioral ability to discriminate a feature in the world can be taken to indicate that a person is, in a very weak sense, conscious of that feature. Saying this much makes a less strong assumption than WDT; one can assume that a person can be (in a very weak sense) conscious of a feature without assuming that they are conscious of it with a conscious mental state. For example, a person with blindsight sees – and is therefore in that sense conscious of – an object moving up, even though the seeing is not conscious. For HOT theory, it is only if the person is aware of seeing that the seeing is a conscious mental state. Some philosophers disagree with this assumption; they prefer to say higher-order thoughts allow introspective or reflective consciousness but are not needed for mental states to be simply conscious. There is no need to quibble over words; clearly, the distinction between those perceptual or learning processes that allow awareness of knowledge and those that do not is interesting, whatever terms one uses (conscious vs. reflectively conscious, etc.). A conscious mental state also may or may not be inseparable from the global availability of the content of the state. However, the availability of information – for example, to figure in intentional control of one's actions – and the fine balance between differentiation and integration, are surely key features of our conscious mental life, regardless of whether one thinks they constitute conscious awareness. The degree of informational differentiation and integration may well be related to the level of consciousness of an individual. In fact, it will help consider a tripartite distinction (adapted from David Rosenthal) of being conscious of the world, being conscious of a mental state, and simply being conscious to some degree.

Behavioral Measures and Findings

Objective Measures

Objective measures refer to the ability to choose accurately under forced choice conditions. If one presupposes WDT, then such ability indicates a conscious mental state; with less strong assumptions, such ability simply indicates that one is conscious of the state of affairs discriminated. Conversely, knowledge is unconscious if a distinction in the world expresses itself only in nonintentional characteristics of behavior (such as its speed), or in physiological characteristics not expressed in behavior at all (e.g., galvanic skin response – how sweaty the person is; or brain activity as revealed by fMRI), or indirectly in other behaviors with related but different contents.

When objective measures indicate that people are not conscious of a masked visual stimulus, such as a word or a number, the impact of that stimulus on behavior and brain is greatly diminished compared to the effects of clearly visible stimuli. Nonetheless, some influences of the

masked stimulus persist. Greenwald and his colleagues have shown that when people are not conscious of masked flashed numbers (presented for 33 ms), the size of the number influences the speed of a subsequent judgment of a consciously presented number: semantic characteristics of the subliminal number are partially accessed, even if semantic access is generally diminished when a person is not conscious of a stimulus. Dehaene and colleagues have shown that when people are not conscious of masked words, nonetheless local field potentials, as revealed by implanted electrodes in the amygdala (part of the brain responsible for emotional perception), distinguish threatening from nonthreatening words. Similarly, when people are not conscious of an image of a naked body, nonetheless spatial attention is drawn to the image location. Skeptics worry that these and similar results may be due to the subject being conscious of the stimuli to a small degree: the inevitable noisy estimate of discriminability never allows knowing when discriminability is exactly zero. In any case, one can conclude that stimuli that one is at most minimally conscious of, can influence reaction times, brain activation, and other physiological measures.

Strategic Control

Strategic control determines the conscious status of knowledge by the person's ability to deliberately use or not use the knowledge according to instructions. In Larry Jacoby's process dissociation procedure, a person tries to avoid using the information (exclusion task) or make sure they do use it (inclusion task); any difference in influence of the stimulus between these conditions is taken to indicate conscious knowledge, and any use of it despite intentions in the exclusion condition is taken to indicate unconscious knowledge. This measure can be motivated by integration theories: the ready availability of information to inference and intentions shows its conscious nature on such theories. On higher-order theories, by contrast, there is no incompatibility between control and the relevant mental states being unconscious (e.g., as in hypnosis, where people can engage in strategic behavior without awareness of their strategies).

People flashed words can be asked to complete word stems either (1) with the flashed word (inclusion instructions) or (2) making sure it is not the flashed word (exclusion instructions). If the word is flashed quickly enough (about 50 ms), then people will complete the stem with the word at above baseline levels even when told not to (i.e., while under exclusion instructions). Although people use the word under inclusion instructions (so they are conscious of the word in a weak sense), they cannot use the information that the word was present in their intentions, as revealed by their inability to exclude the word (thus, there is poor integration of the information with mental activities generally). Similarly,

when words are presented to people under general anesthesia, in the next few days people have a tendency to reproduce the words to word stems even when told to avoid any words they think they heard under anesthesia.

People can gradually acquire knowledge of the structure of an environment (by implicit learning) in such a way that the knowledge does not allow strategic control. For example, in the serial reaction time (SRT) task, used by Axel Cleeremans and others, people have to press one of four buttons depending on whether an 'X' appears in one of four corresponding locations. Unbeknownst to people, there is a predictable sequence of locations. Consequently, people become faster and faster at pressing the buttons. People are subsequently told about the existence of the sequence, and asked to produce a sequence that was not the one they had just been exposed to (exclusion instructions). If the training sequence is not highly predictable and is presented quickly, people cannot help but tend to produce the training sequence in exclusion, that is, even when trying to avoid it.

Subjective Measures

Subjective measures test whether people are aware of the mental states they are in. When a person successfully makes a series of discriminations about the world, to test for awareness of knowing, confidence ratings can be given on each trial. If, on all the trials, when the person says 'guess' nonetheless the discrimination performance is above baseline, then there is evidence that the person has knowledge about the world that they do, not know have: unconscious knowledge according to higher order theories (the guessing criterion). Further, if a person is aware of when they know and when they are just guessing, there should be a relation between confidence and accuracy. Thus, no relation between confidence and accuracy is another indication of unconscious knowledge according to higher-order theories (the zero-correlation criterion). Sometimes methodologically weaker forms of subjective measures are used, for example, only after a block of trials or a whole experiment are people asked to report if they saw anything or knew about a relation.

Right from the very beginning of research into subliminal perception in the nineteenth century, subjective measures were used. The fact that people can be substantially above chance in perceptual judgments (e.g., about what words were displayed) when believing they are just guessing is a robust phenomenon. Such stimuli can produce widespread brain activations and strong effects on choice behavior. Further, flashing unusual shapes that people are not aware of seeing can make people like those shapes more in the future, a phenomenon called 'the mere exposure effect.' Flashing brand names of a thirst-quenching drink can dispose people to choose that brand if they are thirsty. When people are not aware of

seeing stimuli, the stimuli can have further intriguing effects. For example, flashing images of very intelligent people can reduce self-esteem; flashing images of clowns or unattractive people can increase self-esteem. Complicated masking procedures are not necessary to interfere with awareness of seeing; unattended words can influence people who think they did not see any words (so-called inattentional blindness). In contrast to these positive effects, there is no evidence that commercially available subliminal self-help tapes have any effect beyond placebo on study effectiveness, memory ability, or self-esteem.

Various types of brain damage interfere with awareness of seeing. People with blindsight can discriminate shape, size, orientation, and even the emotional expression of faces without being aware of seeing, though processing the meaning of words is limited. People with visual neglect (typically with damage to the right inferior parietal lobe) are often not aware of seeing stimuli in the left side of space. Nonetheless, even the meaning of such stimuli can facilitate processing stimuli on the right-hand side of space for these people.

People can gradually acquire knowledge of the structure of a domain (implicit learning) without being aware of their knowledge. Arthur Reber asked people to memorize strings of letters made by an artificial grammar. When people were subsequently informed about the existence of a grammar, they could classify new strings as obeying the grammar or not without being able to say what the rules were. Indeed, people can classify above chance when they believe that they are literally guessing or using nothing but intuition.

A possible weakness of subjective measures arises when the terms on the scale used are not well defined to people. The term 'guessing' can mean simply "I am not very sure" in everyday life: having knowledge without being absolutely certain does not entail the person is unaware of the knowledge. Nonetheless, in the case of artificial grammar learning at least, people can classify above baseline even when they would bet on a random event such as a coin toss rather than on their own classification decision. In sum, people can have knowledge even when they sincerely believe they do not.

Thus far, the measures have dealt with whether or not a person is conscious of either an event or a mental state (conscious contents). Next we consider some measures that can also bear on the conscious level of an individual.

Brain Measures

EEG Measures

In 1929, Hans Berger discovered that waking consciousness is associated with low-amplitude, irregular electroencephalographic (EEG) activity in the 20–70 Hz range and it

is now known that unconscious conditions such as non-dreaming deep sleep, coma, general anesthesia, and epileptic absence seizures show predominantly low-frequency, regular, and high-amplitude oscillations. Event-related cortical potentials (ERPs) have been used to assess whether a stimulus is consciously perceived or not, though there is dispute about whether early components are most indicative of conscious awareness (i.e., amplitude changes ~ 100 ms vs. ~ 300 ms after stimulus onset). Such ERPs are also associated with other functions beyond consciousness *per se*, for example, in novelty detection, so are unlikely to serve as pure consciousness indices. The proprietary ‘bispectral index’ combines various aspects of the EEG signal to estimate anesthetic depth (conscious level) and hence, probability of accidental waking during surgery. EEG measures of consciousness float free of theory, only gaining purchase through attempted reliable correlations with one or more of the behavioral measures above.

Neural Correlates

A useful way of addressing experimentally the distinction between conscious and unconscious processing is via the influential notion of a ‘neural correlate,’ which refers to patterns of activity in brain regions or groups of neurons that have privileged status in the generation of conscious experience. For example, Stanislas Dehaene and colleagues have shown using fMRI that consciously perceived words evoke widespread cortical activity as compared to equivalent masked stimuli the subject is not conscious of, which evoke only local activity. This evidence is congruent with integration theories such as global workspace theory, though other evidence suggests that being conscious versus unconscious of a mental state may involve a difference in activation only in a small area: the mid-dorsolateral prefrontal cortex. Another ongoing controversy is whether synchronized neural activity, for example, in the so-called ‘gamma’ band (~ 20 – 50 Hz) and especially around 40 Hz, constitutes a dynamical correlate of consciousness; the intuition here is that neural synchrony may account for the ‘binding’ of contents within integrated conscious scenes.

In addition to investigating the neural correlates of conscious contents (of the world or of mental states), it is also possible to examine the neural correlates underlying the conscious level of an individual. Here, ample evidence indicates that normal consciousness is specifically associated with thalamus and cortex. Regions such as the hippocampal system and cerebellum can be damaged without a loss of consciousness *per se*; indeed, in cases such as Rasmussen encephalitis, an entire cortical hemisphere can be surgically removed without loss of consciousness (though not without other effects). Whereas highly localized cortical damage can delete specific conscious contents such as color, shape, and motion, damage to

certain thalamic nuclei or to brainstem regions such as the reticular formation can abolish consciousness permanently. Finally, brain imaging evidence (pioneered by Steven Laureys) has shown that even in apparently unconscious conditions such as the ‘persistent vegetative state,’ stimuli can evoke significant though not widespread cortical responses, as compared to healthy controls. Such imaging studies provide a much needed fine-grained means of diagnosing disturbances of conscious level and establishing recovery potential of brain trauma patients.

Neural Complexity

Several recent measures build on the observation that conscious scenes are distinguished by being simultaneously integrated (each conscious scene is experienced ‘all of a piece’) and differentiated (each conscious scene is composed of many distinguishable components and is therefore different from every other conscious scene). The ‘dynamic core hypothesis’ (DCH) of Gerald Edelman and Giulio Tononi proposes that consciousness arises from neural dynamics in the thalamocortical system with just these features, as measured by the quantity ‘neural complexity’ (C_N). C_N is an information-theoretic measure that is high if each subset of a neural system can take on many different states and if these states make a difference to the rest of the system. The ‘information integration theory of consciousness’ (IITC; proposed by Tononi a few years after the DCH) shares with the DCH the idea that conscious experiences provide informative discriminations among a vast repertoire of possible experiences. In the IITC, the quantity Φ is defined as the ‘information integrated’ across the informational ‘weakest link’ of a system. Importantly, Φ is a measure of the capacity of a neural system to integrate information, whereas C_N is a measure of the actual dynamics of the system. A third measure, ‘causal density,’ measures the fraction of causal interactions among elements of a system that are causally significant; it is low both for highly integrated systems and for collections of independent elements. While complexity measures such as these cannot yet be applied to interactions between all sets of neurons in a whole brain, they can be applied to the summaries of neural activity at multiple sites as provided by, for example, EEG or fMRI. Work employing and developing these measures has only just begun.

The Function of Consciousness

The different theories of consciousness imply different functions. According to WDT, consciousness allows behavioral discrimination in the world; yet, WDT begs the question of why consciousness is required: intuitively

most people accept that automata without consciousness can make discriminations. Integration theory offers clear functions, depending on the theory, to do with flexibility or dealing with novelty. Higher-order theories need to locate the function of consciousness not in the powers of any mental state about the world, but in what can be gained by being aware of such mental states.

Functions for consciousness are easy to suggest though hard to establish. It has been suggested that consciousness allows flexibility, intentional action, or ability to deal with novelty. Yet hypnotizable people can engage in novel intentional actions without being aware of their intentions. For example, they can 'forget' the number 4, counting '1, 2, 3, 5, ...', flexibly overcoming habit, without being aware of any intention or mental state containing the content 'four.' It has been suggested that consciousness allows rationality, yet at least sometimes its reasoning function is only *post hoc*, as revealed by 'choice blindness': people are asked to select the most attractive of two faces and then to describe why they made each selection. On some trials the pictures were switched by sleight of hand immediately after a choice was made; subjects often failed to detect the switch but nevertheless offered a plausible account why they chose a particular face. Indeed, it has been shown that sometimes deliberating on a complex choice leads to worse decisions than being distracted by another task. It has been suggested that consciousness of our own mental states allows us to be conscious of others' mental states, allowing more sophisticated social interactions. Yet we can be affected by the emotion of a face without being conscious of it. Finally, it has been suggested that our rich consciousness of our mental lives arose from sexual selection: it arose

precisely because, similar to the peacock's tail, it is costly yet pointless.

See also: Amnesia; Declarative Memory; Hallucinations in Neuropsychiatry and Drug Abuse: From Phenomenology to Pathophysiology; Implicit Learning and Memory: Psychological and Neural Aspects; Sleep: Learning and Memory; Subjective Experience and the Expression of Emotion in Man.

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Disorders of Face Processing

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Glossary

Capgras syndrome – A disorder in which the patient has the feeling that an impostor has stolen the face and the appearance of a close acquaintance, usually the spouse. This syndrome can sometimes also apply to other objects than faces.

Configural processing – Different types of configural processing are distinguished in the literature on faces. The ability to detect two eyes above a nose and a mouth is called ‘first-order configural processing.’ Holistic configural processing is related to the ability to build a gestalt out of the face elements, with the idea that the whole is more than the parts. Second-order processing is related to the processing of the distance between these elements.

Metamorphopsia – A disorder in which faces or other objects appear distorted.

Person agnosia – A disorder in which patients have a loss of semantic knowledge about people. Consequently, people cannot be recognized through any modality (such as face, voice, or name).

Prosopagnosia – A visual disorder in which familiar faces are not recognized anymore. In the pure form of this disorder, other objects such as tools and animals are recognized normally.

Prosopamnesia – An impaired learning of new faces encountered after brain lesion. In contrast, processing of faces learned premorbidly is normal.

Faces are visual stimuli that are both socially important and complex to process. They are important because a wealth of information is extracted simultaneously from them – not only identity, but also gender, age, expression, intention, gaze, lip reading, trustworthiness, attractiveness, etc. All this information must be extracted quickly and reliably to ensure proper social interactions. Faces, compared to other object categories (e.g., the tool category), are also complex because they are all very similar to each other. Furthermore, faces are usually processed at the exemplar level, that is, each face must be individualized. Information about a face is also extracted from a variety of elements, some of which are subtle, such as eyes–nose–mouth configuration or

zygomatic contracture. Overall, face processing is a challenge to the visual system. Probably due to its social importance and visual complexity, a variety of disorders can affect faces to a greater extent compared to other classes of objects.

In this article, we first describe prosopagnosia, the most well-known disorder of face processing. Following this description, we present several of the key issues that have been debated since prosopagnosia was identified as a specific clinical syndrome. These issues are: varieties of prosopagnosia; overt versus covert recognition; site of the lesions; face versus object recognition; prosopagnosia and facial expression; and finally, mechanisms that are impaired in prosopagnosia. A review of the pathogenesis, epidemiology, and criteria/diagnostic work-up tests follows. In a second section, misidentification delusional syndromes (Capgras, Fregoli, and intermetamorphopsia) and the mirror sign are reported.

Definition and History of Prosopagnosia

The most well-known disorder of face processing is prosopagnosia, a disorder of face identification. Prosopagnosia is an inability to recognize familiar persons from their faces. Prosopagnosic individuals also have difficulties learning new faces. Although this is not systematically the case, visual processing of other objects can be normal, highlighting the specificity of this syndrome. The impairment cannot be explained by a general cognitive dysfunction. Face detection, that is, knowing that a face is a face, is normal. One of the characteristics of prosopagnosics is that they have good skills at using extra-facial visual cues, such as a specific haircut or distinctive trait, gait or posture, further highlighting that it is facial features that they cannot process. Recognition using other modalities such as voice or name is preserved. Prosopagnosia should, therefore, be distinguished from person agnosia (also called cross-modal agnosia for persons), which is characterized by impaired biographical or semantic knowledge about people. In person agnosia, familiar people cannot be identified through any modality (e.g., face, voice, name, verbal description).

Similarly to most neuropsychological syndromes, prosopagnosia does not occur in an all-or-none fashion. In some severe cases, however, the inability to recognize faces can even extend to the patient's close relatives and his/her own face. Patients with prosopagnosia usually tend to hide their deficit, as it is very embarrassing and disabling in daily life. Since familiar persons cannot be recognized from their faces, prosopagnosic patients develop good skills at identifying persons using other cues. They may also develop explicit coping strategies, for example, asking a standard question in order to hear the voice of the person they want to identify.

Prosopagnosia is the most well-known face-processing disorder because of the implications it has in so many social interactions. The cognitive system that subserves face identification may be viewed as a hub providing access (once the face has been properly identified) not only to the name of the person, but also to the affective valence related to that person, to information regarding social hierarchy, to the personal life episodes related to that person, to the plans made with this person, etc. All of this information is crucial for adequate interactions.

Although there have been various reports, from as far back as the nineteenth century, of patients with difficulties in recognizing persons from their face, the name 'prosopagnosia' was coined in 1947 by a German psychiatrist, Joachim Bodamer. He was among the first to argue that some patients may show isolated impairment of face processing, rather than a general impairment in visual object processing. Although there have been some group reports of prosopagnosic patients, neuropsychological single-case studies are more common in the literature on prosopagnosia due to the rarity of this syndrome and also because single-case studies allow an in-depth and extensive investigation of the domains and processes that are impaired or preserved on an individual basis. More recently, brain imaging of prosopagnosic patients has opened new research perspectives such as characterizing the network of brain areas involved in face identification or studying how various categories of faces are processed (such as famous faces vs. parents' faces).

Varieties of Prosopagnosia

Based on the distinction made between apperceptive and associative visual agnosia (a disorder limited to objects other than faces), a similar dissociation between apperceptive and associative prosopagnosia is sometimes proposed in cognitive neuropsychological models. Apperceptive prosopagnosia refers to a difficulty in building a proper percept of a face (i.e., abnormal face perception). Consequently, this (improper) percept cannot be matched to stored

representations of faces in memory and cannot be recognized as familiar or not. On the other hand, face perception is often considered to be normal in associative prosopagnosia, even though intact perceptual representations cannot access corresponding memory representations. Although this taxonomy has some clinical utility in distinguishing patients and in understanding some aspects of face processing, such as covert recognition, it is unclear if this distinction is valid. As mentioned earlier, the apperceptive/associative distinction largely stems from cognitive neuropsychological (box and arrows) models, and, given the current knowledge, it is uncertain how a percept can be processed independently of any memory trace activation or top-down activation. It is also possible that the visuoperceptual abilities of reported cases of associative prosopagnosia have not been exhaustively investigated.

Overt versus Covert Recognition

Prosopagnosic patients show impaired overt (explicit) recognition. There are, however, considerable reports indicating that some of these patients show preserved covert (implicit or unconscious) recognition. A variety of methods have been used to study covert recognition such as skin conductance responses, behavioral methods (reaction times, faster relearning, etc.), or electrophysiological recordings. However, it is not yet clear how findings from these different methods can be reconciled. There are broadly three models accounting for covert recognition. One proposes that recognition is carried out correctly but that the outcome of this computation does not access awareness, awareness being viewed in this case as depending on a separate module. Other lines of evidence have been proposed to support the view that there may be dual processing routes in the brain, one accessible to awareness, the other not. A third approach, supported by both empirical evidence and neurocomputational modeling suggests that covert recognition is in reality only the residual activity of the impaired recognition system used in overt recognition.

Site of the Lesions

Lesions leading to prosopagnosia involve the visual ventral pathway and most notably the ventral occipitotemporal region (lingual, fusiform, and parahippocampal gyri). Prosopagnosia is often related to bilateral lesions. Whether lesions limited to the right hemisphere are sufficient to elicit prosopagnosia has sparked intense debates before brain imaging could be available. It is an important question because if face processing depends largely upon a single (right hemisphere) region, this would be a strong argument that the brain has evolved a specialized system processing faces independently from

other objects. Although there may have been some doubts before brain imaging could be available, recent case studies appear to support the idea that right-hemispheric lesions are sufficient to elicit prosopagnosia. Conversely, lesions limited to the left hemisphere, except perhaps in one patient, never lead to prosopagnosia. These results highlight the importance, and the dominance, of the right hemisphere in face processing. Two locations in the occipitotemporal region appear to be crucial to process faces: the occipital face area (OFA), located in the inferior occipital cortex, and the fusiform face area (FFA), located anteriorly to the OFA along the middle fusiform gyrus. These regions have been identified using functional imaging, as they are regularly activated when faces are presented. The investigation of the role of these regions in prosopagnosic patients with preserved or impaired OFA and FFA has only begun recently.

Face versus Object Processing

Are faces processed by a different system or different mechanisms from other objects? Some authors have indeed argued that prosopagnosia may be an artifactual syndrome related to uncontrolled factors. A host of such uncontrolled factors have been proposed:

1. Faces are usually processed at the exemplar level, that is, a level at which one can say whether a face is familiar or not. This is not the case with other objects, which are usually processed, at least in the lexical domain at the basic (entry) level, that is, an apple is named an apple, not as a single exemplar of its category (Snow White's apple).
2. Pure prosopagnosia, that is, a disorder of face processing with normal object processing, is rare and it is always possible to argue that processing of other object categories has not been properly investigated. In particular, many early reports have relied mainly on accuracy measures without reporting speed of processing.
3. Low-level visual factors, such as intracategory homogeneity, luminance, contrast, spectral density, etc., between faces and other categories of objects have not always been satisfactorily controlled.
4. Face perception requires processing the configuration of the parts (eyes–nose–mouth). Consequently, face perception may be impaired not because of any specific process related to faces (domain-specific process) but because it over-relied on a general configural perception mechanism.
5. Likewise, humans may be viewed as experts at processing faces. A possibility is that it is this expertise (for faces and any other objects with a similar level of expertise), rather than specific face processes, that is impaired in prosopagnosia.

6. In addition, there are only a few case reports of patients presenting with the opposite dissociation, that is, impaired object processing and normal face processing.

Overall, however, various authors working with both acquired and developmental prosopagnosic patients have spent a good deal of effort to convincingly refute these possibilities, particularly in most recent reports.

Prosopagnosia and Facial Expression

Can prosopagnosic patients process facial expression? This important question highlights the notion that different systems may process different aspects of the visual information that can be extracted from a face, an idea that would further support the notion of specificity of the processes carried out to identify faces. There is some agreement that identity and facial expression may be processed by two independent visual pathways, and most models of face processing posit two different pathways that diverge early after a face has been detected, one for identity, and the other for expression processing. Furthermore, some studies indicate that the spared facial expression processing route may help prosopagnosic patients identify a face, suggesting an independent and parallel route. However, this dual-route hypothesis has recently been discussed on the ground that the evidence was not so strong. Alternative models have been proposed, whose merits now have to be assessed.

Mechanisms Impaired in Prosopagnosia

The exact nature of prosopagnosia is still largely controversial. A dominant view suggests that faces are special because some sort of configural processing is essential. Support for this hypothesis comes from the inverted-face effect. Although inverted faces have almost the same physical characteristics as the same faces upright, control subjects are faster and more reliable at processing upright than inverted faces (see also the Thatcher effect, which shows that subjects are much faster at processing facial elements when faces are presented upright than when the same elements are processed on an inverted face). This effect is less important for inverted compared to upright objects. Inverted faces somehow lose their faceness and are then processed as regular objects. Some prosopagnosic patients do not show the inverted-face effect (i.e., they show the same level of effect for both faces and objects). However, an old alternative hypothesis that has recently gained some support is that prosopagnosia may be related to a specific difficulty in processing the eye region (the so-called ocula region). There have also been suggestions that faces may be special because individualization requires precise curvature processing. Overall, it is probable that there are several possible causes of prosopagnosia, the independence of each being largely unknown.

Pathogenesis

Acquired prosopagnosia is mostly caused by infarcts of the posterior cerebral artery, which affect the ventral occipitotemporal region (sometimes only the right). Other common causes are traumatic brain injuries, encephalitis, and dementias, although in these cases prosopagnosia is usually not the only cognitive disorder. Associated symptoms are left hemifield (~25% of prosopagnosic patients), left upper quadrant (~20%), upper field (~10%), or tubular (~10%) defects. Associated achromatopsia is frequent (~60%), as well as visual (object) agnosia (~25%) or visual agnosia and alexia (~35%) and topographical agnosia (a difficulty in recognizing landmarks such as buildings).

Prosopagnosia may also have a developmental origin. In this case, the skills needed to process faces never developed normally, either because of a genetic origin or neonatal injuries. It has been shown that developmental prosopagnosia shares many characteristic features with acquired prosopagnosia. The genes involved in developmental prosopagnosia are not known yet. However, the recent publications of large familial series suggest that some forms are autosomal dominant. Developmental prosopagnosia may have heterogeneous causes. A possibility is that developmental prosopagnosia is related to a defective innate subcortical mechanism that directs attention to faces during childhood. However, other possibilities, such as cortical abnormal development, also have to be considered. Developmental prosopagnosia shares some similarities with dyslexia, dysorthographia, and dyscalculia, as all are related to the abnormal development of very specialized cognitive systems.

Epidemiology

Several studies have compiled data on about a hundred cases each. Although many patients are not documented for various reasons, these data suggest that prosopagnosic patients as a whole are not that uncommon, particularly in association with other visual disorders such as visual agnosia. Acquired pure prosopagnosia, on the other hand (not associated with visual object agnosia or other symptoms), is rare.

Developmental prosopagnosics may not be aware of their difficulties, because they do not know that faces may be individualized and recognized. They usually become aware of their difficulties during the teens. Consequently, the prevalence of developmental prosopagnosia is unknown, and some researchers have proposed that there may be more developmental prosopagnosic patients than acquired prosopagnosic patients.

Criteria and Diagnostic Work-Up/Tests

Diagnosis of prosopagnosia is carried out using neuropsychological tests following a logical order.

Assessment of the Face Recognition Impairment

This step is simply carried out presenting photographs of known faces and asking for identification. Close-ups of famous faces are most often used. If the patient fails to provide the name, the examiner assesses whether the face is familiar or not and asks if any information about the person can be provided. Criteria for prosopagnosia requires that a significant number of faces that should be familiar are found to be unfamiliar compared to matched control subjects.

Verification that the Failure to Recognize Faces Cannot Be Explained by Visual or General Intellectual Impairment

If prosopagnosia is suspected, visual perception must be assessed by an ophthalmologist who will carry out assessment of acuity, visual field, color perception, etc. Some of these tests can, however, be performed clinically, for example, by asking the patient to read from some distance, classify color pens, etc. Some standard neuropsychological tests of visual perception also include low-level visual discrimination subtests (e.g., identify noisy letters on a background of random dots). Similarly, the patient must be able to perform the tasks correctly. Particularly after a brain lesion, it is important to assess general intellectual abilities using, for example, intelligence as well as short- and long-term memory scales.

Assessment of the Specificity of the Findings

Although the patient may not have any low-level visual impairment, it is necessary to verify if he or she is also impaired at processing other objects, that is, if he or she also shows visual agnosia or alexia. This is carried out by using reading and confrontation naming tests as well as standard visuoperceptive and visuospatial batteries. These batteries, specifically designed for this purpose, comprise a variety of subtests enabling the assessment of the perception of shapes, sizes, silhouettes, visual or conceptual matching, three-dimensional rotations, spatial relations, etc.

Another important aspect is to determine whether semantic knowledge about familiar persons is preserved. Simultaneously, recognition using other modalities will also be assessed, for example, asking the patient what he knows about some famous people whose names are

provided either orally or better, visually. Access to this knowledge from the name, voice, or verbal description must be preserved for a diagnosis of prosopagnosia.

It should be possible following this step to determine if the patient presents with pure prosopagnosia or prosopagnosia with other associated syndromes.

Characterization of the Prosopagnosia

When a diagnosis of prosopagnosia has been made, it may be useful, at least clinically, to know whether it is more apperceptive or associative in nature. A standard test consists in presenting the face of a person and asking the patient to recognize the face of that person while it is presented from a different view among distractors. In this condition, the patient must build a unique representation of the face and be able to recognize it independently of the perspective, which implies being able to use (possibly) domain-specific processes such as configuration. This test does not require using memory as both the exemplar and target are presented simultaneously. If the patient fails, this suggests he or she shows apperceptive prosopagnosia; conversely, if the patient succeeds, this suggests he or she is able to build a correct percept out of the face and that the patient shows associative prosopagnosia. Such tests, although popular, have been criticized because patients may perform this test based on feature processing (e.g., shape of the eyebrows or haircut) and also because although they may succeed, they usually take much more time than control subjects. Other means to characterize the prosopagnosia further is to determine how facial and other objects configurations are processed, if the patient shows the inverted-face effect, to assess covert recognition, to assess memory for new faces, to assess visual imagery of premorbid familiar faces, etc.

Management

Severity usually decreases during the few months following brain injury in acquired prosopagnosia. However, it is difficult, if not impossible, to rehabilitate face processing if there is any defect left. Management thus consists in helping the patient to cope with his problem, assisting in identifying the specific features that may help in recognizing other persons.

Other Disorders of Face Processing

There are other disorders of face processing than prosopagnosia and their variety suggests that faces may hold a special status in cognition.

Metamorphopsia

Metamorphopsia is a syndrome in which the shape of objects appears distorted. It can be permanent, affect parts of the visual field, and may have a retinal, cortical, or even subcortical origin. In some instances, however, metamorphopsia may be restricted to faces, either permanently or transiently when associated with epilepsy. Faces or parts of the face are then seen as larger (macropsia) or smaller (micropsia), facial elements may be misaligned or turned sideways by several degrees, etc. Metamorphopsia does not necessarily prevent identification. It is usually considered a visuosensory deficit. As for prosopagnosia, whether face metamorphopsia is related to face-specific mechanisms or not is matter of debate.

Prosopamnesia

Prosopagnosia refers to a difficulty in learning new faces in the absence of the problem with face perception and recognition of previously familiar faces. Only a few patients presenting with prosopamnesia have been reported. The existence of prosopamnesia suggests that learning mechanisms of new faces are distinct from pre-morbid stored representations of faces.

Misidentification Delusional Syndromes

Misidentification delusional syndromes include Capgras, Fregoli, and intermetamorphosis syndromes. In the Capgras syndrome, the face, and identity, of a highly familiar person, usually the spouse, is thought to have been stolen by an impostor (or a double, a duplicate that takes the form of a thief, an alien, etc.). It is sometimes referred to as a 'hypofamiliarity syndrome.' This syndrome appears to predominate in the visual modality and there have been some reports of a dissociation between the face, which is thought to have been duplicated, and the voice, which is not subject to delusion. This situation is very distressing for both the patient and family, sometimes leading to complicated situations. It is observed in dementias (e.g., Lewy body disease and Alzheimer's dementia) and in psychotic patients. In dementia disorders, this condition usually fluctuates during the day, being enhanced at dusk. It has been shown that Capgras patients did not show normal skin conductance response when presented with photographs of the subject of their delusion. This finding has led to the suggestion that Capgras patients showed normal overt recognition but abnormal affective recognition, leading to a double dissociation with prosopagnosic patients showing abnormal overt recognition but normal skin conductance response and covert recognition. Capgras syndrome is most often associated to humans but may also be related to highly affective objects (e.g., a dog).

In contrast to the Capgras syndrome, the Fregoli syndrome (in reference to the famous Italian quick-change artist) refers to a 'hyperfamiliarity syndrome.' It is the delusional belief that a significant person (e.g., family member) is incarnated in other persons. These persons may be unfamiliar and any one of them may be the subject of the delusion. Intermetamorphosis refers to the situation when a significant relative is replaced by another significant relative (e.g., the husband is replaced by the father). This double delusion distinguishes intermetamorphosis from the Fregoli syndrome, although they are not always easy to disambiguate and are sometimes confused in the literature. Both may be accompanied by reduplicative paramnesia (the feeling that a place such as the hospital or home has been reduplicated).

The status of face perception versus person knowledge in misidentification delusional syndromes is not always clear and merits further exploration.

Mirror Sign

The mirror sign is the phenomenon of not recognizing one's own image when looking at oneself in a mirror. It is sometimes called 'negative heautoscopy,' in contrast to 'positive heautoscopy,' a reduplicative hallucination of seeing one's own body at some distance ('out-of-body experience'). The person seen in the mirror is mistaken for a relative or a stranger. Semantic knowledge of what a mirror is and of its properties is usually preserved. Other persons' reflections may be correctly identified. Associated behavior may be to grope at or behind the mirror or to attempt to engage in a dialog with the (unrecognized) reflection. The mirror sign is usually observed in dementing illnesses, particularly Alzheimer's dementia as well as in psychotic patients.

See also: Agnosia; Amnesia; Animal Models of Learning and Memory; Declarative Memory; Social Relationships and Social Knowledge; Temporal Lobe and Object Recognition.

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Relevant Websites

- <http://www.faceblind.org> – Faceblind.Org.
<http://www.face-rec.org> – Face Recognition Homepage.

Dyslexia (Developmental)

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Glossary

Case studies – The single patient methodology consists in providing an exhaustive analysis of a single patient performance. The overall performance on a set of tasks that jointly relate to various processing mechanisms is used to constrain the interpretation of the pattern of impairments. Caramazza (1986) argued that the use of impaired performance to constrain cognitive theory can only be meaningful in the setting of single patient methodology.

Categorical perception – Auditory perception is said to be categorical because discrimination is better for stimuli belonging to different phonemes (e.g., /p/-/b/) than for stimuli belonging to acoustic variants of the same phoneme, even though physical differences are the same in both the cases. Developmental dyslexics show poorer categorical perception than normal readers.

Dyslexia subtypes – Distinct subtypes of developmental dyslexia have been described which correspond to specific patterns of reading (and spelling) performance. A strong version of subtypes would require each behaviorally defined subtype to further demonstrate distinct cognitive disorder and distinct neural dysfunction.

Group studies – The average performance of a group of patient is interpreted. Group research is motivated by the assumption that members of the group show an important set of common characteristics. However, it has been argued that grouping patients with similar symptoms does not insure that the patients in the group are homogeneous in terms of the components of processing that are impaired in these patients.

Irregular word – The phonological form of the written word cannot be obtained by application of grapheme-phoneme mapping rules. Reading depends on some degree of partly arbitrary associative learning between orthography and phonology. Specific impairment of irregular word reading is a defining feature of developmental surface dyslexia.

Magnocellular system – The magnocellular system corresponds to the cortical dorsal stream of the visual pathways. Visual magnocells have large receptive fields, respond in a fast transient fashion, have broadband wavelength sensitivity, prefer low spatial frequencies, and are sensitive to low-contrast stimuli. Anatomical, neuro-imaging, and behavioral studies have been

provided in support of a magnocellular stream deficit in dyslexic individuals, leading to the magnocellular theory of developmental dyslexia.

Phonological awareness – Sensitivity to the sound structure of spoken words. Phonological awareness is assessed through tasks requiring the identification and manipulation of phonological units such as syllables, rhymes, or phonemes. Phoneme awareness, the sensitivity to phonemes, more strongly relates to reading performance and is typically impaired in individuals with phonological dyslexia.

Pseudo-word – A letter sequence that corresponds to none of the lexical forms in a given language but is pronounceable. Reading pseudo-words depends on a process that allows generalization from mappings between orthography and phonology. Specific pseudo-word reading impairment is a defining feature of developmental phonological dyslexia.

Visual attention span – The visual attention (VA) span corresponds to the number of distinct visual elements which can be processed simultaneously in a multi-element display. With respect to reading, the VA span corresponds to the number of orthographic units simultaneously processed in a letter string. A visual attention span disorder typically dissociates from phonological disorders and might play a critical role in the acquisition of specific orthographic knowledge.

Definition

Developmental dyslexia (or specific reading disability) is a specific learning disability characterized by an unexpected difficulty in learning to read in children who have at least average intelligence, who do not have general learning difficulties, and whose reading problems are not due to extraneous factors that might interfere with learning to read (such as sensory acuity deficits, severe emotional problems, acquired brain damage, or inadequate educational opportunity).

Semiology

Reading problems in developmental dyslexia are manifested in extreme difficulties in acquiring single word identification and decoding skills. Reading comprehension

skill may be affected as a consequence of poor word identification but listening comprehension is preserved. Spelling difficulties are frequently associated with specific reading disorders and often remain into adolescence even after some progress in reading has been made. Associated emotional and behavioral disturbances are common. In most English countries, the diagnosis of dyslexia proceeds on the basis of a discrepancy between reading accuracy and aptitude as measured by IQ tests. However, the validity of this discrepancy definition is becoming increasingly debated. Whereas measures of oral reading accuracy are typically used in English, timed tests of word and pseudo-word reading are standard practice in non-English countries.

Prevalence

Dyslexia is a common developmental disorder, with prevalence rates ranging from 3% to 17% of school age children. This variability is partly due to methodological differences between the studies. Using a one standard deviation cutoff, 16% of school-age children are diagnosed as dyslexic readers; they are no more than 2.5% when using a two standard deviation cutoff. The frequency of developmental dyslexia also differs according to the characteristics of the language in which children are taught to read. Most current knowledge about developmental dyslexia derives from studies conducted on English speakers and diagnosis is more difficult in more transparent languages such as Italian or German.

Persistence

Developmental dyslexia is a persistent condition. Although the reading performance of dyslexic readers improves with time, the gap between dyslexic and non-dyslexic readers remains all across the life span. The clinical features of dyslexia however vary with age. In deep languages in which dyslexic children exhibit low accuracy scores in reading, adolescents and young adults may be able to read words accurately but reading remains effortful and slow. Spelling problems may be useful clinically in differentiating dyslexic from average readers in older individuals.

Cognitive Feature

Assuming that phonological awareness is a core deficit in developmental dyslexia, some definitions of developmental dyslexia have been proposed that include the phonological deficit as a defining feature. However, there is evidence indicating that phoneme awareness is script dependent, so that phonological deficits are harder to detect in children who have learned to read in transparent languages. In nonalphabetic languages such as

Chinese or Japanese, a rapid naming deficit, not the phonological deficit, is the most dominant type of cognitive deficit in dyslexic children. Finally even in deep languages, cases of developmental dyslexia have been reported who exhibit no associated phonological disorders, and the existence of nonphonological core deficits in developmental dyslexia is still hotly debated. Based on current knowledge, general definitions which do not include cognitive-based criteria seem more parsimonious.

Heterogeneity

A distinction has been drawn between different subtypes of developmental dyslexia whose reading profiles parallel those previously described in acquired dyslexia. Some rare cases of deep dyslexia (poor word and pseudo-word reading, concreteness effect, and semantic errors), visual dyslexia (all reading errors are visual), and neglect dyslexia (neglect the left or right side of horizontally presented words) have been described in children. However, the two varieties of developmental phonological dyslexia and surface dyslexia are more frequent and better documented.

Case studies

Developmental phonological dyslexia

Prototypical cases of developmental phonological dyslexia show selective difficulties in pseudo-word reading but relatively preserved irregular word reading. Lexicalization errors (e.g., chait → chart; a phonologically similar word is produced instead of the target pseudo-word) are relatively common responses. A similar pattern is observed in writing with selective poor pseudo-word spelling but preserved word spelling abilities. A phonological processing disorder has been found in all reported cases of developmental phonological dyslexia. All exhibited poor phoneme awareness and poor verbal short term memory. Cases of phonological dyslexia have been described in children in a number of different languages, but diagnosis of phonological dyslexia may be more difficult in transparent languages in which phoneme awareness is acquired more easily.

Developmental surface dyslexia

In developmental surface dyslexia, reading of irregularly spelled words is impaired but reading accuracy is adequate for regular words and pseudo-words. Regularization errors resulting from the application of a rule-based system are typically observed (e.g., bear → beer) as well as visual paralexias (e.g., orchestra → orchard). Individuals with developmental surface dyslexia also display confusions between words with the same pronunciation but different spellings. Irregular word spelling is severely impaired but

the phonological aspects of words are preserved leading to phonologically plausible errors (e.g., whistle → wisle). All reported cases of developmental surface dyslexia showed preserved phonological abilities. Their phoneme awareness and verbal short-term memory skills were within the normal range of age-matched controls. In transparent languages, the diagnosis of surface dyslexia lies on difficulties in homophone heterograph comprehension and stress assignment.

Group Studies

In group studies, subtypes of reading disability have been identified based on the relative imbalances on the tasks of irregular word and pseudo-word reading. Regression techniques have been used to identify dyslexics with larger-than-expected discrepancies between irregular word and pseudo-word reading, based on the linear relationship between irregular words and pseudo-words in the control group. Individuals with unusually impaired irregular word reading relative to pseudo-words were labeled surface dyslexics. The opposite pattern defined the phonological dyslexia subtype. Using this procedure, two groups of phonological and surface dyslexic children have been identified as compared to age-matched controls. However, only a third of the children exhibited discrepancies between irregular word and pseudo-word reading and most were impaired on both types of items, thus showing a pattern of mixed dyslexia. Furthermore, the surface dyslexics' performance was very similar to that of younger normal readers, so that the surface dyslexic group virtually disappeared when a reading-age control group was employed. In contrast, substantial numbers of phonological dyslexics were identified in the comparison with reading-age-matched but younger controls. Such results were interpreted as showing that children with surface dyslexia in the chronological age comparison actually exhibited a developmental lag and that only those with phonological dyslexia reflected true developmental deviance.

Cognitive Bases

In most research, despite the heterogeneity of the dyslexic population, the tendency was to treat developmental dyslexia as a unitary syndrome with a single underlying cognitive disorder. However, a few attempts rather consider that the cognitive origin of developmental dyslexia is multifactorial. In this line, different cognitive disorders would independently contribute to the poor reading outcome of dyslexic children.

Phonological Deficit

Behavioral evidence

Phonological awareness – the sensitivity to the sound structure of spoken words – has been consistently reported as impaired in children with developmental dyslexia. This ability can be evaluated at the syllable level by testing rhyming abilities or at the phoneme level through tasks such as phoneme deletion (say cat without the /k/) or spoonerisms (basket-lemon → lasket-bemon). Other evidence for a phonological processing disorder comes from studies showing that dyslexics are poor at verbal learning and pseudo-word repetition. They have poor verbal short-term memory and have difficulties in object naming. Difficulties in acquiring phonological awareness and skill in alphabetic coding have been viewed as the consequence of poorly specified phonological representations. Some results however suggest that phonological representations may be intact and that the phonological deficit primarily surfaces in tasks requiring verbal short-term memory.

Causal relationship

The phonological theory postulates that a phonological disorder is the cognitive basis of developmental dyslexia. The most compelling evidence for a causal relationship between poor phonological skills and developmental dyslexia is provided by training and intervention studies. Indeed, direct instruction designed to facilitate phonological awareness and letter-sound mapping improves learning to read. Studies on normal reading acquisition further support a causal relationship in showing that phonological awareness strongly relates to reading progress and that children's knowledge of the phonological structure of language prior to literacy instruction is a good predictor of later reading ability. Early assessment of phonological abilities (phonological awareness, verbal short-term memory, and letter sound knowledge) thus allows identifying children at risk to develop reading problems.

Consequence on reading

By impairing the development of grapheme–phoneme skills, the phonological disorder is expected to primarily impact pseudo-word reading. Accordingly, dyslexic readers as a group typically show poor pseudo-word reading. The self-teaching hypothesis further claims that phonological recoding is critical to the acquisition of word-specific orthographic knowledge. Poor phonological abilities might therefore more drastically impact development of the reading system. This is compatible with the observation that most dyslexic readers are impaired in both pseudo-word and word reading.

Allophonic Perception

Studies using speech perception tasks typically evaluated categorical perception. They showed evidence that dyslexic readers perceive phonetic boundaries less sharply than normal readers do, mainly because they are better at discriminating acoustic differences between stimuli belonging to the same phoneme category. Interestingly, some studies used sine wave analogs of speech that can be either perceived as electronic sounds resembling whistles (acoustic perception mode) or recognized as syllables, after debriefing (speech perception mode). They revealed that the deficit was speech specific. Ill-specified phoneme representations in developmental dyslexia might explain the deficit in phoneme awareness and could interfere with the establishment of unambiguous connections between graphemes and phonemes.

Naming Speed Disorder

Behavioral evidence

A growing number of data point to naming speed deficits in developmental dyslexia. Naming speed is investigated through tasks of rapid automatized naming (RAN) that require children to name arrays of familiar items – letters, digits, colors, or objects – as quickly as possible. Performance on the colors and objects versions of the task predicts later reading performance in preliterate children, but the relationship between RAN and reading tends to be stronger for letter/digit tasks in older children. Along with phonological awareness, performance in RAN is one of the most powerful predictors of reading in the English language. It is the strongest predictor of reading performance in transparent orthographies and nonalphabetic languages.

Relationship with phonology

For some scholars, performance in RAN depends on the speed with which phonological information corresponding to letter names can be accessed from memory. According to this view, RAN performance is just another measure of phonological abilities. In contrast, other scholars consider that RAN performance is independent from phonology and that the cognitive deficits that lead to poor RAN performance are an independent source of reading disability. This assumption is supported by evidence showing that RAN performance accounts for unique variance in reading beyond that explained by phonological skills. Furthermore, phoneme awareness and rapid naming are differentially related to reading subskills: the former more strongly correlates with reading accuracy and performance in pseudo-word reading, the latter with reading speed and measures of orthographic knowledge. However, the cognitive processes which underlie RAN are not yet well understood. RAN is a complex task

that involves several different processes – from the uptake of visual information to the articulation of the spoken response – many of which are also necessary for reading. Current knowledge does not provide strong evidence that performance on the RAN task just reflects phonological processing skills and is not influenced by other cognitive dimensions.

Motor Problems

Dyslexics have been described as having balance and motor-coordination disorders. Their writing is appalling and they show soft signs of a cerebellar disorder, such as reach and gaze overshoot and muscle hypotonia. Given the role of the cerebellum in motor control and automation, a cerebellar dysfunction would affect speech articulation. This would lead to poor phonological representations and poor phonological skills which would be directly responsible for reading acquisition disorders. The cerebellar deficit hypothesis was presented as a biological explanation of the co-occurrence of phonological deficits and low level motor impairments in developmental dyslexia. However, motor impairments are only found in a subset of dyslexic individuals.

Visual Deficits

Congenital word blindness

At the end of the nineteenth century, severe reading acquisition disorders were described as congenital word blindness. It was thus agreed that reading difficulties were the result of a visual processing disorder. This hypothesis was largely contested in the mid-twentieth century, and the phonological hypothesis now dominates the scene. Nevertheless, there is evidence that at least some aspects of visual processing are impaired in many dyslexic individuals.

Low-level visual disorders

Low-level visual processing deficits have been reported in developmental dyslexia. These low-level visual deficits have been linked to functional anomalies in the magnocellular visual subsystem. They manifest themselves by poor motion perception, atypical eye movements, and reduced contrast sensitivity at low spatial and high temporal frequencies. However, poor performance in low-level visual tasks relates to pseudo-word reading and is typically associated with phonological disorders. The amodal version of the magnocellular theory of developmental dyslexia was proposed to account for the co-occurrence of phonological and low-level visual deficits in dyslexic children. This theory postulates that magnocellular temporal processing deficits result in basic visual and auditory processing impairments. The impairment in low-level auditory transient processing

would entail problems with phonological analysis which remain the most plausible proximal source of the reading problem. Accordingly, magnocellular deficits have been reported in the context of phonological dyslexia but not in the surface dyslexia subtype.

Visual attention span deficit

Deficits in processing strings of visual elements presented simultaneously have been reported in developmental dyslexia. It has been hypothesized that impaired multi-element processing might reflect deficits in allocating attention across letter or symbol strings, thus limiting the number of elements that can be processed in parallel during reading. This disorder was interpreted as reflecting a visual attention (VA) span disorder, namely a reduction in the number of distinct visual elements that can be processed in parallel in a visual display. Different subgroups of dyslexic children have been identified characterized by either a single phonological disorder or a single VA span deficit, or the two disorders, or none of them. The VA span deficit was found to account for the reading performance of dyslexic children, independently of their phonological skills. Moreover, data suggest that a majority of dyslexic children exhibit a single disorder, thus providing additional support for the hypothesis that the phonological and VA span deficits might contribute independently to developmental dyslexia. Further evidence were reported that the visual attention span disorder is specific to parallel processing and affects the number of letters processed at each fixation during text reading. The visual attention span was found to be impaired in case studies of developmental surface dyslexia but preserved in developmental phonological dyslexia.

Visual attention span abilities correlate with the reading outcome of dyslexic children. Strong relationships have been found with both irregular word and pseudo-word reading accuracy and speed. In limiting the amount of orthographic information extracted during reading, a visual attention span reduction would interfere with normal development of orthographic knowledge, thus leading to poor irregular word reading and spelling, i. e., a pattern of developmental surface dyslexia. Regular word and pseudo-word would remain unaffected as far as the visual attention span is large enough to process groups of letters that correspond to relevant orthographic units. However, a severe reduction impacting the processing of relevant sublexical orthographic units would end up affecting regular words and pseudo-words as well.

Impaired Temporal Processing

Defects in phonological processing have been speculated to derive from a basic deficit in temporal resolution of rapidly changing auditory stimuli. Although auditory

stimuli are well detected by dyslexic individuals when presented in isolation, performance is impaired when they are displayed in rapid succession. Actually, similar findings have been reported in the visual (and tactile) modality suggesting that the disorder might follow from a more general difficulty to rapidly allocate perceptual attention to sequences of stimuli in any sensory modality. The sluggish attentional shifting theory of developmental dyslexia assumes that the rapid stimulus sequence processing deficit is secondary to weakened parietal-lobe-supported attentional capture. The attention of dyslexic individuals once engaged cannot easily disengage. The sluggish attentional capture and prolonged attentional dwell time would impair speech perception and reading skills via distorted phonological representations. Accordingly, the temporal processing disorder was typically reported in dyslexic participants with a phonological deficit.

Neural Mechanisms

Developmental dyslexia is a neurobiological disorder. Postmortem studies, anatomical magnetic resonance imaging (aMRI) and diffusion tensor imaging (DTI) methods have been used to examine anatomical differences between dyslexic and nondyslexic brains. Methods based on positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have led to identify those of the cortical areas involved in reading which manifest atypical functioning in developmental dyslexia. Electroencephalography (EEG) and magnetoencephalography (MEG) techniques revealed hemodynamic deviances in developmental dyslexia.

Brain Structure

Postmortem microscopic evaluations of brains of people with lifelong reading disability revealed small, focal malformations such as ectopias (nests of cells in unusual locations) and dysplasias (focally distorted cortical lamination) located mainly in the left perisylvian regions. However, such malformations which reflect cortical neural migration anomalies do not necessarily characterize the whole dyslexic population inasmuch as only a few brains were investigated, some of which belonged to individuals who probably met the diagnostic criteria for specific language impairment. Nevertheless, a number of other structural particularities have been found in developmental dyslexia. The Heschl gyrus and planum temporale atypical symmetry suggest a language laterality disorder. The planum parietale which is the core of the supramarginal gyrus seems to have a different morphology and less supramarginal gray matter was reported in dyslexic individuals. The frontal lobe also shows atypical

asymmetry and decreased gray matter density, in particular in Broca's area. Differences have also been found in the cerebellum, and voxel-based morphometry further revealed abnormal gray matter density in the left fusiform gyrus, a key region for reading. Microscopic examinations of the thalamus further revealed that the magnocellular layers of the lateral geniculate nucleus are disordered and the magnocells over 27% smaller in dyslexic brains. The disorder is selective inasmuch as no particularities were reported in the parvocellular system. Auditory magnocells in the medial geniculate nucleus also differ in dyslexic brains, in accordance with an amodal magnocellular dysfunction.

In summary, a great number of cortical regions are, at least, more variable in morphology in dyslexic individuals. Such structural differences have sometimes been associated with specific behavioral impairments, suggesting that the relationship might be causal. However, no single neuroanatomical marker distinguishes dyslexic from control individuals. Multiple anatomical differences have to be taken into account suggesting that there may be different neurobiological subtypes of dyslexia.

Brain Function

Brain imaging investigations have demonstrated differences in activation patterns between typical and dyslexic readers at all ages. Dyslexic individuals demonstrate atypical activations in the three left-hemisphere neural systems of reading, namely the inferior frontal, temporo-parietal, and temporo-occipital regions. Although our knowledge of the neurobiological bases of developmental dyslexia greatly improved in the last decades, it is noteworthy that inconsistencies between the studies are frequently observed which might result from the heterogeneity of the dyslexic population and/or differences in investigation methods.

The frontoparietal network

Atypical activations have been reported in the region of the left inferior frontal gyrus (including Broca's area) which is believed to serve articulation, verbal short-term rehearsal, and phonological analysis during reading. This region seems to be less active in dyslexic readers in some studies but others reported higher than normal activation. Because it is more likely in older, mostly compensated individuals, enhanced activity in the left inferior frontal gyrus might represent a compensatory response to overcome failure of phonological processing in more posterior areas.

More generally, defect of activation in the left inferior frontal and supramarginal gyri is viewed as reflecting the dysfunction of a left-sided functional network related to phonological processing and verbal working memory. PET examination of the neural activity of these two left perisylvian regions during a discrimination task using sine

wave analogs of speech further revealed that they were less active during the speech perception mode than the acoustic mode in a group of dyslexic individuals with poor phoneme awareness. Because the two modes involved physically identical auditory stimuli, poor activation restricted to the speech mode suggests specific difficulties to engage phonological processes. It has further been hypothesized that absence of activation in the insula linking the anterior and posterior language areas might relate to a disconnection within the left perisylvian network. The role of these regions in reading and phonological processing is comforted by results from intervention studies. After intense phonological intervention, a significant increase in the activation of the left hemisphere neural circuits of reading was observed in the dyslexics. This increase in brain activity was associated with an improvement of reading performance.

The temporo-parietal areas

Deficient activation in the left angular gyrus during pseudo-word reading tasks was further reported in developmental dyslexia. This area and nearby temporal and parietal areas further show less activity during rhyme judgment and short-term memory tasks, suggesting that the angular gyrus is poorly connected with other areas involved in the mediation of reading in dyslexic readers. A failure of activation in the angular gyrus/temporo-parietal junction bilaterally during motion detection tasks was interpreted as evidence for a dysfunction of the visual magnocellular system. Activity in this region correlates with reading performance but reduced left temporo-parietal activation remains when the dyslexic children are compared to reading-age-matched controls, suggesting that reduced activity in this region is not just the consequence of their poor reading level. The temporo-parietal junction appears as one of the principal loci of cerebral dysfunction in dyslexia. This could be due to the role of the left temporo-parietal areas in reading-related attentional processing, that is, those attentional processes involved in graphemic parsing during pseudo-word reading and in the integration of visual features in a coherent whole as required for word recognition by sight.

The occipito-temporal region

Finally, a failure of a left occipito-temporal region named the visual word form area (VWFA) to function properly during reading is viewed as the neural signature of developmental dyslexia. A deficit in activation of this region was reported in well-compensated adult dyslexics irrespective of language (French, English, or Italian). This area which is involved in the rapid, effortless recognition of familiar words is a visual unimodal area that encodes the abstract identity of strings of visual letters and develops progressively during learning to read. Poor development of the VWFA in developmental dyslexia

might thus be the consequence rather than the cause of the reading disorder.

Genetic Component

Dyslexia is an etiologically complex disorder with a strong genetic basis. Linkage studies have provided chromosomal sites that might harbor factors involved in dyslexia predisposition. Four genes have been identified (DYX1C1 on 15q21, KIAA0319 or DCDC2 on 6p22, ROBO1 on 3p12) which are not specific to reading-related neuronal circuits but are involved in brain development (neuronal migration or connectivity). Because a family history of dyslexia confers an increased risk of developing reading problems, it is possible to investigate dyslexia prospectively. The identification of at-risk children thus allows the prediction and prevention of reading disabilities.

Remediation

The use of phonology-based training methods, acoustically modified speech training and nonverbal audio-visual training, showed increased activation in the brain regions involved in reading. Change in brain activity correlated with improved phonological or reading performance. However and whereas improvement in phonological abilities was observed after intensive phonological training, generalization to reading was inconsistent and some children did not respond to remediation. Furthermore even when intensive training emphasizing phonological processing improved reading performance, the remediation effect was restricted to reading accuracy and hardly extended to reading rate.

See also: Animal Models of Learning and Memory; Attention and Speed of Information Processing; Brain Imaging; Development and Language; Developmental Neurogenesis; Neural Basis of Working Memory; Short-Term Memory: Psychological and Neural Aspects.

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Emotion–Cognition Interactions

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Glossary

Attentional blink – A research paradigm in which participants are instructed to respond to two targets embedded in a stream of stimuli that occur in rapid succession. If the second target occurs close in time to the first target, its occurrence is not reported and attention is said to have ‘blinked’.

Avoidance learning – A form of motivated learning in which organisms generate correct responses in order to avoid receiving negative reinforcers.

Dimensional theories – Theories of emotion which postulate that individual emotions are organized along two fundamental dimensions of valence and arousal.

Emotional perseveration – The persistence of an affective state or emotional reaction despite a change in context indicating that the emotion is no longer appropriate.

Reversal learning – A form of associative learning in which cue-outcome contingencies are reversed in their reward value.

Social referencing – The use of nonverbal social communicative cues, such as arm gestures, eye gaze shifts, or facial expressions, to instruct someone where to direct their attention and how to react to stimuli in the environment.

Information processing capabilities of neurocognitive systems evolved to solve socio-ecological problems to ensure reproductive fitness and survival of the species. Such problems necessarily entailed emotional considerations inherent in courtship and mating, territorial defense, maternal behaviors, predator-prey interactions, and bodily sustenance. Emotions serve to prioritize behavior in accordance with such biologically relevant goals, and brain systems that mediate emotional functions are intimately connected with others dedicated to domain-specific cognitive processes and executive control. Although Western philosophical traditions sometimes cast the relationship between cognition and emotion as antagonistic, the nascent field of affective neuroscience illustrates how emotion acts iteratively and integratively with various cognitive domains to produce complex human behaviors.

Emotion–Cognition Convergence Zones within the Limbic and Paralimbic Forebrain

Emotions arise from somatic signals in response to sensory triggers that signify the presence of a salient event in the environment. The limbic and paralimbic regions of the forebrain are anatomically poised to integrate information from both the internal milieu and the external world, and these structures combine information from parallel cortical and subcortical processing pathways ([Figure 1](#)). Subcortical afferent pathways that carry information about visceral activity and sensory features of the world can swiftly initiate emotional behaviors. At the same time, cortical pathways elaborate the meaning of afferent input and compare it to stored knowledge accumulated from prior experience. Afferent sensory pathways converge on particular limbic and paralimbic forebrain structures – including the amygdala, insula, anterior cingulate cortex, and orbitofrontal cortex – that integrate the information and guide executive control regions to select appropriate responses ([Figure 1](#)). These structures communicate to each other and send efferent projections back to sensory and association cortices involved in relevant cognitive functions, such as attention and memory, to modify ongoing actions, fill in perceptual details, and update representations of the significance of features of the environment. The interplay between cognitive and emotional processes is therefore fluid and iterative during the management of complex behaviors.

Role of the Amygdala

The amygdala is an almond-shaped collection of approximately a dozen subcortical nuclei that reside in the anterior portion of medial temporal lobe. These nuclei have been implicated in a variety of affective, social, motivational, visceral, olfactory, and cognitive functions. The amygdala is the most densely connected structure in the primate forebrain and exerts powerful influences over processing in other brain areas. For instance, the amygdala has feedback projections to almost all stages of the ventral visual cortical stream, including the primary visual cortex (V1). The amygdala receives direct sensory input from the thalamus that bypasses the primary sensory cortices, which provides a rapid route to detect emotionally salient stimuli in the environment and to

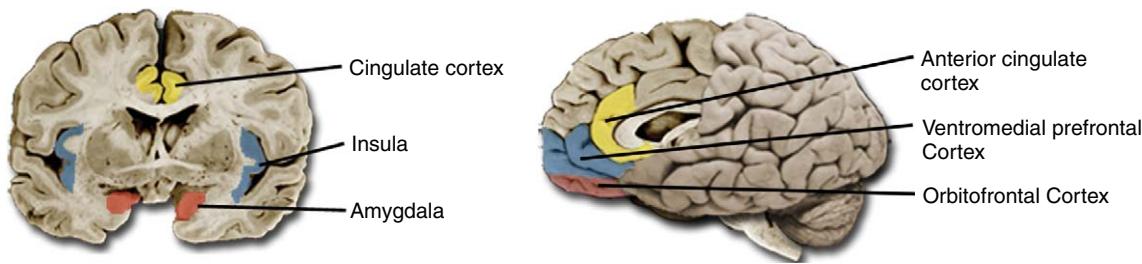


Figure 1 Limbic and paralimbic brain regions important for emotion–cognition interactions. The anterior cingulate cortex is the cortical component of the Papez circuit for emotion, which is based on control of the hypothalamus, and has dense interconnections with the amygdala, insula, and orbitofrontal and ventromedial cortices.

initiate emotional reactions. Because this subcortical route provides only crude sensory information, the amygdala combines this information with that arising from more refined cortical processing pathways. The amygdala orchestrates emotional output via connections to the hypothalamus, which controls the autonomic nervous system and release of stress hormones, as well as brain stem motor nuclei involved in emotional expression and defensive reflexes. Output projections to the basal ganglia and prefrontal cortex initiate coping responses to emotion-eliciting situations.

Role of the Insula

The insula is an evolutionarily older portion of the cortex that is covered by the frontoparietal and temporal opercula on the lateral surface of the brain. The insula contains multiple functional components, including a portion of primary gustatory cortex, secondary somatosensory cortex, sensory association areas, and visceromotor cortex. The insula serves as a convergent point of interoceptive processing from somatic afferents, which are integrated in a more anterior segment in the right hemisphere. From there, viscerosensory information is relayed to the orbitofrontal cortex, anterior cingulate cortex, and amygdala. The insula is important for the transmission of pain and has been implicated in awareness of somatic states. The anterior insula and anterior cingulate cortex contain neurons with a unique spindle morphology (von Economo neurons) that are found in hominoid primates, elephants, and whales, but whose function is unknown.

Role of the Anterior Cingulate Cortex

The cingulate cortex lies at the base of the medial wall of the forebrain and surrounds the corpus callosum. The anterior division has a more primitive cytoarchitecture, is divided into subgenual and rostral divisions, and is anatomically connected to limbic and autonomic motor structures. The subgenual division is implicated in mood disorders and visceromotor activation, whereas the rostral

division is associated with multiple functions, including the affective component of pain, motivational aspects of attention, expressing and evaluating emotional states, emotional learning, and integration of affect during demanding cognitive processing. Bidirectional connections with the posterior cingulate and its dorsal frontoparietal and hippocampal connections provide links to visuospatial, mnemonic, and attentional processing.

Role of the Orbitofrontal and Ventromedial Prefrontal Cortices

The orbitofrontal cortex lines the ventral surface of the frontal lobe. It contains a portion of secondary gustatory and olfactory cortices and its posterior sector is densely connected with diencephalic, striatal, and limbic/paralimbic structures. Dorsocaudally, the orbitofrontal cortex blends with paracingulate and dorsolateral heteromodal prefrontal cortex, and the confluence of these areas constitutes the ventromedial prefrontal cortex. One of the key functions of the orbitofrontal cortex is the representation of motivational value that is used during decision-making and contingency learning to guide goal-directed behavior. The medial orbital sector also signals pleasurable aspects of sensation while the ventromedial prefrontal cortex is engaged during internalized thoughts and feelings. In humans, damage to the orbitofrontal and ventromedial prefrontal cortices often cooccurs and leads to disorders of personality, affect, motivation, impulsive behavior, and social cognition and comportment.

Emotional Influences on Perception and Attention

Emotional Biases in Perception

Prioritizing stimuli with potential emotional significance has adaptive value to the organism, particularly for engaging defensive reflexes. Several perceptual and attentional mechanisms contribute to the rapid detection of emotional stimuli, including reflexive orienting, top-down attentional biases, and engagement of subcortical

processing pathways that are selectively tuned to emotional sensory features. Stimuli with high threat potential, such as snakes or angry facial expressions, elicit autonomic responses and activation of limbic forebrain structures even when presented subliminally (without conscious awareness). Under these circumstances, the amygdala interacts with other subcortical areas to a greater degree than when emotional stimuli are presented supraliminally. Emotional stimuli also are detected more rapidly than neutral stimuli on an attentional blink paradigm, in which participants report two target words separated by a short lag within a stream of other words. This performance benefit is reduced in amygdala-lesioned patients, which suggests that the amygdala can override capacity-limited perceptual encoding processes to allow emotional triggers to reach awareness. In neglect syndrome patients, emotional stimuli are detected in the contralesional visual field more often than neutral stimuli, presumably by recruiting these subcortical circuits.

The amygdala also modulates processing along cortical visual pathways as a function of emotional significance. For instance, emotional facial expressions elicit greater activation in occipitotemporal cortices, including the fusiform gyrus and superior temporal sulcus, relative to neutral expressions. However, amygdala-lesioned patients exhibit reduced emotional modulation of fusiform face activity, implicating a role for amygdalocortical feedback projections in facial affect perception. In anxiety disorders, prepotent threat signals are readily detected, and, accordingly, sensory-limbic pathways are hyperactivated in response to emotional elicitors. Even relatively innocuous social stimuli (e.g., the face of an unknown individual with neutral expression) can yield potentiated amygdala responses in social phobia. Such observations suggest that appraisal mechanisms combined with plasticity in corticolimbic circuits contribute to the manifestation of perceptual biases in affective disorders.

Emotional Interactions with Attentional Systems

Once detected, emotional stimuli recruit additional attentional processes to elaborate the meaning of the stimulus and its spatiotemporal context. Changes in the autonomic nervous system alert the organism to the presence of a potential emotional trigger which, in turn, redirects processing resources to react appropriately. When presented in complex environments, emotional stimuli bias attentional orienting and sustained attention to help localize the relevant stimulus and determine any change in its meaning. For instance, eye and head movements will be directed toward a lurking predator to initially locate it, and subsequent eye fixations will be spatially distributed to make inferences about its direction of movement and to identify possible escape routes. Positive emotional states tend to expand attentional focus and make individuals

more open to novel sensory stimulation and social interactions, whereas negative emotional states tend to focus attention on specific spatial locations and induce vigilance and social withdrawal. During social interactions, facial expressions, gestures, eye gaze, and vocalizations are used to communicate to others the emotional status of the environment (social referencing), and these cues must be attended and interpreted appropriately.

In response to emotional cues, amygdala efferents to the nucleus basalis of Meynert in the basal forebrain facilitate acetylcholine release that broadly targets neocortical receptor sites, including those in the frontoparietal attentional control system. Under these cholinergic influences, synaptic plasticity is enhanced and cortical neurons reduce their threshold for firing, implicating a readiness for information processing associated with high attentional states. Amygdala damage in rodents leads to failures in the cholinergic regulation of attentional responses to conditioned cues that predict rewarding outcomes. Other connections between the amygdala and dorsal frontoparietal cortices occur through feedback projections to sensory cortices or through frontal interfaces (e.g., anterior cingulate, orbitofrontal cortex, inferior frontal gyrus, or insula) (**Figure 2**). The amygdala and ventral visual processing areas are also the targets of modulation by attentional control circuits in response to emotional stimuli. For instance, hemodynamic responses in the amygdala and fusiform gyrus are sensitive to the amount of visual exploration allocated to facial expressions of emotion, and directing amygdala-lesioned patients to attend local facial features (such as the eye region for fear expressions) can improve their recognition abilities under some circumstances, presumably via attentional modulation of processing in face-sensitive occipitotemporal cortices.

The rostral anterior cingulate and adjacent medial prefrontal cortex have been particularly noted for their role in linking emotional evaluation with attentional control circuits. On an emotional oddball task in which individuals are presented with task-irrelevant emotionally arousing distractors while responding to interleaved neutral attentional targets, the rostral anterior cingulate responds equally to both stimulus categories. However, if the task instructions are switched and the arousing stimuli become the attentional targets, activity in the rostral anterior cingulate doubles in magnitude, which suggests a convergence of attentional–emotional processing. Moreover, when individuals attend to their emotional experience in response to negatively arousing pictures, activity in the medial prefrontal cortex increases relative to when they attend to the surface features of the pictures. In neurobiological models of depression, these regions are hypothesized to maintain a balance between dorsal attentional systems and ventral emotional systems to regulate mood states.

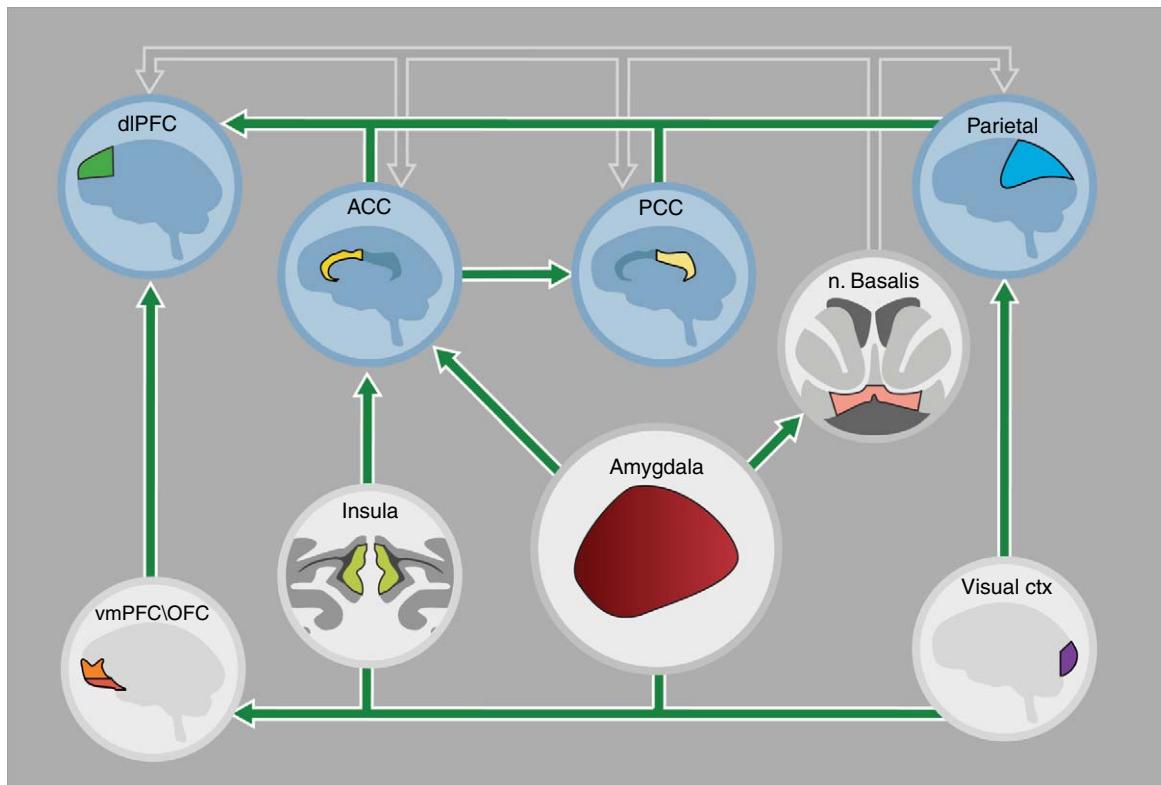


Figure 2 A neural circuit model of emotional interactions with visuospatial attentional control circuits. Visual input activates parallel pathways leading to the amygdala for emotion recognition and the parietal lobes for action preparation. Engagement of the cholinergic system in the nucleus basalis (n. Basalis) increases cortical arousal broadly. Targeted projections via cortical interfaces, including the ventromedial prefrontal cortex (vmPFC), orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), and insula, bias processing in the dorsal fronto-cingulo-parietal attentional control system (shaded in blue). Feedback projections are equally important for the cognitive control of emotion (not indicated). Striato-thalamic projections are eliminated for simplicity. ctx = cortex, dlPFC = dorsolateral prefrontal cortex, PCC = posterior cingulate cortex.

When attention is socially directed by a combination of emotional expressions and eye gaze, these facial features are processed independently until approximately 300 ms after stimulus onset, at which time the two cues interact to guide spatially-directed attentional orienting. Facial expression activity in the amygdala is altered by gaze direction, and one hypothesis suggests that its activity is enhanced when the meaning of the gaze-expression combination is ambiguous. For instance, greater amygdala activity is seen to angry facial expressions with averted gaze (and no peripheral target present) relative to gaze directed at the viewer. In the former case, the source of the anger is unknown and additional information is needed to evaluate the meaning of the attention-directing cue. Furthermore, the superior temporal sulcus codes for violations of expectancies when viewing eye gaze shifts in others. When individuals watch someone fail to spatially track a peripheral target through misappropriated gaze shifts, activity in the superior temporal sulcus is enhanced relative to when the target is correctly foveated. These findings provide a foundation upon which to delineate how attention modulates activity in cortico-limbic

structures to interpret the intended meaning of others' nonverbal actions during social exchanges.

Emotional Influences on Learning and Memory

Conditioned Fear Learning

One of the most fundamental ways in which organisms learn about the emotional significance of events in the environment is through the process of classical (Pavlovian) conditioning. When appetitive or aversive reinforcers are present in the environment, it is important to determine which sensory cues and contexts reliably predict their occurrence. During fear conditioning, a relatively innocuous conditioned stimulus such as a tone is presented just prior to an innately (unconditioned) aversive reinforcer, such as an electric shock. Following several tone-shock pairings, the tone acquires negative value through its predictive association with the impending shock and begins to elicit characteristic defensive responses indicative of a state of fear, including startle

reflex potentiation, behavioral immobility, and increased sweating. These conditioned fear responses also transfer to features of the global environment in which the shock occurs even in the absence of the cue itself (contextual conditioning). In humans, fear conditioning can be acquired even when individuals are not consciously aware of the cue-outcome contingency, particularly when the cues themselves are fear-relevant (e.g., angry faces). Thus, simple forms of conditioned fear learning are implicit in nature, although more complex fear associations depend on explicit knowledge of the predictive relationships among the cue, reinforcer, and their spatio-temporal context. The strength of learning generally depends on the intensity of the reinforcer, which shows that emotional arousal is critical to the acquisition of such conditioned behaviors.

A wealth of research in humans and nonhuman animals has elucidated the neurobiological mechanisms of this form of emotional learning (Figure 3). Across species, the amygdala has been implicated as a key structure that performs computations that underlie associative learning during fear conditioning. Rodents or humans with amygdala damage are impaired in conditioned fear learning, despite having normal fear responses to the reinforcer itself. In humans, amygdala damage impairs physiological expression of conditioned fear learning even when the patients have intact explicit knowledge regarding the cue-reinforcer contingency. Neuroimaging studies have consistently shown that activity in the thalamus, amygdala, anterior cingulate gyrus, and sensory cortex increase during the acquisition of conditioned fear. Of these structures, the amygdala is most tightly linked to individual differences in the physiological expression of fear learning, and

its activity tends to be greatest during the early portion of training when the emotional associations are initially formed. In posttraumatic stress disorder, the amygdala is hyperactive during conditioned fear learning, which suggests that chronic stress potentiates sensory associations to threatening stimuli. Maladaptive expression of conditioned behaviors also contributes to pathophysiological changes underlying other psychiatric conditions, such as specific phobias, in which there is aberrant regulation or generalization of the fear potential of cues that signal danger.

Amnesic patients with selective hippocampal damage show the opposite pattern as amygdala-lesioned patients – they exhibit normal physiological acquisition of conditioned fear on simple cue-reinforcer paradigms but fail to explicitly remember the contingency pattern. However, physiological impairments arise in amnesia when the training paradigm is more complex, such as trace conditioning, wherein a retention interval separates the end of the cue and the onset of the reinforcer. Moreover, amnesic patients have difficulty forming contextual associations to the reinforcers and thus fail to exhibit a return of fear when they re-encounter a conditioned stimulus in a place that was previously associated with shock. Neuroimaging studies have confirmed the role of the human hippocampus in healthy adults who undergo contextual and trace fear-conditioning regimens.

Emotional Modulation of Explicit Memory

Although the amygdala and hippocampus exhibit partially independent functions during conditioned learning, they cooperate during the formation and retrieval of explicit (declarative or conscious) emotional memories. Emotionally arousing stimuli coded by the

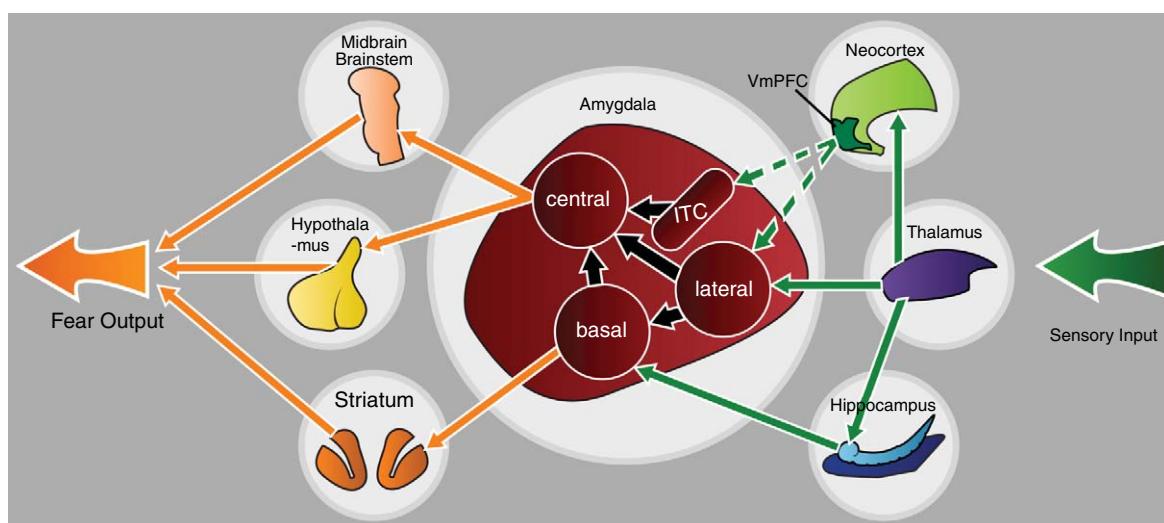


Figure 3 A neural circuit model of fear conditioning. Fearful stimuli activate the amygdala via both direct thalamic and indirect thalamo-cortical routes. Fearful behavior is initiated through brainstem, hypothalamic, and fronto-striatal outputs from the amygdala. Extinction of fear is mediated by inhibitory influences from the ventromedial prefrontal cortex (vmPFC). Contextual modulation of fear is mediated by the hippocampus. Feedback projections are not shown. ITC = intercalated cell group.

amygdala interact with hippocampal-dependent encoding and consolidation processes through two routes. First, bidirectional neural pathways link the amygdala and hippocampus directly and indirectly via adjacent rhinal cortex interfaces, which provide rapid communication channels for coupling their activity, including high-frequency oscillatory firing, to boost memory for emotional events. Second, the amygdala controls the peripheral release of stress hormones, including noradrenaline and cortisol, in response to emotionally arousing events by engaging the hypothalamic–pituitary–adrenal axis. These hormones feed back onto receptor sites located throughout the cerebrum, including the hippocampus and the amygdala itself, although such effects take time to unfold relative to the direct neural interactions. Studies in nonhuman animals have shown that stress hormone-dependent processing in the amygdala and hippocampus is important for enhancing memory consolidation for emotionally arousing tasks, such as avoidance learning.

In humans, enhanced functional coupling among the amygdala, hippocampus, and rhinal cortex has been observed in neuroimaging paradigms that examine subsequent memory for emotionally arousing material. Encoding-related activity in and functional interactions among these regions increase for arousing stimuli that are later remembered relative to those that are later forgotten, and this successful encoding activity is greater for arousing than for neutral material. Epileptic patients with partial amygdala lesions exhibit reduced encoding-related activity for emotional stimuli in the hippocampus and vice versa, which indicates a functional reciprocity between these regions to support emotional memory encoding. These patients also fail to show less forgetting over time of emotionally arousing material relative to neutral material, which is a behavioral hallmark of arousal-mediated memory consolidation in healthy individuals. Hence, when the amygdala is engaged concurrently with memory-related structures in the medial temporal lobe, there is a selective long-term retention boost for emotionally arousing information. Other regions make contributions to emotional memory formation, including lateral prefrontal and sensory cortices, which implicates a distributed network to support encoding processes that are enhanced in the presence of an emotionally evocative stimulus.

Human evidence also implicates a role of stress hormones in enhancing memory for arousing events. When individuals are presented a short story with emotional and neutral segments, their memory for the emotional segment is enhanced relative to the neutral portions of the story. However, patients with bilateral amygdala damage and healthy adults under the influence of propranolol, a beta-adrenergic blocker, do not exhibit a boost in retention for the emotional portion of the story. Direct influences of propranolol on amygdala activity have also been reported, but it is unclear whether these effects

selectively affect hippocampal interactions that support emotional memory formation. The effects of cortisol on human emotional memory have not been as well-established in terms of neural mechanisms, although moderate stress-induced increases in cortisol levels generally produce beneficial effects during encoding but impairing effects during retrieval.

While studies in nonhuman animals have emphasized the role of the amygdala and adjacent medial temporal lobe structures during the encoding and consolidation stages of memory, human research has shown that these regions also play important roles during retrieval. Functional coupling among the amygdala, rhinal cortex, and hippocampus is enhanced during the successful retrieval of emotionally arousing stimuli relative to neutral stimuli. Moreover, the amygdala and hippocampal activity during retrieval is selectively associated with a particular retrieval operation – recollection – that is accompanied by a vivid sense of traveling back in time to re-experience the event and retrieve contextual details associated with it. These structures are also engaged and functionally coupled with activity in right ventral prefrontal cortex during the recall of autobiographical memories, and the degree of amygdala activity during retrieval predicts the level of emotional intensity associated with the recovered memory. Relative to trauma-exposed individuals, patients with posttraumatic stress disorder exhibit exaggerated responses in limbic forebrain regions, including the anterior cingulate and amygdala, in response to reminders of their traumatic experience, although chronic stress yields structural damage to the hippocampus, which may result in less contextual specification of traumatic memories. Thus, medial temporal lobe structures, in consort with prefrontal and sensory cortices and stress hormones, interact during both encoding and retrieval to yield explicit retention advantages for emotionally arousing events to ensure their selective retention in long-term memory. The amygdala and its hormonal targets influence other brain regions to support implicit forms of memory, such as the cerebellum for motor learning and the striatum for procedural learning (**Figure 4**).

Whereas amygdala–hippocampal coupling engenders memory advantages based on the arousal aspects of emotional stimuli and experiences, additional mnemonic boosts are provided by affective valence. Affective valence (pleasantness) and arousal (intensity) are construed as orthogonal dimensions of emotion which may exert independent effects on cognition. Stimuli that are low in arousal but nonetheless affective in meaning tend to be more semantically or thematically related than randomly selected neutral stimuli. Amygdala-lesioned patients typically show normal retention advantages for these affectively valent, low-arousing stimuli, and neuroimaging studies have shown that successful encoding of

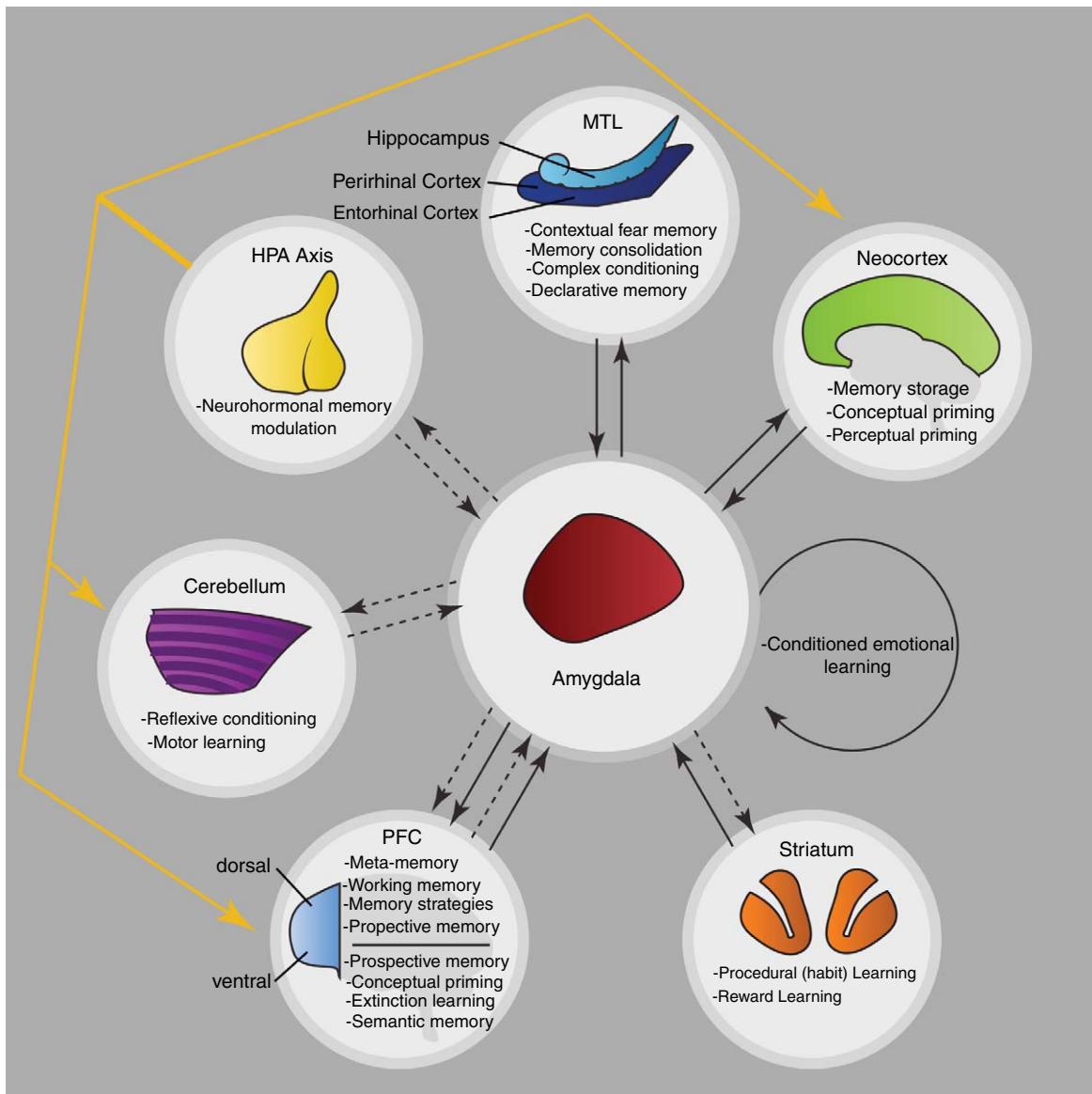


Figure 4 A neural circuit model of arousal-mediated memory effects. Distributed brain systems that support specific forms of memory interact with the amygdala either directly (solid lines) or indirectly (dashed lines). When an emotionally arousing stimulus is detected by the amygdala, various memory processes are engaged, depending on the behavioral goal, to facilitate remembering the circumstances of the event and reacting appropriately. Engagement of stress hormones via the hypothalamic–pituitary–adrenal (HPA) axis further enhances memory consolidation via feedback projections to cortical sites (yellow lines). Memory effects of emotion in the absence of high arousal are not amygdala dependent but instead engage semantic memory networks in frontotemporal cortical areas (not shown). MTL = medial temporal lobe, PFC = prefrontal cortex.

these stimuli yields greater activity in the left hippocampus and a region of left ventrolateral prefrontal cortex associated with deep semantic processing. Theories of memory biases in mood disorders emphasize a contribution of spreading activation in semantic networks along emotion nodes or attractors, which helps organize information in memory. Semantic and organizational encoding benefits do not involve the amygdala but instead engage left fronto-temporal cortices to process and relate information more deeply when a common valence is shared among items.

Cognitive Control of Emotion

Extinction and Reversal Contingency Learning

The emotional significance of a sensory stimulus is often malleable and contextualized so that its meaning varies across time or place. One way in which changes in emotional significance can be learned is through alterations in stimulus–outcome associations. For instance, if a conditioned fear stimulus no longer predicts an impending aversive event, then fear responses should subside and a new safety memory formed (extinction learning).

Alternatively, a conditioned fear stimulus may instead begin to predict a pleasurable outcome, yielding a switch in its affective valence (reversal learning). Such outcome-dependent changes in affective value critically involve the ventromedial prefrontal cortex and its inhibitory influences over the amygdala (Figure 2). Stimulating the ventromedial prefrontal cortex in rodents elicits reductions in amygdala responses to conditioned fear stimuli and a concomitant suppression of fear behavior. Human neuroimaging studies have confirmed that during fear extinction training, activity in the amygdala decreases, and activity in the ventromedial prefrontal cortex reflects the behavioral expression of extinction learning. Posttraumatic stress disorder patients fail to engage these prefrontal control circuits during fear extinction, which may partly explain why they continue to respond to traumatic reminders even in safe environments. Neurologic patients with orbital and ventromedial prefrontal cortex damage are impaired in reversal learning – they persist in responding to conditioned cues in a stereotyped way, despite knowing that emotional outcomes are no longer likely to occur (emotional perseveration). The lack of emotional flexibility in these patients adversely affects their interpersonal relationships and livelihood as they do not appropriately update their expectations and actions according to changes in the value of behavioral outcomes. A fundamental tenet of exposure and counterconditioning therapies is that outcome-based control mechanisms implemented in the ventromedial prefrontal cortex can be trained to help patients with affective disorders overcome maladaptive conditioned behaviors.

Cognitive Reappraisal

An alternative method to teach individuals to modify their affective responses is to use cognitive resources to lessen the negative impact of an emotional event (cognitive behavioral therapy). Several techniques can be effective in this regard. For instance, individuals can be instructed to reframe the meaning of a stressful situation by mentally imagining a more positive outcome while reflecting on it. Other techniques ask individuals to imagine being less personally involved in the event or to reallocate attentional resources away from emotional aspects of the situation. In all of these examples, cognitive resources, such as working memory, attentional set shifting, and mental imagery, are used in an effort to change one's emotional experience. During cognitive reappraisal of negative emotion, activity in dorsal frontoparietal and dorsal anterior cingulate regions increases, whereas activity in the insula, amygdala,

and other limbic areas decreases in accordance with self-reported reductions in negative affect. Such studies of emotion regulation show that the relationship between emotional and attentional control systems is reciprocal, depending on the behavioral goal (Figure 2). Although the specific areas recruited depend on the cognitive operations used, in general these investigations illustrate how executive control regions restore a balance across cognitive and affective systems to regulate behavior in response to the vagaries of life experiences.

See also: Active Avoidance and Escape Learning; Animal Models of Learning and Memory; Cognition: Attention and Impulsivity; Cognition: Learning and Memory: Pavlovian; Emotions; Evolution of Emotions; Fear Conditioning; Implicit Learning and Memory; Psychological and Neural Aspects; Stress and Emotionality.

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Evolutionary and Developmental Issues in Cognitive Neuroscience

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Glossary

Diffusion tensor imaging (DTI) – A magnetic resonance imaging (MRI) technique that measures water diffusion through biological tissues. This noninvasive method can be used to visualize white matter tracts in the brain.

MRI morphometry – An analytic approach to structural MRI data analysis that enables the comparison of the size and shape of specific brain structures, for example, at different ages or across different species.

Ontogeny – The development of an organism from the fertilized egg to its mature form.

Phylogeny – The evolutionary development of a species.

Theory of mind – The ability to attribute mental states – beliefs, intents, desires, pretending, knowledge, and so on – to oneself and others, and to understand that others have beliefs, desires, and intentions that are different from one's own.

Saying that evolution involves changes in the genome really does not tell us much by itself. To make meaningful contact with the organism and its life history, it is important to consider genetic changes in the context of development. For example, building a bigger brain could entail adding more neurons or, alternatively, increasing connectivity and synapse number while maintaining neuron number. We know that all neocortical neurons are generated early in development. Thus, increasing brain size by adding more neurons would require changing gene activity early in development. Tinkering with the genome at this early stage could have a variety of secondary effects, both positive and negative, such as extending the life span. In contrast, enlarging the brain by increasing synapse number could be effected by changing the actions of genes expressed later in development, even as late as adulthood. There may be a bias toward modifications late in development, given that modifications early in development could have deleterious effects that extend across the rest of the life span.

Second, both developmental and evolutionary researchers explore the relationship between variations in brain structure and variations in brain function. Stemming directly from Darwin's doctrine of continuity, there is a strong tradition in the neurosciences and psychology that the brain and mind of humans differ only quantitatively from those of our relatives. As Darwin put it, the differences are matters of degree and not of kind. Darwin's doctrine of continuity does not fit well with our modern understanding of evolutionary change, but it remains popular – in part because it serves as a rationale for viewing model animals as simplified versions of human beings. Yet, humans are not simply macaque monkeys with additional components or layers of complexity – macaques and humans are both specialized endpoints of evolutionary history, each having changed in particular ways since their divergence about 25 million years ago. Increasingly, experimental psychologists are embracing significant species differences in psychological organization, although there is still a lack of consensus about the nature of human cognitive specializations, as discussed below.

Similarly, in development, children are often – but overly simplistically – viewed as simplified versions of adults. Infants exhibit certain behaviors that are adaptive

Most cognitive neuroscience research to date has focused on the relationships between the brain and behavior in the human adult. We can gain additional insights into these relationships by considering how this finely tuned organ came to be, both from an evolutionary and a developmental perspective.

The tradition of linking evolution and development together dates back to 1866 to the well-known 'biogenetic law' of Ernst Haeckel: "Ontogeny recapitulates phylogeny." For Haeckel, ontogeny and phylogeny were two aspects of the same process. The biogenetic law was discredited long ago as a general principle of biology. It assumes that evolution acts only at the endpoints – that is, adult stages – of development, whereas evolution can, and does, modify organisms at every stage of development. The process of development is not merely a series of architectural changes that are required to yield the mature organism: the intervening organizations have to be functionally viable in the social and ecological contexts in which immature animals find themselves.

There are, however, other reasons to consider development and evolution together. One reason is that evolutionary changes are modifications of developmental programs – a concept explored in evolutionary developmental biology, a field commonly known as 'evo-devo.'

at their stage of life, but that are no longer needed later in life, such as grasping and suckling reflexes. Infants also exhibit cognitive capacities that adults do not; they can discriminate subtle phonetic differences, even between sounds that are not used to distinguish between words in their native language – a capacity that allows them to learn language readily. Adolescents, too, are not merely adults with diminished capacity. They exhibit, strongly, reward-driven behavior and high levels of novelty seeking – behaviors that maximize the potential for learning. Such behaviors are associated with tangible risks, however, and after a period of exploration and learning during the teen years, it might make sense to curb these exploratory tendencies to a certain extent.

In summary, comparative studies show that while there are many similarities between humans and other animals, in particular other primates – which is expected because we do share common ancestors – there are also important differences. These differences are manifested not only in the highest-order cognitive and behavioral functions, but also at a variety of levels of organization and a variety of points in development. On the flipside, developmental studies do provide evidence of emerging levels of complexity from infancy to adulthood, but at each stage of development we are adapted to our particular circumstances.

Below, we provide a brief overview of current topics in evolution and development that relate to higher-order cognitive functions and the brain regions that support them, given that these are the functions for which the cross-species and developmental differences are most salient. We first consider ideas about the evolution and development of behavior, then brain anatomy, and finally brain function.

Comparative and Developmental Psychology

There have been several efforts to characterize the nature of the differences between humans and the great apes – chimpanzees, bonobos, gorillas, and orangutans – the animals most closely related to us. Tomasello and colleagues, for example, see the differences mainly in terms of the elaboration of social cognition in humans, as compared to cognition related to the physical world. Povinelli and colleagues, on the other hand, see a deeper distinction involving capacities for abstract representation that cuts across the social/physical distinction. For the latter view, what is uniquely human is the emergence, during childhood, of symbolic systems, including language as well as the ability to represent unseen causes and abstract categories.

The idea that language is a human specialization is no longer highly controversial. Additionally, humans, but not

nonhuman primates, understand physical interactions of objects in terms of abstract or unobservable causes or relations – a way of understanding the world known as ‘folk physics.’ Similarly, humans understand the knowledge states of other individuals – so-called ‘theory of mind’ – which is another example of how we understand the world in terms of abstract causes or relations. Povinelli and colleagues have argued that chimpanzees (1) do not understand that other individuals – or they themselves – possess minds and (2) do not understand how minds work. Thus, for example, chimpanzees do not understand that one has to have certain kinds of perceptual experiences to possess certain kinds of knowledge. In contrast, starting around 4 years of age, children performing the Sally-Anne test begin to demonstrate understanding of what knowledge others have access to.

Humans have the ability to extract abstract relationships between multiple mental representations. This ability, referred to as ‘relational reasoning,’ allows us to represent higher-order relationships between sets of mental representations. Analogical reasoning is a form of relational reasoning that involves abstracting a relationship between familiar items and applying it to novel representations. Forming analogies allows us to determine general principles from specific examples, and to establish connections between previously unrelated pieces of information. Analogical thought is an important means by which cognition develops. For example, children use analogies to learn new words and concepts by association with previously learned information. Penn, Holyoak, and Povinelli have argued that nonhuman primates lack the ability to integrate multiple mental relations, an ability that is considered a key component of human cognition.

Comparative and Developmental Neuroscience

Comparative Neuroanatomy

Human brain evolution is remarkably poorly understood, although it is beginning to yield its secrets to modern comparative studies. It is clear that evolution affected the human brain at many levels of organization, from the molecular biology of neurons to patterns of long-range cortico-cortical connectivity. Moreover, human evolutionary specializations are not limited to brain regions that are usually associated with higher-order cognitive functions. There are, for example, differences in the morphology and biochemistry of neurons in anterior cingulate area 24, a limbic region implicated in the affective regulation of behavior. Additionally, there are cellular and modular organizations of primary visual cortex that may be reflected in changes in the response properties of higher-order visual cortex. Thus, human specializations

are observed at the cellular level even in terms of basic visual processing.

Brain Size and Proportions of Lobes and Regions

The most conspicuous and well-known specialization of the human brain, however, is its remarkable size. Our closest relatives are chimpanzees, and average adult-human-body size is only slightly larger than that of a chimpanzee; yet, our brains are about 3 times larger: chimpanzee brains average a little less than 400 cc, while human brains average approximately 1400 cc. Most of the difference reflects an expansion of the neocortex and its associated white matter in human evolution. Although the human and chimpanzee lineages diverged some 6–7 million years ago, paleontological evidence indicates that most of this increase in brain size occurred over the last 2 million years, a point at which hominins may have begun to incorporate large amounts of animal flesh in their diet.

Traditionally, this large increase in size was thought to reflect the addition of tissue to the classical higher-order association regions, not only including the prefrontal cortex, but also the temporal and parietal association regions. The conclusion that prefrontal and other higher-order cortical regions disproportionately expanded in humans has more recently been challenged by Semendeferi and colleagues. Using MRI morphometry, they reported that the relative proportions of the cerebral mantle occupied by the frontal, parietal, and temporal lobes are about the same in humans and great apes, despite the much larger absolute size of the human brain. It must be remembered, however, that prefrontal cortex is not the same as frontal cortex: the frontal lobe contains motor and premotor cortex, in addition to prefrontal cortex. Similarly, the parietal and temporal lobes contain primary somatosensory and auditory areas, respectively, as well as association cortex. As it happens, the sizes of the primary, sensory, and motor areas in humans are, in absolute terms, very similar to those of great apes, while the association cortical regions are vastly larger in humans. Comparisons of sensory versus association nuclei of the thalamus yield similar results. Thus, the available evidence supports the classical conclusion that humans underwent an enormous evolutionary expansion of association cortex, although it is important to note that the evidence comes mainly from a handful of very old studies.

The evolutionary enlargement of the human brain could have had a number of effects on the internal organization of the brain. Many researchers have followed Brodmann in concluding that the human brain has cortical divisions (areas) that are not present in smaller-brained primates. Therefore, for example, his human map contains areas 44 and 45 – which collectively

constitute a large part of Broca's area – in the human frontal lobe, areas that are missing in his monkey maps. This is intuitively appealing, since one might expect that humans evolved new cortical areas to support novel human functions. The truth of the matter would seem to be more complicated, however: there is reasonable (if not definitive) evidence that homologs of Broca's and Wernicke's language areas exist in nonhuman primates, their lack of language notwithstanding. One might argue, more generally, that the expansion of cortex in human evolution should be accompanied by the addition of new cortical areas, since larger-brained mammals typically have more cortical subdivisions than smaller-brain mammals, but we currently lack the detailed and reliable cortical maps for humans and great apes that would allow us to definitively identify homologous areas across species and determine whether humans possess areas that nonhuman primates lack. The example of language cortex should, however, prompt us to take seriously the idea that human brain evolution was not simply an add-on process, but that it also involved modifying preexisting structures to support novel functions.

There is, in fact, evidence that the evolution of language involved physical changes in the brain other than the addition of new areas. Buxhoeveden and colleagues have described differences in the size and spacing of cortical microcolumns between the left and right Wernicke's areas of humans, differences not observed in chimpanzees or macaques. Recently, Rilling and colleagues used diffusion tensor imaging (DTI) to compare the organization of white matter pathways that interconnect Wernicke's and Broca's areas in humans, chimpanzees, and macaques. In humans, it has long been supposed that Wernicke's and Broca's areas are linked by a specific fiber bundle, the arcuate fasciculus. Using DTI, Rilling and colleagues found that the arcuate fasciculus of humans, but not chimpanzees, carries fibers from a broad region of middle and inferior temporal cortex, ventral to Wernicke's area, which are known to represent word meaning.

The advent of DTI, which enables the noninvasive tracking of fiber pathways, is a landmark in comparative neuroscience. For the first time in the history of neuroscience, it is possible to directly compare the connectivity of the human brain to that of chimpanzees and other nonhuman primates, and to explore how evolution reorganized the system of white matter fascicles that bind cortical areas into higher-order neurocognitive systems. It seems likely that the arcuate fasciculus findings will be the first of many demonstrations of evolutionary specializations of human brain connectivity.

Of course, fiber organization and long-range cortical connectivity, as important as they are, represent just one level of brain organization. As noted above, evolutionary specializations of the human brain have now been

documented at finer levels of organization, such as micro-architectural organization of neuronal groups and the biochemical and morphological characteristics of different cell types. Evidence from recent comparative genomic studies points to the possibility of rather profound differences in the organization of the molecular machinery that regulates cerebral energy metabolism and the formation and stabilization of synapses.

There remain major unanswered questions in our understanding of human brain evolution. For example, while it seems likely that the genetic program regulating the generation of neurons in embryonic life was modified in human evolution, we do not know which genes were involved. Moreover, it has been argued that human postnatal development is not simply a stretched-out version of ape development, but also includes uniquely human pre-adult developmental stages. We know very little about the genetic and neurodevelopmental correlates of such changes. There is, as noted above, evidence from gene-expression studies suggesting that energy metabolism and synaptic biology of adult human cortex were modified in human evolution; what are the psychological counterparts of these changes? Finally, we still know very little about the nature of changes in the large-scale regional organization and connectivity of human cerebral cortex that support distinctively human higher-order cognitive functions.

Fortunately, we now have methods, such as DTI and various molecular techniques, that – in the context of comparative studies – will enable us to progress on these fundamental questions about human nature. Unfortunately, comparative studies have historically not enjoyed very strong support from funding agencies, and the US government recently decided to end support for its chimpanzee facilities. Without the ability to compare humans to chimpanzees, our closest relatives, our ability to understand what it is about our minds and brains that make us human will be severely compromised.

Human Brain Development

Just as a comparison of brain structure between humans and other species can provide clues regarding possible differences in mental capacity, so can it be helpful to examine how the brain changes over development. A healthy human child is born with a brain that looks remarkably like the brain of an adult, albeit 3.5 times smaller. Our brains undergo substantial structural changes during infancy and childhood. The number of synapses in the brain reaches a maximum density of about 150% that of the adult level in the first year of life, with pruning of excess synapses taking place over the rest of childhood and adolescence. Long-range projections between brain regions are established, and the axons that functionally connect these distant regions become myelinated, leading

to faster neuronal transmission. By around age 9, a child's brain is approximately the same volume as that of an adult. However, upon closer examination, structural differences between these brains can still be observed.

During the second decade of life, changes in brain structure are less dramatic but still important. Cortical gray matter volume, which reflects neuronal density and the number of connections between neurons, peaks at around age 10–12, and then begins to decline. Gray matter loss occurs at different rates in different subregions of the brain. By the metric of rate of gray matter reduction, both prefrontal and parietal cortices exhibit protracted developmental time courses. During this second decade, steady increases are observed in white matter volume – which reflects myelination and increased axon thickness. Greater coherence of white matter tracts over development is associated with better performance on tasks that require interaction between regions connected by these tracts. In summary, both cortical pruning within brain regions and increased neuronal connectivity within and between regions underlie changes in brain function over development.

The finding that gray matter peaks at a different age for different brain regions shows that the brain matures in a piecemeal manner. The fact that brain development unfolds quite similarly across individuals invites the speculation that this piecemeal maturation is finely orchestrated. Indeed, as noted previously, evolutionary pressures come into play at all stages of development, such that brain function at each stage should be age appropriate.

Although brain maturation may, in broad strokes, unfold in a fairly stereotyped way, there are significant individual differences between children in brain structure and in the trajectory of brain maturation. These differences can provide insight into structure–function relationships. Interestingly, recent longitudinal work indicates that the dynamics of gray matter increases and decreases over childhood are associated with differences in intellectual ability, particularly in anterior PFC.

Developmental Functional Brain Imaging Research

The pattern of developmental changes in brain activation over childhood and adolescence has been generally characterized as a shift from diffuse to focal activation and from posterior to anterior activation. Differences are usually quantitative, with one age group engaging a region more strongly or extensively than another, but sometimes qualitative, with a shift in reliance on one set of brain regions to another.

Importantly, the precise pattern of change observed depends not only on the ages being examined and the brain region in question, but also on the task being performed during functional brain imaging. We have found

that a region may exhibit adult-like patterns of activation during middle childhood in one cognitive task, but not another. Thus, a region's cellular architecture and pattern of connections may be sufficiently developed to contribute effectively to a neural circuit underlying one task or cognitive function, but not to another. An important current and future direction for developmental neuroimaging studies, and also for comparative neuroimaging studies, is to examine developmental changes in the pathways and functional interactions between brain regions.

Conclusion

Both evolutionary and developmental approaches to cognitive neuroscience explore the differences in brain structure and function between organisms. Developmental cognitive neuroscience has focused on age-related differences in the function of a brain region or the organization of a brain network. In contrast, evolutionary neuroscience has, until quite recently, emphasized the similarities rather than differences between species, in particular between humans and nonhuman primates. Developmental and individual differences research shows us that, even within a species, there can be salient differences in structural and functional organization; it stands to reason, therefore, that differences should be observable across species.

We remain profoundly ignorant about how the human brain changed in evolution, but going by current evidence, it is clear that the human brain is not simply an enlarged monkey or ape brain: it is a different brain. Similarly, we still know relatively little about how the human brain develops and changes over the life span, but it is important to keep in mind that the adult brain is not merely the child brain with additional functions and circuits. Each stage of development must be functionally viable, and it is of interest to consider not only the adult brain as an endpoint, but also the specializations of the infant, child, or adolescent brain.

See also: Brain Evolution in Vertebrates; Brain Imaging; Human Evolutionary Genetics; Language and

Communication – Brain Substrate; Neural Basis of Working Memory; Physical Cognition and Reasoning; Primate Origins of Human Behavior; Social Cognition: From Behavior-Reading to Mind-Reading.

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Sex Hormones, Mood, and Cognition

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Glossary

Androgens – A natural or synthetic steroid hormone that stimulates or controls the development and maintenance of masculine characteristics in vertebrates. Endogenous or natural forms are produced chiefly in the testes. The primary endogenous mammalian androgen is testosterone.

Aromatase – Aromatase is an enzyme that is responsible for a key step in the biosynthesis of estrogens (estradiol or estrone) from androgens (testosterone and androstenedione, respectively).

C19 steroids – C19 steroids are a subset of androgens synthesized in the adrenal gland (adrenal androgens), that have 19 carbons and include dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S), and androstenedione.

Estrogens – A natural or synthetic steroid that stimulates or controls the development and maintenance of female secondary sex characteristics. Endogenous or natural forms are produced chiefly in the ovaries. The primary endogenous mammalian estrogen is estradiol.

Neurosteroids – Neurosteroids are neuroactive steroids which are synthesized in the central and peripheral nervous system that can rapidly alter neuronal excitability through neurotransmitter-gated ion channels and alter gene expression through intracellular steroid hormone receptors.

Progesterone – Progestins are synthetic progestogens (e.g., medroxyprogesterone acetate (MPA)), are often combined with estrogens in contraceptive regimens or postmenopausal hormonal therapies, and may differ from progesterone in their behavioral effects.

Progestogens – Progestogens are steroid hormones that maintain the uterine lining and are thus important in maintaining pregnancy. Progesterone is the most important and the only naturally occurring human progestogen. ‘Allopregnanolone’ is derived from progesterone and affects mood and cognition.

‘progestogens’ (**Table 1**). Aromatase is the enzyme that converts testosterone and other C19 steroids (androgens with 19 carbon atoms) to estradiol. Because of this, it may be unclear, in some studies, whether testosterone has direct effects on the central nervous system (CNS) or whether it has been converted to estradiol which then affects CNS function. Aromatase is expressed in the brain, and is classically thought to regulate neuroendocrine processes and behaviors important to reproduction. However, recent studies suggest that aromatase also affects mood and cognition and thus will be considered here. Progesterone is the most important and the only naturally occurring human progestogen. Allopregnanolone is derived from progesterone and affects mood and cognition. Synthetic progestogens, herein termed progestins, are often combined with estrogens in hormone therapies and may differ from progesterone in their behavioral effects. In general, androgens are considered male sex hormones as the greatest single source of androgens are the testes and they typically have masculinizing effects. Estrogens and progestogens are considered female sex hormones as their greatest single source is the ovary. However, all of these hormones are present in each gender, albeit at different levels. Sex steroids are usually thought of as being produced in the gonads. However, some – notably estradiol, progesterone, and allopregnanolone – are produced in the CNS and these are termed neuroactive steroids or ‘neurosteroids’.

Two Major Roles of Sex Hormones

The two major ways in which sex hormones affect the CNS, mood, and cognition are through organizational and activational mechanisms. Organizational effects are achieved by developmental influences of sex hormones during critical periods that result in generally permanent sexually dimorphic differentiation of brain morphology that gives rise to sexually dimorphic physiology and behavior in adulthood. Activational effects are acute effects of gonadal hormones on the fully developed nervous system and, in general, serve to maintain sexually dimorphic physiology and behavior in adulthood.

Major Classes of Sex Hormones that Effect Mood and Cognition

The three main classes of sex steroids are ‘androgens’ and ‘estrogens’ – of which the most important human derivatives are ‘testosterone’ and ‘estradiol’, respectively – and

Table 1 Sex hormones known to affect mood and cognition

Androgens	Estrogens	Progesterogens
Testosterone	Estradiol	Progesterone
Androstanedione	Estrone	Allopregnanolone
Dihydrotestosterone		Progrestins
Dehydroepiandrosterone		
Anabolic steroids		Aromatase

Organizational Effects aka Developmental Programming by Sex Hormones

Androgens

Testosterone exposure early in life accounts for most of the known sex differences in brain structure, physiology, and behavior in rodents and nonhuman primates. Discrete periods of endogenous testosterone surges occurring pre- and postnatally suggest that these may be critical periods of organizational effects. Removal of testosterone in males by the administration of antiandrogens or castration increases female-typical behavior, whereas early administration of testosterone to females increases male-typical behavior. Behaviors that may be affected include juvenile play, grooming, aggression, sexual behavior, and maze performance (spatial ability). Sexually dimorphic behavior in the human is apparent in juvenile play (playmate and toy preferences), perhaps spatial ability, aggression, sexual orientation, gender identity, and the prevalence of behavioral pathologies such as autism, schizophrenia, and depression and anxiety disorders. Thus, sex-hormone exposure early in life may have lifelong influences on behaviors relevant to cognitive function and mood.

Estrogens

Since testosterone and other C19 steroids are converted to estradiol in the brain via aromatase, estradiol may have organizational effects in the CNS and on behavior. However, there is little specific data available about organizational effects of estrogens on mood or cognitive function.

Progesterogens

There is little evidence of organizational effects of progestogens on mood or cognitive function.

Activational Effects

Mood

Androgens

Testosterone administration in male and female rats reduces fear- and anxiety-related behavior. Mood changes, including dysthymia, depression, anxiety, and

irritability, may be found in men with low testosterone levels or hypogonadism. Classic hypogonadism may be observed in young and middle-aged men, and exogenous testosterone treatment may improve mood. Men with refractory depression may improve with exogenous testosterone therapy. Age-related hypogonadism is a part of normal aging in the later life of men. Scores on tests of depression may increase with decreasing testosterone levels. Thus, an association between testosterone and mood in men is supported by the observations that depression may be associated with reduced testosterone concentrations, mood in hypogonadal men may be improved by exogenous testosterone administration, and testosterone itself may have antidepressant properties.

The ovaries contribute about 50% of circulating testosterone either by direct secretion or by precursor production. Thus, the decline in ovarian function at menopause is accompanied by reductions in testosterone as well as estrogen. Testosterone supplementation improves the overall sense of well-being, as well as mood, suggesting that testosterone affects mood in women as well as men.

Aromatase

Understanding of the effects of androgens and estrogens on mood and cognition is complicated by aromatase – the enzyme that converts testosterone and other C19 steroids to estradiol. Animal studies suggest that hormone-related depression may involve the effects of estradiol on the neural serotonergic system which is perturbed in depression. In female rats, estradiol induces gene expression of the 5-hydroxytryptamine (serotonin (5-HT)) a receptor and the serotonin transporter (SERT) in the dorsal raphe nucleus, both of which are targeted by antidepressant pharmacotherapy with selective serotonin reuptake inhibitors (SSRIs). This results in increased densities of 5-HT_a receptors and SERTs in forebrain regions which in the human are involved in the control of mood, cognition, and emotion. In male rats, castration decreases, while testosterone or estradiol increases, 5-HT_{2a} receptor and SERT gene expression. 5 α -dihydrotestosterone has similar actions as testosterone; however, it cannot be converted to estradiol by aromatase. 5 α -dihydrotestosterone does not affect the 5-HT_{1a} receptor or SERT gene expression, suggesting that the effects of testosterone are due to its conversion to estradiol by aromatase.

Because of this conversion, it is unclear in many studies whether testosterone has direct effects on the CNS or whether it has been converted to estradiol which affects CNS function. Aromatase is expressed in the brain, and recent studies suggest that aromatase affects mood and cognition.

Aromatase knockout female, but not male, mice show increased depressive-like behaviors. Polymorphisms in the cyp19 gene, which encodes the aromatase enzyme,

are associated with depressive symptoms in women. Modifications in brain aromatase activity may affect how steroids such as estrogens modulate aggressive behavior. In animal studies, previous social experience regulates aromatase activity in the brain, and may modulate the effects of aromatization on the likelihood of aggression or components thereof such as hostility or irritability. Thus, aromatase activity may modulate certain aspects of mood.

Ovarian sex hormones

The endogenous cyclicity of ovarian sex hormone production affects emotional behavior and involves both estradiol and progesterone. Anxiety disorders such as generalized anxiety disorder, posttraumatic stress disorder (PTSD), and panic disorder are about twice as common in women than men. Anxiety represents a dysregulation of the fear circuitry. In female rats, fear- and anxiety-related behaviors vary with the estrous cycle – with lower levels than those of males during the afternoon of proestrous when estrogens and progestins are at their peak.

Depressive disorders, including dysthymia, depression, atypical depression, and seasonal depression, are about twice as common in women than in men. The risk of depression in women increases shortly after puberty and remains elevated through menopause. Women are prone to depression during times of reproductive hormone change such as puberty, the postpartum period, the premenstrual phase of the menstrual cycle, and the perimenopause, as well as during oral contraceptive use. Thus, the female reproductive system function modulates mood; however, the mechanisms are not well understood.

Not all women experience changes in mood with changing reproductive hormone milieu, suggesting a subset of vulnerable individuals. Premenstrual dysphoric disorder (PMD) is a cluster of both negative mood symptoms and physical symptoms that occur during the luteal phase of the menstrual cycle and disappear after the onset of menstruation. Women with and without PMD do not differ in gonadotropin or gonadal steroid production. Pharmacological suppression of ovarian function eliminates PMD; adding back estradiol or progesterone results in the return of PMD symptoms within 2 weeks that remit by the fourth week. The same pharmacological manipulations in women without PMD result in no effects on mood. Thus, PMD appears to be an abnormal response to normal hormonal changes and levels.

Likewise, for some women, pregnancy is a time of elevated risk for depressive and anxiety disorders. Women at greater risk of depression during pregnancy are younger, from lower socioeconomic strata, not married, and are less educated. A clinical conundrum concerns whether to treat depression during pregnancy – as antidepressants may be teratogenic – while untreated maternal depression increases the risk of poor obstetric

and neonatal outcomes. Abrupt changes in the hormonal milieu appear to be instrumental in the heightened risk for depression during the postpartum period.

During the perimenopause, the best predictor of a depressive episode is a prior history of depression. However, there is also increased risk of a first episode of depression during the perimenopause. A history of PMD or poor sleep patterns are risk factors for perimenopausal depression. The prevalence of vasomotor symptoms (hot flushes) is also associated with increased risk for depression during the perimenopause. Hot flushes may disrupt sleep-exacerbating mood disturbances, or they may reflect a particular underlying physiology that enhances risk.

Taken together, there appears to be a subset of women at risk for deleterious effects on mood of a changing reproductive hormone milieu. Factors that contribute to individual vulnerability are not understood; however, genetic factors are implicated. Monozygotic twins are highly concordant for PMD, whereas dizygotic twins are not. Women whose mothers had PMD are much more likely to have PMD themselves.

Estrogens

In general, estrogens are associated with positive effects on mood. Estrogen receptors are ubiquitous in brain areas important in mood. Estradiol regulates neurotransmitter systems implicated in depression including serotonin, norepinephrine, dopamine, acetylcholine, γ -aminobutyric acid (GABA), and glutamate, signal transduction systems important to cell survival, and has immediate effects on neuronal excitability. As mentioned above, estradiol induces gene expression of the 5-HT_{2a} receptor and the SERT in the dorsal raphe nucleus both of which are targeted by antidepressant pharmacotherapy with SSRIs. This results in increased densities of 5-HT_{2a} receptors and SERTs in forebrain regions, which, in the human, are involved in the control of mood. Estradiol also increases dendritic branching and synapse formation in the hypothalamus and hippocampus during the rat estrous cycle, and dendritic pruning follows when estrogen levels fall. As the estrous cycle is only 3–4 days in length, these represent rapid changes in neuroarchitecture and implicate synaptic remodeling in the modulation of mood by estrogens. Thus, a plethora of neural mechanisms are available through which estrogens may modulate mood.

Anxiety represents a dysregulation of the fear circuitry. Estradiol has been found to both facilitate and disrupt fear learning. These apparently contradictory findings may be due to the antagonistic roles of different estradiol receptors. There is considerable evidence that estrogens regulate aggressive behavior in many species, including the human. However, this relationship is influenced by several other important variables such as aromatase activity, estrogen receptor expression and related cofactors, and social experience. Estrogen replacement therapy in

surgically or naturally postmenopausal women has positive influences on mood and well-being. However, these positive effects on mood and well-being are attenuated by the addition of a progestogen. In animal studies, estradiol has synergistic effects with SSRIs on behavior and serotonin neurochemistry, and in surgically or naturally postmenopausal women estrogen replacement therapy can facilitate the antidepressant actions of SSRIs.

Progestogens

Progesterone may affect mood directly, primarily through interaction with progesterone receptors, or via the progesterone derivative allopregnanolone which is produced peripherally and centrally. In the brain, allopregnanolone is thought to have no effect on progesterone receptors but instead acts as a positive modulator of the GABA-A receptor. Corticotropin-releasing factor (CRF) has numerous functions in the brain including coordination of stress responses by the hypothalamic–pituitary–adrenal (HPA) axis and many other aspects of emotional arousal. In female rats, progesterone administration reduces responsiveness to CRF, and systemic administration of progesterone or allopregnanolone has anxiolytic effects.

Allopregnanolone accumulates in the brain and plasma during the luteal phase of the menstrual cycle. The increase in progesterone levels during the luteal phase of the menstrual cycle is considered to be partly responsible for the negative mood changes in PMD. Although ovarian steroids are required for the onset of premenstrual symptoms, women with PMD may also have altered GABA-A receptor sensitivity. Likewise, postmenopausal women have significantly greater negative mood symptoms during progesterone treatment than during treatment with estradiol or placebo if their circulating allopregnanolone levels are similar to those observed in the midluteal phase of the menstrual cycle, but not if they are either higher or lower. These observations suggest that optimal levels of allopregnanolone are critical for mood.

Postmenopausal hormonal therapies include a progestogen when prescribed to women with a uterus to reduce endometrial proliferation and the associated cancer risk. One of the more common progestogens in postmenopausal hormonal therapies is the progestin medroxyprogesterone acetate (MPA). In postmenopausal women, hormonal therapy using micronized progesterone has more beneficial effects on vasomotor symptoms, somatic complaints, anxiety, and depressive symptoms than MPA-containing hormonal therapies.

Cognition

Androgens

Cognitive ability including memory, attention, language, and visuospatial ability declines with age. In men, the average free testosterone index (the ratio of serum total

testosterone to sex hormone-binding globulin (SHBG)) may decline by as much as 50% between 30 and 80 years of age. Older men with higher levels of the free testosterone index have better scores in tests of visual memory, verbal memory, visuospatial function, and visuomotor scanning; whereas men who are hypogonadal have lower scores on tests of memory and visuospatial function, and a faster decline in visual memory. There is some evidence that age-related decrements in testosterone and at least some aspects of cognition may be causally related. Exogenous testosterone therapy in older men has been observed to improve certain aspects of cognitive function, notably spatial ability. Several studies also have found that men with mild cognitive impairment and men with Alzheimer's disease have lower circulating total or bioavailable testosterone. Furthermore, low testosterone levels are a risk factor for the development of Alzheimer's disease as much as 10 years in advance. Testosterone-replacement therapy in men with mild cognitive impairment or Alzheimer's disease has been the focus of a few small studies. The results have been mixed, but deserve further study.

Aromatase

Some experimental and clinical studies also suggest that aromatase activity impacts cognitive function. In songbirds, local aromatization of testosterone to estradiol enhances hippocampal-dependent processes, including spatial memory. In male rats, inhibition of brain aromatase ameliorates the spatial learning impairment induced by exogenous testosterone administered into the hippocampus, and systemic administration of an aromatase inhibitor facilitates working memory. Aromatase inhibition has been observed in some clinical studies, but not others, to prevent the improvements in memory produced by testosterone administration. Aromatase inhibitors used in cancer treatment have been observed in some studies to impair verbal and visual learning in women. Thus, aromatase activity may improve or impair specific cognitive processes, most likely by regulating estradiol levels.

Estrogens

Of all the sex hormones, the evidence for estrogen effects on cognitive function is most abundant. Estrogen acts across a broad range of neurotransmitter systems including acetylcholine, catecholamine, and GABA in animals and human beings. Estrogen receptors are present in many limbic regions involved in learning and memory, including the hippocampal formation and amygdala, and the cerebral cortex. Estradiol enhances synaptic plasticity, neurite growth, hippocampal neurogenesis, and long-term potentiation. The broad range of these effects suggests that estrogen may act as a conductor, orchestrating the functions of multiple systems important to cognitive function.

Cognitive function varies with menstrual cycle phase. In general, estrogen has been shown to enhance performance in working memory tasks, and delayed match-to-sample tasks. However, decreased performance on a delayed match-to-sample working memory task has been observed in association with the high estradiol levels of the follicular phase of the menstrual cycle when specific emotional facial expressions are used as stimuli. Thus, the effects of estradiol on memory for certain emotional information may be different. In general, male mammals appear to have better spatial ability than females. Performance on spatial-delayed-recognition span tests is poorer in primates when estradiol levels are high at mid-cycle than in the follicular and luteal phases, suggesting that estradiol influences spatial ability.

Decrements in cognitive function are associated with menopause. Estrogen therapy initiated in the early postmenopausal period improves cognitive function, particularly memory, and may decrease risk of Alzheimer's disease. Estrogen therapy initiated late in the menopause has no beneficial effects on cognitive function, and may increase the risk of Alzheimer's disease, suggesting a critical window for beneficial effects of postmenopausal hormonal therapy on cognitive function.

Progestogens

The majority of research on sex hormone effects on cognition has focused on estrogens. However, progestogens have powerful effects on a broad range of neural systems, and these effects may be progestogen specific. While progesterone has important neuroprotective properties that may protect against cognitive decline with age, other progestogens may not share these characteristics. In rats, acute treatment with allopregnanolone has GABA_A-agonist effects that impair learning and memory. In women with PMD, difficulty in concentration and impairment in working memory accompanies mood changes during the luteal phase when progesterone and allopregnanolone concentrations are high. Cognitive disturbances are common during pregnancy and tend to become apparent in the second trimester when progesterone and allopregnanolone levels are high in the brain. In normal, healthy, cycling women, exogenous progesterone administration impairs facial recognition accuracy. Progestogens, most commonly progestins, are combined with estrogens in hormonal therapy for postmenopausal women with a uterus. The inclusion of a progestin may antagonize the beneficial effects of estrogens on cognition.

Combined hormonal therapy initiated late in the menopause increases Alzheimer's disease risk over that reported for estrogen therapy alone. Further investigation is needed to clarify the effects of progestogen on cognitive function.

See also: Aging and Cognition; Animal Models of Learning and Memory; Cognition: Learning and Memory: Spatial; Declarative Memory; Depression; Emotion-Cognition Interactions; Hormones and Memory; Human Fear and Anxiety; Neural Substrates of Unconditioned Fear, Defense, and Anxiety; Neuropsychological Aspects of Anxiety Disorders; Offensive and Defensive Aggression.

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Social Cognition: From Behavior-Reading to Mind-Reading

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Glossary

Convergent evolution (homoplasy) – The acquisition of similar traits in unrelated lineages; that is, two lineages share a trait, but not because of common descent with shared ancestry.

Gaze-following – Looking in the direction that others are looking based on various cues including eye, head, and body position.

Mutualism – A type of cooperative interaction in which two or more individuals obtain a benefit from working together that they would not have obtained alone

Perspective-taking – The ability to reason about what others perceive, given that others may perceive aspects of the world differently than oneself. For example, an individual standing in one location may not be able to see everything that is visible to an observer in a different location.

Phylogeny – The pattern of relatedness between species and taxonomic groups, representing the evolutionary branching pattern of speciation leading to extant (living) species.

Social cognition – Processing social information. Social-cognitive skills can depend on the information provided the overt behavioral cues of others (in what direction are they looking or moving?) or depend on more mentalistic processes (what do others see or want?).

Social tolerance – A measure of how individuals in a species interact. Species with high tolerance tend to have more relaxed dominance hierarchies, and can sit in close proximity and even co-feed without much aggression. Species exhibiting less tolerance may have more ‘despotic’ hierarchies and show more aggression when interacting.

Theory of mind – The ability to attribute various mental states – such as beliefs, desires, and knowledge – to other individuals. Perspective-taking is one component of theory of mind.

as a driving force in primate cognitive evolution. This basic thesis – that the sophisticated cognitive abilities of primates have evolved for a social function – has spurred experimental and theoretical investigations for over 40 years. Although, most early proposals of the social intelligence hypothesis were inspired by observations of seemingly complex social behaviors across the primates, however, the psychological mechanisms underlying these behaviors were not well understood. It was not until relatively recently that research began to address the cognitive abilities primates actually use when interacting with others, such as whether other primates share capacities like theory of mind with humans.

In this article, we highlight a selection of complex behaviors that primates exhibit when interacting with others, with special attention to the cognitive mechanisms supporting those behaviors. Fundamental to the study of comparative cognition is the idea that many species may exhibit behaviors that appear similar, even though the psychology underlying those behaviors may differ across taxa. While this distinction may be methodologically frustrating – behavioral observations are a rich source of knowledge about animal psychology, and experimental tests of cognition may not always be viable – in fact, it is a testament to the ingenuity of evolution: the hard social problems that primates face get solved, even if the solution is not always the same! This distinction highlights the importance of thinking about primate social interactions not only in the context of behavioral evolution – the special things that primates (and humans) do – but also in terms of cognitive evolution – the special ways that primates think. We use this framework to analyze primate social behavior, and the differing psychologies underlying this behavior, in three areas: gaze-following, food competition, and mutualistic cooperation. The ultimate challenge of such analyses will be to understand why such different cognitive mechanisms have evolved across species.

Gaze-Following: Reflective Orienting or Understanding of Attention?

Imagine a small group of chimpanzees foraging in close proximity in a forest. One chimpanzee hears something in the trees, and looks up. The other chimpanzees follow suit. What cues lead the other chimpanzees to look up: the change the first chimpanzee’s head position, or more

Introduction

The social world has long been thought to be a major force shaping primate cognition: the social lives of primates are thought to be sufficiently complex to have acted

specifically a change in that chimpanzee's eye position? Did they follow gaze because they thought that the first chimpanzee had seen something, or was the process more reflexive, relying on an egocentric mechanism? Now imagine the same occurrence in a group of capuchins. Did these very different primates use the same cues as the chimpanzees? Do they understand the situation in the same way?

Gaze-following or co-orienting behaviors like this allow individuals to apprehend important objects and events that others have detected in the environment, including food sources, predators, and conspecifics. Thus, gaze-following allows individuals to exploit the information that others have acquired about the world. At the most basic level, diverse species of primates spontaneously follow the gaze (face direction) of human experimenters or conspecifics. Species including chimpanzees and the other great apes; Old World monkeys such as various macaques, mangabeys, and baboons; New World monkeys including capuchins, cotton-top tamarins, and common marmosets, and even some lemur species, all follow gaze, at least to a certain degree in some contexts.

Although gaze-following behaviors are widely shared across the primate order, the psychological basis of these co-orienting behaviors seem to vary widely, especially in how flexibly this behavior is deployed. First, different species may vary in what cues they use to follow gaze. For some species, there is evidence that individuals can successfully follow eye position alone (e.g., apes). Other species, in contrast, appear highly dependent on shifts in the position of the face, head, or even entire body (such as capuchins, tamarins, and lemurs). Interesting, this variation may be due to variation in the amount of information that the eye carries due to differences in eye morphology across different taxa.

Secondly, gaze-following appears to reflect understanding of attention in some species, but may be more of a reflexive response in others. For example, gaze-following in chimpanzees and other great apes suggests that individuals of these species follow gaze because they understand something about the nature of seeing. Apes not only direct their own gaze in the direction of others, but also follow gaze around barriers and past distracting objects that are not the target of another's gaze, sometimes by physically reorienting their own bodies. They may also check back with the actor in attempt to verify the direction of the other's gaze or quickly stop following the gaze cues when they cannot locate the target of the other's gaze. These flexible shifts in behavior across contexts suggest that apes follow the gaze of others because they expect there to be something interesting to see.

The evidence for such behaviors in more distantly related primate species is less complete, mostly because few studies have been conducted. Macaques, like apes,

habituate to repeated gaze cues when they repeatedly cannot locate the target of another's gaze. However, studies of New World monkeys and lemurs suggest that the co-orienting behaviors in some of these species are more reflexive and used in more limited circumstances. For example, cotton-top tamarins will co-orient with conspecifics at high rates during natural interactions (although the cause of this co-orienting is unclear), but fail to follow the explicit gaze cues provided in controlled experimental settings. Similarly, some lemur species co-orient with conspecifics during their natural behaviors, but seem less able to follow gaze in experimental contexts. Thus, although behavioral co-orienting may be common to all primates, not all primates necessarily follow gaze because they understand that others see things. Thus, observations of different species of primates altering the direction of their gaze in response to another individual doing the same does not mean that this behavior is modulated by the same cognitive mechanism.

Competition for Food: Behavioral Predictions or Visual Perspective-Taking?

Imagine the same group of chimpanzees foraging in the forest. As most of the group is distracted, busy following the gaze of that first chimpanzee, a young male realizes that the group has come upon a fig tree with many ripe fruits. He knows that if he has to compete with more dominant individuals for this food, he will likely lose out. Luckily, he spots a large fig that is hidden behind a fallen log and thus out of sight of the others, and quickly runs to take it. What cues led him to target that piece of food, and not another in full view of the other group members? What did he think when he did so? What would it mean if a capuchin was spotted doing the same thing?

Food competition like this is an important consequence of the fact that primates are social living animals: although being gregarious has benefits, it also has costs. Dealing with competition with conspecifics is, therefore, a major problem that primates have to solve. However, there are different ways to deal with such competition that might be quite successful. One possibility is to become a skilled behaviorist, or an expert in using the actions of others in order to decide what to do. In this case, the young chimpanzee might be especially sensitive to the orientation his competitors head and body, as well as any indication that the competitor was going to approach another piece of fruit. A soon as the competitor headed one way, the male headed the other – in this case, to the fruit behind a log. An alternative strategy is to become a mentalist. Rather than depend on the actions of others when making social decisions, a mentalist might form representations of what others perceive – or even

know – and act based on this model of what is going on in the other individual's head. In this case, the male might have realized that, even though he could see the fallen fruit, the competitor could not do so from his different spatial position, and thus it was safe to take the fruit.

These two potential mechanisms might look the same in a naturalistic context, leaving even the most skilled observer of wild chimpanzees and capuchins in a tough predicament: how to determine if chimpanzees and capuchins are approaching this problem in the same way? Luckily, these different underlying psychologies predict very different outcomes in experimental manipulations. For example, a behavior-reading strategy might function perfectly well in most natural situations in which the behavior of others is readily apparent. However, this kind of cognition should break down when such behavioral cues are not available for the formation of predictions. A mentalist strategy, in contrast, should be functional in this context whenever there are cues to what another agent perceives, because a mentalistic can infer how seeing is linked to future behavior – even if the competitor has not yet undertaken any actions to observe and react to. This type of cognition, consequently, would potentially allow animals to integrate different types of behavior and contextual cues into a representation of an unobservable phenomenon – another's psychological state – without limiting behavioral predictions to only specific types of input from the environment (the current behavior of that individual, such as the direction in which they are facing at the moment).

These different hypothetical mechanisms for predicting other's behavior has been tested in chimpanzees in a situation where two chimpanzees must compete for access to food. As in the example above, the two chimpanzees had differing knowledge about the food that was available: the subordinate could see both pieces, but only one was visible to the dominant. When both individuals were released into this room, the subordinate targeted the piece that only she could see – a result that is consistent with either cognitive mechanism. Support for a mentalistic view, however, is provided by a condition where the subordinate was released before the dominant. Even though monitoring the other chimpanzee's actions would not be successful in this context, the subordinate still preferred the hidden piece. This suggests they predicted what the competitor would do, instead of simply reacting to what she was doing. A second set of studies further supported the hypothesis that chimpanzees used a mentalistic strategy. Here, both the subordinate and a dominant watched the food items being baited, but sometimes the dominant was switched with another dominant before the competition began; that is, although a dominant always saw the baiting, and a dominant always was competing for the food, sometimes the current competitor had not witnessed the baiting and thus did not know

where the food was located. The subordinates made more attempts to obtain the food when the current dominant had not been present during baiting than when no switch occurred. Thus, the chimpanzees behaved as though they understood that seeing the baiting led to knowing where the food was located – even without overt behavioral cues on the part of the dominant.

Following these initial studies, several experiments using competitive paradigms have assessed similar perspective-taking skills both in apes and monkeys. For example, when rhesus macaques can choose to steal food from one of two human experimenters, they show sensitivity to variations in visual access and prefer to approach food in front of an experimenter with limited visual access (e.g., because their eyes are covered) than food in front of an experimenter with unimpeded visual access (e.g., their mouth is covered in a similar fashion, but they still can see). Studies with chimpanzees have similarly shown that they prefer to retrieve a piece of food that a competitive human cannot see, even attempting to disguise their interest in the food as they approach. Critically, in both of these examples the experimenters provide no direct behavioral cues to what they can and cannot see, such as by touching or approaching some of the food. Rather, primates in these studies must infer which food option is 'safe' from the direction of the humans' gaze and whether the gaze is impeded by barriers – suggesting that these primates do not simply react to the behaviors that they observe, but rather predict what others will do by reasoning about their unobservable psychological states in a flexible manner.

Moreover, some evidence suggests that the perspective-taking that chimpanzees and rhesus monkeys engage in extends to the auditory modality – providing further evidence that these species can integrate many disparate cues to reason about psychological states. For example, when rhesus macaques are confronted with a human competitor sitting in front of two boxes containing food, they preferentially steal food from the box that is silent, and do so only when the competitor cannot already see their actions. This suggests that rhesus monkeys recognize how their behavior will alter the psychological state of the human: if the human cannot see them, then the noise will alert him to their presence. If the human can already see them, then noise will have no impact on the human's knowledge about their behavior. Chimpanzees similarly prefer a silent approach over a noisy one when competing with a human over food.

This mentalistic strategy, however, does not appear to be shared by all primates. For example, both capuchins and marmosets have been tested in versions of the conspecific competition paradigm. Subordinate capuchins are quite successful when released concurrently with the dominant: like chimpanzees, they preferentially target the hidden piece of food. However, in the critical test in

which the subordinate is given a head start and must make a decision in the absence of any behavioral cues, capuchins are flummoxed – in fact, it seems they are not sure which piece to approach until they can see the direction the dominant will take first. Similarly, marmosets target the hidden food in the basic food-competition task, but a series of additional tests suggests that they lack any real understanding of visual access, supporting the conclusion that their successful behavior when competing is due to a behavior-reading mechanism. That is, both species appear to depend heavily on the behavior of the competitor, rather than reasoning about what the competitor sees or knows, when competing for contested food.

Thus, although chimpanzees, rhesus macaques, capuchins, and marmosets all show the same complex behavior in the naturalistic context – targeting the hidden piece of food and thus successfully competing with a dominant – they appear to do so in very different ways: chimpanzees and rhesus depend on a mentalistic interpretation of the situation ('she can not see the food, thus it is safe for me to approach and take it'), whereas capuchins and marmosets depend on the behavior of their competitor to make their social decisions ('she is heading toward that piece, thus I will try to take the other').

Mutualistic Cooperation: Working Together or Working Alone In Proximity?

As the same group of chimpanzees is foraging in the forest, it becomes clear that the earlier noise in the trees was not incidental – it is a group of red colobus monkeys that have (unfortunately for them!) strolled too close to their ape predators. The chimpanzees become silent, and then a subset of the group takes off after the monkeys, some in the trees and some on the ground. After a lengthy pursuit, one of the adult males makes a kill, and later shares meat from the carcass with one of his close allies. What did the chimpanzees understand about the pursuit as it was happening, and how did they view their individual behavior in the context of the group's behavior as a whole? Why did the successful male share meat with his friend? If several capuchin monkeys are spotted chasing and catching a squirrel in a similar fashion, would such behavior be under the control of similar cognitive mechanisms as those seen in chimpanzees?

Group-level cooperative activities such as this are increasingly thought to be important for understanding human cognitive evolution: one thing that seems to distinguish us from even our closest phylogenetic relatives may be the ability to pool individual resources to reach a common goal. In many ways, humans are the ultra-social species! Consequently, examples of complex cooperation in other primates are often quite telling, giving researchers hints of what might have changed in the

human lineage. Cooperation is certainly not something that is unique to the human, however – indeed, like food competition, cooperation in primate societies results from the fact that primates are gregarious. Thus, many primates have been studied in experimental paradigms that examine the cognitive abilities underlying cooperative behaviors.

Human-like cooperation seems to involve many interacting components: in cooperative situations, people typically take on different, mutually reinforcing roles (division of labor), account for what others meant to do, as opposed to what they ultimately ended up doing (attend to the intentions of others), and choose their partners based on how they acted previously in cooperative contexts (reputation and image scoring). One possibility is that some primate species might understand their particular cooperative endeavors like humans. Other primates, however, might cooperate quite successfully without possessing all those components – or, indeed, any of these components. For example, did the pursuing chimpanzees view themselves as part of a group effort to obtain meat, or did each individual have his own strategy to obtain a monkey (but happened to act simultaneously with several other individuals with the same idea?). Did the male who made the kill share meat because he understood that his friend played a crucial role in capturing the monkey, or did something else (such as a desire to prevent intrusive begging) drive his generosity?

We distinguish two broad types of strategies from successful cooperation – recognizing, of course, that many other variants undoubtedly exist. One type of cooperation stems from high levels of inter-individual tolerance: because individuals of such species tend to be in proximity to one another, they show high rates of by-product cooperation just by virtue of time spent together in close affiliation. For example, the chimpanzees in the hunting example may like to groom and sit near each other; thus, they tend to be together when a group of colobus appears. Although their actions in the pursuit appear mutually reinforcing toward the goal of capturing the monkey, the goal is not shared from the perspective of individual chimpanzees. Rather each individual tries to catch the monkey on his own without regard for what other individuals are doing. An alternative is cognitive cooperation: species that have some understanding of the cooperative act itself and the role of their partners in that act. For example, the hunting chimpanzees might actually have realized that hunts are more likely to be successful if different individuals pursue the monkey from different directions, even playing different roles in the pursuit (e.g., one chimpanzee chasing the monkey in the clutches of another). As with gaze-following and food competition, experimental tests of cooperation can help to disentangle these two solutions to the problem of cooperation.

Tolerance appears to play a critical role in facilitating cooperative behavior across a number of species, and

variation in tolerance predicts successful outcomes in cooperative tasks both within and between species. For example, in one study, rhesus and Tonkean macaques were confronted with a problem: there was food placed under a large stone, but no single individual monkey was strong enough to move it. Two monkeys working in tandem, however, could displace the rock and access the food. Only the Tonkean macaques succeeded at doing so, however, and the cause for this differential success seems to be different levels of tolerance in these two species. Rhesus have a ‘despotic’ social structure with strict dominance hierarchies, whereas Tonkean macaques have a more relaxed dominance system. Higher levels of tolerance result in a higher likelihood that a pair of Tonkean macaques would sit in close proximity and attempt to move the stone in tandem. Notably, there was no evidence that the Tonkean macaques understood the problem better than did their close phylogenetic relatives; rather, the Tonkean macaques were just more likely to engage in individual attempts to move the rock at the same time that another individual was doing the same thing.

Results from other primate species confirm that cooperative success can depend heavily on the nature of the relationships that exist between individuals, rather than cognitive abilities *per se*. For example, when pairs of capuchins are presented with an apparatus in which two handles must be pulled simultaneously to acquire food, but the handles are too far apart for one individual to pull both alone, the monkeys show a high rate of success. However, an examination of what the capuchins actually did during the operation of this apparatus indicated that the individuals seemed to pull the handle without regard for what their partner was doing: that is, both pulled at a high rate and thus succeeded basically by accident, but the monkeys did not try specifically to pull in concert with their partner. Similarly, when marmosets were presented with an instrumental cooperative task in which one individual had to pull a lever so that another could grasp a bowl of food, success depended on the role that the dominant and subordinate took. Although individuals had previously shown proficiency in the components of the task when tested alone, only those pairs where the subordinate pulled the lever and the dominant took the food showed any success when working together.

Personal relationships appear to play a similar role in ape cooperation. In a series of experiments, pairs of chimpanzees were confronted with a rope attached to an out-of-reach tray of food. If two chimpanzees pulled both rope ends together, they could eat the food; if one individual tried to pull the rope alone, the rope came unthreaded. The level of tolerance between any two individuals – as measured by their ability to share food in the absence of the cooperative component – was the major determinant of whether that pair would successfully cooperate.

Similarly, bonobos – the sister species of chimpanzees with greater levels of inter-individual tolerance – appear to be more successful than chimpanzees on a version of this task. However, unlike some of the studies with monkeys described above, chimpanzees do not seem to cooperate merely as a by-product of this tolerance. While chimpanzee cooperation is constrained by personal relationships between individuals, it also has a complex cognitive component. For example, chimpanzees know that they need a partner for the cooperative task, and are more likely to recruit that partner (by unlocking a door and allowing the other individual access to the room) when the task requires two individuals than when it can be solved alone. Moreover, chimpanzees prefer to recruit partners that are more successful at the task than those that are less skilled, and recruit those that have recruited them in the past over those that have failed to do so. Thus, chimpanzees seem to remember the past cooperative behavior of others and understand how that influences their own possibility of success now. These results suggest that while tolerance does constrain chimpanzee cooperation, chimpanzees are also cognitive cooperators that understand the role their partner plays in the joint endeavor.

Conclusions: How Does Social-Cognition Evolve?

In this article, we have attempted to highlight some major social problems that primates face, as well as the potential diversity of cognitive solutions to these problems. These particular examples, however, are by no means the only social problems where primates show complex behaviors. Indeed, there are many other examples of primates being quite sophisticated in navigating their social world, in social learning, communication, deception, and knowledge of relationships. Similarly, the cognitive ‘solutions’ to these problems that we have highlighted are not the only ones that primates may use: research on the psychology of most primate species is extremely limited, and it is likely that the future will reveal a much wider range of cognitive mechanisms than research thus far has addressed. However, the examples discussed here serve as important reminders that behavioral complexity can be driven by a diverse set of underlying mechanisms.

In the end, two big questions remain: to what extent do humans and other primates solve social problems in a similar fashion, and why do different species (including humans) solve similar behavioral problems with different cognitive solutions? The first question is a fundamentally phylogenetic one, and research on chimpanzees and bonobos – our two closest primate relatives – have begun to pinpoint how the social cognitive abilities of these apes are shared or differ from our own. As the

studies of gaze-following, perspective-taking, and cooperation mentioned here reveal, in many ways they are surprisingly similar, challenging researchers to come up with new hypotheses about what cognitive traits are unique to our species. The second question is an evolutionary one, and may prove to be the more challenging of the two because it requires reconstructing historical events – evolutionary change and the forces that drove it – that cannot be directly observed. For this question, comparative studies of cognition like those described here, in which the performance of closely related species are examined in similar contexts, are a powerful tool. This is especially the case when such studies are conducted with a focus on the ecological or social variables that may have driven cognitive evolution across taxa.

Studies of social cognition in primates thus provide an important lesson for the cognitive sciences as a whole: there is no such thing as ‘primate social cognition’! Although shared phylogenetic history and convergence does result in cognitive traits that are similar across species, comparative research has increasingly revealed the diversity of primate social psychology. Thus, in many ways it is more appropriate to talk about ‘chimpanzee social cognition,’ ‘rhesus macaque social cognition,’ or ‘tufted capuchin social cognition.’ Notably, this is not just true for primates: research has begun to reveal social-cognitive mechanisms in other groups of organisms as well. For example, wild spotted hyenas live in large social group with monkey-like linear dominance hierarchies and engage in cooperative hunting behaviors. Accordingly, these carnivores (but possibly not their close relatives – the relatively less social brown hyena) appear to possess sophisticated social-cognitive skills that deal with their social landscape, such as understanding of third-party relationships. Canids have emerged as an important model for the evolution of communicative gestures comprehension: in contrast to wolves, dogs are skillful at using points and gaze cues to find hidden food, possibly because of selection during domestication. The most sophisticated social-cognitive skills are found in corvids – a group including jays, ravens, and crows. Studies of these birds have revealed startling parallels with the abilities of primates. Specifically, corvids appear to employ many social cognitive skills (such as perspective-taking or encoding the ‘reputation’ of potential

competitors) to protect their food stores when they engage in caching behaviors.

Such an emphasis on the differences between species can challenge approaches that use a few ‘model’ species for understanding cognitive traits. However, these instances of convergence among distantly related species, like primates and birds, provide opportunities for biologists to test evolutionary hypotheses about the function of specific social-cognitive skills. A complete understanding of the evolutionary pressures leading to complex social cognition requires that scientists look beyond primates to determine the causes and origins of complex cognition. Thus, we hope that we have piqued interest as to what the causes and origins of social cognitive mechanisms might be.

See also: Behavior Adaptation and Selection; Brain Evolution in Vertebrates; Cooperation; Emotion–Cognition Interactions; Evolution of Emotions; Evolutionary and Developmental Issues in Cognitive Neuroscience; Mirror Neuron Mechanism; Personality, Temperament, and Behavioral Syndromes; Primate Origins of Human Behavior; Social Communication; Social Competition and Conflict Resolution; Social Learning and Behavior Transmission; Social Relationships and Social Knowledge.

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Sleep: Medical Disorders

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Introduction

The growing recognition of the complex, multisystem nature of normal sleep physiology, which reached a peak in the 1970s, was associated with the appreciation that, far from the traditional view of sleep as benign and innocent, dysfunctional possibilities abounded – and many were soon documented. By the early 1990s the sleep disorders medicine movement had taken shape, and now it is a worldwide process which has changed the interface between internal medicine, neurology, and psychiatry forever.

Today, sleep disorders medicine is a large and still growing field that is testimony to the power of one unique principle: physiology is state dependent. Because the brain undergoes such dramatic reorganization over both the circadian and ultradian phases of its operation, the central control of virtually every bodily function is mechanistically altered over time. As a consequence functions like respiration – once considered stable – are changed beyond recognition as the brain changes its state. In the case of respiration, it is thus quite normal for breathing rate, depth, and efficacy to change as waking gives way to non-rapid eye movement (NREM) and then rapid eye movement (REM) sleep. Airway patency is also normally altered in sleep. Furthermore, inspiratory neurons, defined in waking, may no longer be inspiratory neurons in sleep. Sensitivity to blood gases such as oxygen (O_2) and carbon dioxide (CO_2) may change or even reverse in sleep.

Because sleep disorders medicine is already so complex a field, it may be helpful to approach its many manifestations from the vantage point of general principles. The result of such a re-ordering of data may seem oversimplified to the specialist but can be clarifying for the uninitiated. The principled approach is the one taken here. Readers, and especially medical professionals who seek state-of-the-art details should consult a more detailed and definitive text such as Kryger *et al.*

State Dependence and Pathophysiology

To understand sleep disorders, it is important to recognize the consequences of normal changes in brain-state control systems. Some of these are detailed in the main

entry: sleep. Only the consequences of sleep disorders will be emphasized here.

Any system with changing activation levels and patterns is subject to alterations of its functional capacity according to the ‘level-setting function’ of its control system. The sleep disorders that can be expected are the insomnias (when the level set for waking is too high) and the hypersomnias (when the level set for waking is too low). We now know that level setting is, in part, a function of brainstem aminergic systems. This recognition helps us intuitively link the insomnias to anxiety disorders via the common ground of aminergic hyperactivation. This is a key point because many people who consult sleep disorders specialists (or any other caregiver) are often anxious worriers who have trouble falling asleep. If they are at the opposite end of the arousal spectrum (and feel sleepy all the time), they are more likely to be depressed and lethargic rather than anxious and hyperactive.

These same contrasts and extremes distinguish between otherwise normal short sleepers and constitutionally constructed long sleepers. We do not expect everyone to be 5'8" tall even though that is the mean height. We will not be surprised to find 7' plus individuals (on the high end) and 5'8" or even 4'6" on the short end. We do not advocate shrinking the tall or stretching the short, so why do we make such a fuss about normalizing sleep length? The answer is that administrative and social assumptions work against biodiversity. We want the same work and school schedules for everyone but nor everyone is ready to get up – or go to bed – at the same time.

The brainstem control systems for sleep and wake are not only set at different levels but undergo different rates of change in time. Thus, it may take some people 35 min to fall asleep while others accomplish the task in 30 s. Short sleepers tend to be hyperactive and to postpone sleep onset so that when they do go to bed, they are sleepy as a natural consequence of deprivation and sleep-ready in terms of fatigue. Long sleepers do just the opposite, so spending more time in bed awake.

Complementing the changes in level setting are ‘timing errors.’ These errors may arise in the input–output gating systems that make response to external stimuli more or less likely over the sleep–waking cycle. Gating systems also determine the likelihood that internal stimuli will be generated. These systems change level radically in synchrony with the NREM–REM sleep-cycle oscillation.

They too produce differential effects according to the level at which they are set and give rise to a new kind of dynamic problem. For example, it is not a good idea to turn on motor-pattern generators in sleep before quelling muscle tone. An unwanted consequence of such timing error could be motor activity such as is seen in the so-called parasomnias, including sleep walking, sleep talking, or the REM sleep behavior disorder (RBD). Similarly, it is not a good idea to turn on the visual hallucinations of dreaming before sleep begins or keep them going after awakening. This is the cause of hypnagogic and hypnopompic hallucinations, respectively. These hallucinations are very frightening to affected subjects who assume that they may be psychotic.

Life Cycle Changes in Sleep

The dynamics of sleep propensity and coordination are impressively great for any individual at any time of life, but there are also prominent changes in sleep across the life cycle. Young people sleep longer, more deeply, and feel more refreshed than the elderly who may complain bitterly about their shallow, fragmented, and unrefreshing sleep. Throughout life, sleep is thus always getting worse with respect to itself. Old persons do not sleep like babies anymore than tennis champions play for more than 5 years. In midlife, this conversion from long-and-deep to short-and-shallow sleep begins with the normal loss of slow-wave sleep (SWS) at about age 40. There is an associated fall in anabolic hormone release so that fat/protein ratios increase. This unfortunate progression is not insomnia. It is physiologically less deep sleep and should be accepted as such.

Neurobiological Basis of Sleep Disorders

Sleep and waking are controlled by brainstem neuronal oscillators which must interact in a coordinated way to produce orderly sequences of behavior. Chief among the control systems is the circadian oscillator in the hypothalamus, which programs rest and activity in coordination with ambient light levels. Its activity is also synchronized with body temperature and energy regulation via food and metabolic caloric control (see Figure 1).

The circadian clock normally suppresses the pontine NREM-REM sleep oscillator via an orexinergic signal from the hypothalamus to the aminergic neurones of the pons. This signal keeps those cells active in waking. When aminergic activity reciprocally declines, cholinergic activity increases ultimately leading to REM sleep. The hypothalamic and pontine oscillators, both, interact with the respiratory center in the medulla giving rise to the idea of the brainstem as a set of coupled oscillators whose

interaction is crucial to normal and abnormal sleep. The problems associated with the set points and timing of the neuronal components of these systems have already been discussed here.

Too Little Sleep: The Insomnias

Many factors can influence the necessary reduction of aminergic activity that permits sleep to occur. Chief among them are signals that the thalamocortical system is still in the waking mode. In the vernacular, this is what is meant by ‘taking a problem to bed with you.’ As long as obsessive rumination over uncompleted or conflictual wake-state behavior persists, aminergic activity remains high and sleep cannot be initiated.

If the level of aminergic activity remains high, sleep may be interrupted as maintenance of sleep fails. This leads to early-morning awakening. The best antidote to aminergic overactivity is a combination of relaxation training, self-induced sleep deprivation, and exercise – three physiological processes which favor sleep. We recognize three main timing defects as afar as insomnias are concerned: initial, central, and terminal insomnias.

Difficulty falling asleep with a sleep latency of over 30 min (initial insomnia) is usually related to psychological, psychiatric, or situational problems; it may be due to environmental factors and inadequate sleep hygiene. Central insomnia is, instead, due to a tendency to hyperarousal. External insomnia may be linked to medical or psychological problems ranging from primary sleep disorders, chronic pain from arthritis or cancer, and metabolic disorders such as diabetes and hypertension. Early awakening from sleep (terminal insomnia) is strongly associated with mood fluctuation or it may be due to circadian rhythm alterations such as the advanced sleep phase typical of many elderly patients who have little else to do, go to bed earlier and earlier, and hence awaken in the early morning.

There are acute adjustment insomnias and chronic, idiopathic, or psychophysiologic insomnias when patients report evidence of conditioned sleep difficulty and/or heightened arousal in bed due to somatic or mental tension. This kind of insomnia is aggravated by unfamiliar and irregular sleep-timing settings.

Paradoxical insomnia refers to the complaint of severe insomnia unsupported by objective evidence of sleep disturbance and without commensurate daytime impairment.

The overestimation of sleep difficulties contrasting with polysomnographic (PSG) evidence of adequate sleep represents the key feature of this nosological entity. Drugs or substances of abuse (e.g., nicotine, cocaine, alcohol) may also interfere with the normal physiology of sleep. Therapeutically prescribed drugs responsible for iatrogenic insomnia include antidepressants such as the

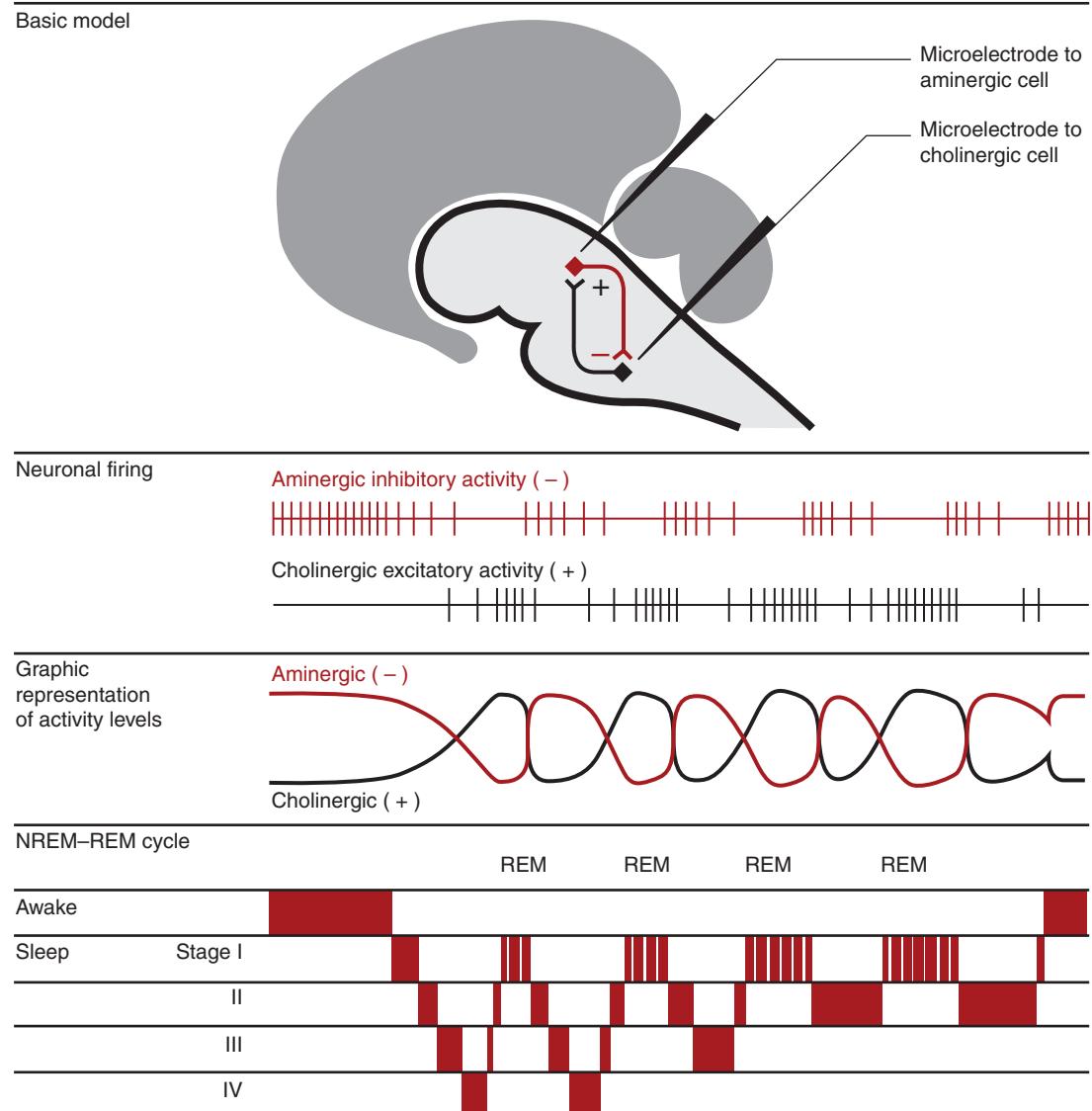


Figure 1 Reciprocal interaction model of state control. This depiction of one night of sleep, based on EEG recordings which differentiate the various stages of sleep, reveals some characteristics of normal sleep. First, sleep is 'deepest' early in the cycle and tends to become shallower. Second, the REM periods tend to be short early in the night and tend to be progressively longer as the night goes on. Animal studies indicate that this rhythm is generated by the alternating activity of specific nuclei of neurons in the brainstem. Some of these nuclei are strongly inhibitory and mediated by serotonin and norepinephrine; their actions are opposed by a group of excitatory cholinergic and glutaminergic neurons. By using microelectrodes, the activity of the various nuclei can be tracked at the level of single neurons, and neuronal firing can be recorded. By plotting the intensity of firing on a vertical axis, the neuronal activity over time reveals a sinusoidal pattern which matches the rhythm of the EEG record.

selective serotoninreuptake inhibitors (SSRIs), clomipramine, antihypertensive medications, the statins, antibiotics, and theophylline.

Unless an organic condition such as sleep apnea or periodic leg movement is suspected, nocturnal polygraphic recording is rarely needed for diagnosis and should be reserved for the insomnias that are refractory to therapeutic trials. Sleep diaries and ambulatory actigraphic devices may help to distinguish the validity of reported symptoms and different patterns of behavior (see

the concluding section titled 'Diagnosis and treatment of sleep disorders').

Pharmacological treatment by hypnotics (benzodiazepines with short or medium half-life such as triazolam and estezolam), and γ -aminobutyric acid (GABA-A) modulator hypnotics (e.g., zolpidem, eszopiclone, or melatonergic agonists such as ramelteon) are sometimes indicated in the acute or subacute conditions for periods <1 month. Sedative antidepressants such as mirtazapine, amitriptyline, trazodone, maprotryptiline, mianserine, doxepine, or

beta-blockers can be helpful in chronic insomnias. The effective use of these drugs must always be supported by attention to sleep hygiene and by cognitive behavioral therapy.

Too Much Sleep: The Hypersomnias

When aminergic activity is low or unsustained by orexin, cholinergic drive increases with consequent feelings of lethargy, fatigue, and even depression. This physiology is associated with sleepiness and a tendency to fall asleep at unwanted times during waking. Among the most common specific causes of hypersomnia are narcolepsy and depression.

Narcolepsy

This rare but instructive sleep disorder is caused by the almost complete loss of hypothalamic neurons containing hypocretin (a.k.a. orexin) – a neuropeptide essential to the maintenance of wakefulness. As a consequence of inadequate facilitation of aminergic neurons in the locus ceruleus and other pontine aminergic nuclei, the REM sleep oscillator is triggered during the daytime. Strong emotion may contribute to this triggering process, which explains why narcolepsy was so long thought to be psychogenic.

Afflicted individuals have compelling attacks of sleep often with sudden loss of muscle tone (cataplexy). These two wake-state symptoms are manifestations of level setting changes in the REM sleep-generator circuits. Narcoleptic patients may also show examples of timing errors in such sleep-related symptoms as sleep-onset REM periods (SOREMPs) with dream-like visions called hypnagogic and with hypnopompic hallucinations and sleep paralysis (the persistence of dream imagery and atonia into the postsleep awakening period; see Figure 2).

Confirming the clinical symptoms, the diagnosis of narcolepsy requires nocturnal polygraphic recording revealing of two or more SOREMPs and a short mean sleep latency, usually less than 2 min. Sleep laboratory testing is followed by daytime assessment of sleepiness by the Multiple Sleep Latency Test (MSLT). Orexin levels in the cerebrospinal fluid (CSF) may be obtained and found to be less than one-third of mean control values. The presence of a human leukocyte antigen (HLA) DQB1 0602 typing is a supportive, but not diagnostic, criteria which may be found in 90% of patients with narcolepsy/cataplexy.

Nowhere in sleep medicine can one find a more complete overlap between the symptoms of a disorder and the underlying pathophysiology. Complementing this intellectual triumph is therapeutic efficacy using drugs known to suppress REM sleep. Anticataplectic drugs include

sodium oxybate (Xyrem) which promotes sleep consolidation and an increase in SWS, during the night, at the expenses of REM sleep. Antidepressants (e.g., venlafaxine, clomipramine, protryptiline, and SSRIs) may also be useful. As for vigilance-promoting agents, the most currently used drug, modafinil, is a proaminergic drug mainly supporting noradrenergic transmission.

Other Hypersomnias

Other central nervous system (CNS) hypersomnias include the idiopathic type (which is not related to REM-sleep alteration but to a tendency to oversleep in NREM) and recurrent types including the Klein–Levin syndrome (KLS) and menstrual-related hypersomnia. KLS is characterized by recurrent episodes of hypersomnia occurring weeks or months apart and associated with overeating, hypersexuality, aggressiveness, and cognitive abnormalities. Alterations in the hypothalamus have been reported. An autoimmune mechanism for this disorder is suggested both clinically and by the HLA association with the DQB1*02 haplotype.

Depression

The pathogenesis of the affective disorders is less well understood than that of narcolepsy, but most models of mood control specify decreased aminergic drive (the ergotropic factor mediating energy availability and release associated with feeling good or even elated mood) and/or increased cholinergic drive (the trophotropic factor favoring energy conservation, rest, sleep, and depressed mood).

Instead of a tendency for the REM-sleep attacks seen in narcolepsy, depressed patients want to go to bed early and stay there for a longer time. Their sleep efficiency is very low because they spend long times awake in bed. They complain accordingly of insomnia. NREM sleep in such patients is typically curtailed and the time of onset of the first REM period is reduced. This change is referred to as a shortened REM-sleep latency.

The first REM period may be both longer and stronger than usual as if the cholinergic REM-sleep drive were acting unopposed. These abnormal sleep features respond promptly to SSRIs (e.g., fluoxetine, sertraline, paroxetine, citalopram) or to mixed reuptake inhibitors (e.g., venlafaxine, duloxetin, mirtazapine, reboxetine) and the widely used tricyclics (e.g., clomipramine, imipramine). A positive response of sleep features to drug initiation is a good predictor of a later benefit in mood regulation. This delay is not well understood but suggests that sleep may have its correlation with mood via a long-term downstream mechanism linking the membrane events to gene expression.

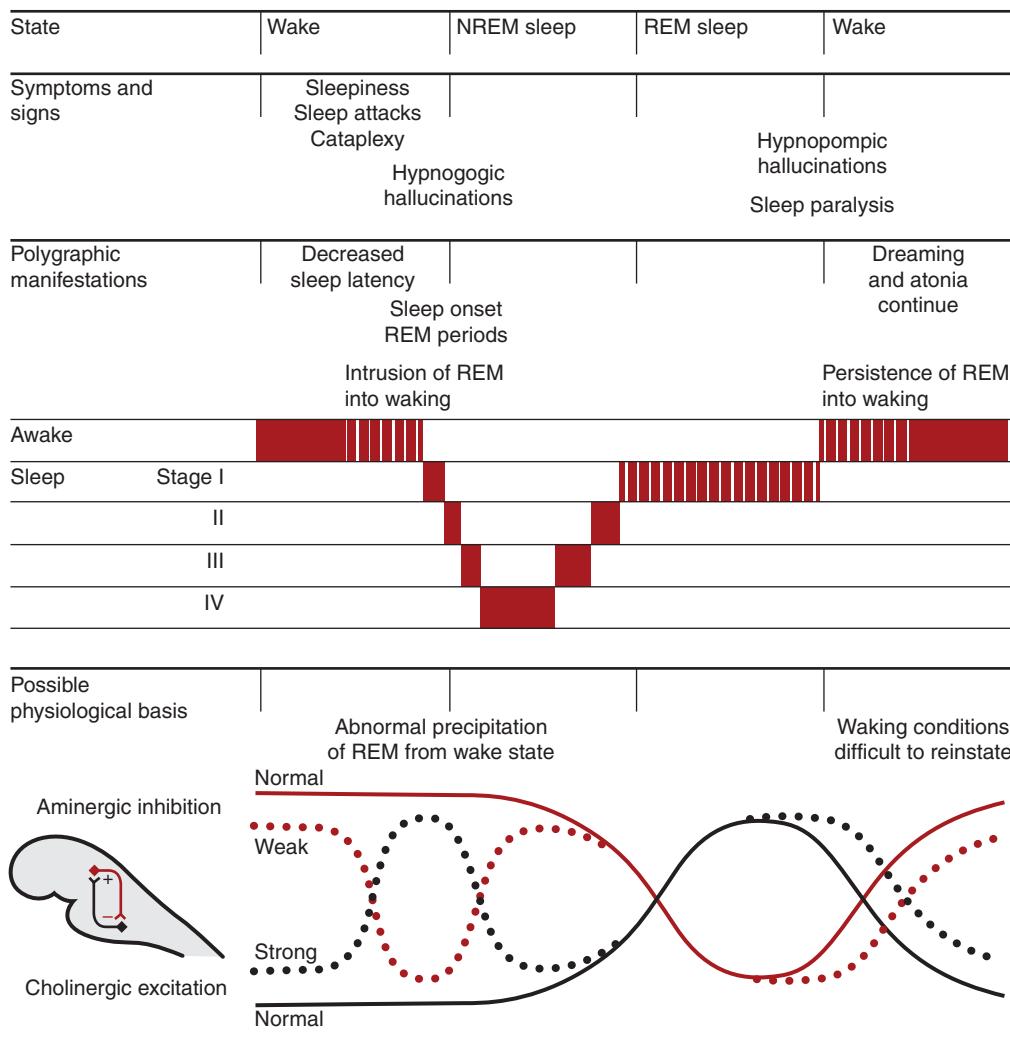


Figure 2 Pathophysiology of narcolepsy. Narcoleptic patients have four abnormalities of sleep–wake-state control that apparently result from changes in the set-point of the REM-state oscillator. They are known as Gelineau's Tetrad honoring the French neurologist who first described them in the nineteenth century. During waking, signs of REM sleep may intrude as sleepiness, sleep attacks, and cataplexy. A sustained REM period or hypnagogic hallucination may occur at the onset of sleep unlike the normal rapid passing through stage I and the first NREM–REM duration may be decreased (measured as shortened REM latency). On arousal, REM physiology and dream psychology may persist as hypnopompic hallucinations and/or motor inhibition (sleep paralysis). Following the first cycle, the system then resets and operates normally except that at the end of REM when there may be a lag in the reinstatement of waking-state conditions and REM phenomena may again escape their normal temporal bounds. The reciprocal interaction model accounts for all these phenomena by hypothesizing either a decreased level of aminergic inhibition (red dotted line) and/or increased level of cholinergic excitation (black dotted line) in the pontine oscillator. The clinical efficacy of aminergic agonists such as amphetamine or amine-reuptake blockers such as imipramine may be due to a resetting of aminergic inhibition back up to level (heavy red line). By reciprocal interaction, this would also reset the level of the cholinergic generator back down to its normal level (heavy gray line).

Sleep Apnea Syndromes

As pointed out in the introduction, it is normal, though still surprising and unexpected, to realize that normal males may omit as many as 50 breathing efforts per night. As a function of the lowered set point of aminergic and related activation systems, the respiratory oscillator of the medulla simply fails to issue breathing commands.

A decreased activation of the respiratory oscillator is normal at sleep onset and throughout NREM sleep. At the

same time and for the same reason, the muscle tone of the airway declines leading both to snoring and less efficient blood oxygenation. The partial pressure of O₂ may decline and that of CO₂ may rise at the same time that brainstem responsiveness to those blood gas levels declines. The result may be central hypoxia and/or hypopnea (Figure 3).

With the onset of REM sleep, there is further aminergic demodulation. There is a concomitant increase in activation probably mediated by cholinergic systems

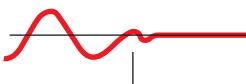
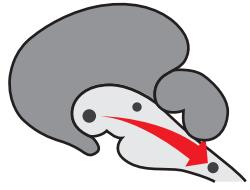
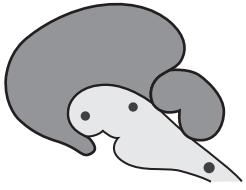
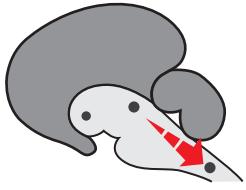
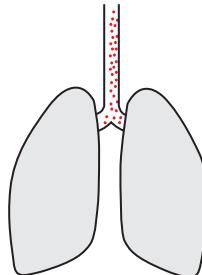
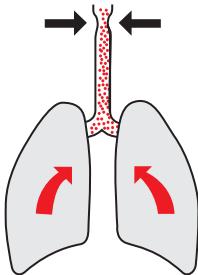
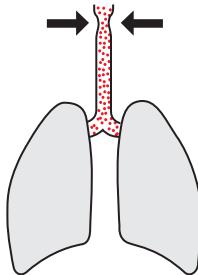
State	Wake	NREM sleep	REM sleep
Breathing pattern	Eupnea/hyperpnea	Hypopnea/apnea	Hyperpnea/apnea
	 Voluntary or metabolic response	 Obstruction	 Desynchronization of respiratory oscillator
Central component	Strong tonic neural drive	Decreased tonic neural drive	Increased tonic and phasic neural drive
			
Peripheral	Muscle tone high Airway patent	Muscle tone decreased Airway collapses	Muscle tone absent Airway collapses
			
		Forced expiration causes obstruction	

Figure 3 Pathophysiology of the sleep apnea syndrome. During waking, the respiratory oscillator of the medulla receives tonic drive from other neural structures and can respond to voluntary and metabolic signals to change the breathing pattern. Tonic drive to the oropharyngeal musculature actively maintains the airway and assures ventilation. In NREM, disinhibition decreases tonic drive on both the respiratory oscillator and the peripheral muscles, thus diminishing respiratory rate and amplitude as well as predisposing the airway to collapse. If obstruction occurs, the forced expiratory effect may actually aggravate this cycle and cause prolonged apneas with marked hypoventilation and hypoxia. During REM sleep, tonic and phasic drive from the pontine generator neurons may desynchronize the respiratory oscillator, leading to hyperapnea and/or apnea. Worse yet, the medullary oscillator can become unresponsive to metabolic signals. These processes may multiply the deleterious effects on ventilation, especially in those patients who are subject to airway collapse.

which are disinhibited as aminergic activity plummets. Neurons, sensitive to O₂ or CO₂ levels in waking, no longer have that propensity and the very definition of inspiratory and expiratory neurons may change or even reverse! With the genesis of REMs and ponto-geniculocolic (PGO) waves, unexpected impingements of neuronal signals may activate or block the respiratory oscillator producing ineffective ventilation via hypopnea or apnea of central origin.

Obesity, male gender, and age conspire to make matters worse. The airway may collapse and obstruct when

breathing efforts attempt to overcome anoxia. The number of microarousals may run to 400 per night and are entirely unrecognized by the patient. Cardiovascular complications arise early and frequently in the course of the disorder. They range from hypertension, cardiac arrhythmia with increased risk of metabolic syndrome, coronary artery disease, and stroke. Because the sleep-apnea subject wakes up often to breathe, he may suffer from extreme daytime sleepiness or he may consider himself insomniac. In fact, he is both! Given this grim picture, it is a wonder that anyone benefits from a night of sleep!

The grunting sound of forced expiratory effort against the obstructed airway can be imitated or tape-recorded by allies of the patient. Bed partners can also turn on the light or use a flashlight to determine if cyanosis is yet present. Any such severely affected subject should be referred on an emergency basis to a sleep laboratory for definitive diagnosis and treatment.

Sleep apnea is effectively countered by continuous positive airway pressure (CPAP), which helps to hold the airway open and by dietary weight reduction. Maxillary surgery may be specifically indicated for patients with relevant local anatomic factors impinging on airway patency. The success of these interventions is greatly increased by a team effort which involves bed partners and family members. Sleep apnea may sometimes overlap with chronic obstructive pulmonary disease – a condition predisposing to severe desaturation during REM sleep when the accessory respiratory muscles are silent and cannot help to diminish the airway deficiency.

Central sleep apnea refers to a recurrent cessation of breathing during sleep. It is thought to be linked to the instability of the respiratory control system in the transition from wakefulness to sleep. It is mainly seen either in elderly patients or in individuals with heightened ventilatory responsiveness to CO₂.

Wrong Kind of Sleep: The Parasomnias

The term parasomnia refers to unusual motor phenomena accompanying sleep. These abnormal movements are either linked to specific sleep stages such as NREM (disorders of arousal such as enuresis, night terrors or sleep walking, etc.) or to REM (such as REM behavior disorder (RBD), nightmares, and sleep paralysis). Some parasomnias have a preference for sleep-stage transitions (such as enuresis and grunting respiration).

A variety of disorders, commonly seen in adolescents, evince what Roger Broughton has called “disorders of arousal.” Awakening from deep NREM sleep is fraught with difficulty because of sleep inertia – a process identified by David Dinges as related to a persistent NREM-sleep drive that counteracts the effort to wake up from deep sleep. Thus, sleep-walking, sleep-talking, enuresis, and night terrors are all sensorimotor disturbances related to this difficulty in waking up. In these conditions, the brainstem and corticothalamic mediators of motility and perception show timing errors with respect to each other.

As stressed by Mark Mahowald, the parasomnias are dissociations by definition and par excellence. For example, in sleep-walking, subjects are awake as far as lower brain centers are concerned but asleep in their upper brains. In sleep-walking, subjects may navigate quite well despite the effect that their cortical

electroencephalogram (EEG) remains in stage IV SWS throughout the episode. Children who awaken, in panic, and evince night terrors, experience intense and unopposed autonomic activation without being able to become fully awake. Less activation with more complex behavioral and cognitive abnormalities are experienced during confusional arousals – another form of commonly occurring sleep disorder, especially in older adults and following sleep deprivation. Disorders of arousal tend to have a familial occurrence and a high prevalence in childhood. They all tend to occur in the first part of the night when NREM sleep prevails.

REM Sleep Behavior Disorder

The most typical and disrupting behavior among the REM parasomnias is RBD. In contrast to sleep-walking, this sleep disorder occurs in REM sleep and represents the unopposed read-out of motor commands normally quelled by the REM inhibition of spinal motoneurons that results in electromyogram (EMG) suppression and sleep atonia. This condition is most commonly seen in middle-aged males whose sleep is suddenly and dramatically interrupted by dream enactment. The movements may be fragmentary or behaviorally complex and are often associated with dreams of threat or attack. The precise details of the association of specific movements to specific aspects of dream content remain to be determined.

A similar condition can be produced in experimental animals by making lesions between the pontine locus ceruleus and pontine motor-pattern-generator centers. Cats with such lesions which enter REM sleep may suddenly jump up and express what appears to be a series of defense and attack postures. Experimental RBD was first described by Jouvet and Delorme. Morrison, who confirmed this observation, called this state REM sleep without atonia. It is another, striking example of a radical state-dissociation.

Males suffering from RBD may develop the condition spontaneously or they may have a history of exposure to SSRI medication or some other REM-sleep-suppressing pharmacological agent. RBD may also be secondary to several neurological conditions including multiple sclerosis, brainstem tumor, Alzheimer’s disease, cerebrovascular disease, and the synucleinopathies. Among the latter, Parkinson’s disease and multiple system atrophy are particularly prevalent. RBD may anticipate clinical parkinsonism by several years. Dopamine transporter (DAT) scans may reveal bilateral alterations intermediate between parkinsonism and normality. Diagnostic screening must include video-enhanced polysomnography to record both the lack of REM atonia and the excessive muscle phasic activity during REM sleep leading to violent behavior outbursts during sleep. Clonazepam, a benzodiazepine

with serotonergic properties, is the drug of choice for RBD but desipramine, melatonin, and dopaminergic agents have also been employed successfully.

Movement Disorders of Sleep

One of the great surprises of basic sleep research is that dopaminergic neurons do not obey the general rule of slowing in NREM sleep and firing arrest in REM sleep. Instead, the dopaminergic neurons of the substantia nigra continue to discharge throughout both NREM and REM sleep. The descriptive details and significance of this exceptional finding remain to be investigated but there are several sleep disorders which may reflect an alteration of dopamine itself or dopaminergic interaction with the other aminergic neuromodulators. The co-activation of cholinergic and dopaminergic neuromodulators in normal motor control is well known.

Sleep-related movement disorders include bruxism (tooth grinding), rhythmic movement disorder (head and body rolling), and periodic leg-movement disorder (PLM). One observes 5–90-s interval, brief (0.5–5 s) movements of the limbs in PLM which cause severe disruption of sleep continuity. As a consequence, there

is an impairment of daytime vigilance as well as interruptions of nocturnal sleep (Figures 4 and 5).

Restless Legs Syndrome

Restless leg syndrome (RLS) is characterized by an irresistible urge to move the legs accompanied by uncomfortable paresthesias. It impairs a smooth sleep transition, severely delaying sleep onset and often interfering with sleep maintenance. Once sleep is finally established, RLS may promote multiple arousals. Abnormal iron metabolism, dopamine levels, and genetics appear to contribute to the pathology of RLS.

Familial forms of RLS tend to occur early in life and are transmitted via an autosomal dominant genetic pattern. Iron deficiency is one of the most common secondary causes and brain iron deficiency may be a primary factor for the occurrence of RLS. There are a few direct and many indirect signs pointing to altered DAT in RLS. Both DAT scan and postsynaptic alterations have been reported.

Dopamine-promoting agents represent the therapy of choice (via drugs like pramipexole, ropinirole, and rotigotine). Gabapentin, pregabalin, and opioids are still used especially in secondary RLS, such as is seen in diabetes or end-stage renal disease. The differential diagnosis of RLS

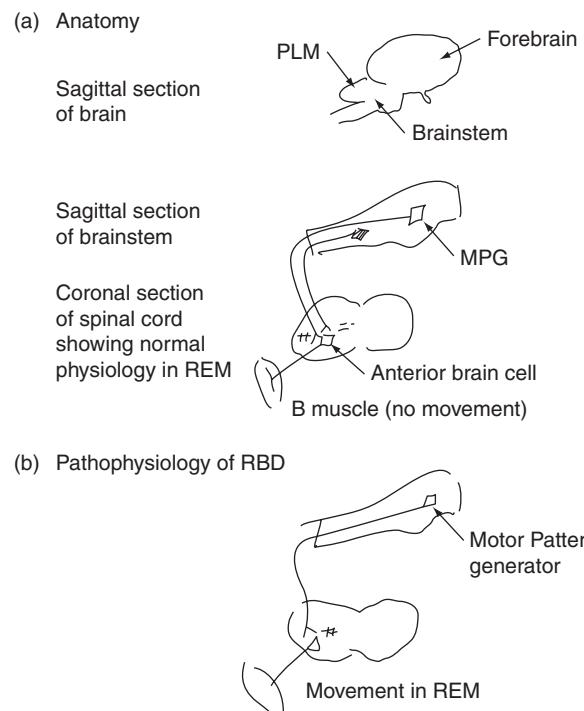


Figure 4 REM sleep behavior disorder (RBD). In the RBD, as in experimental REM sleep without atonia, there is a failure to inhibit final common-path motoneurons in the spinal cord and/or a facilitation of motor-pattern generators in the pontomedullary brainstem. In both cases, the result is a failure to effect the motor paralysis which normally prevents motor behavior in REM. In both cases, there is likely to be a loss of interaction between motor-pattern-generator neurons (MPG) and aminergic neurons (LC).

Conditions	EMG
Bruxism	(submental muscles)  Repetition but aperiodic
Rhythmic movement disorder	(neck muscles)  Repetition and periodic
Periodic leg movement	(anterior tibialis muscles)  Periodic with long silent epochs
Restless legs syndrome	(anterior tibialis muscles) Wake Sleep  Aperiodic Periodic

Figure 5 Sleep-related movement disorders. Note that recordings of EMG are from different muscles and that the patterns differ among conditions.

includes secondary forms. Leg cramps (akathesia) and positional leg discomfort are experienced. RLS is a risk factor for depression, hypertension, and cerebrovascular disease and is comorbid with several dopaminergic disorders including attention-deficit disorder, migraine headaches, sleep-related eating disorder, narcolepsy, and Parkinson's disease.

Diagnosis and Treatment of Sleep Disorders

Further details of pharmacological treatment of the conditions discussed here may be found in relevant medical texts. In this section, instead, we emphasize a natural history approach to the understanding and treatment of sleep disorders.

Home-Based Evaluation versus Sleep-Lab Diagnosis

Most sleep disorders including the most severe and life-threatening sleep apnea syndromes can be assessed by the astute clinician with the help of observant bed partners, family members, and self-observation training. Sleep logs and simple devices such as flashlights, sleep logs, and hand-held-type recorders are useful. Home-based electrographic studies can also be evaluated using portable polygraphic and movement-based measurement systems such as the Nightcap.

Sleep-Lab Referral

Severe cases, especially patients with narcolepsy, sleep apnea syndromes, RBD, and RLS are best evaluated and treated in sleep disorders centers at tertiary care facilities. Most sleep disorders will, nonetheless, be managed by family doctors in group or solo practice. Since much of the knowledge of sleep medicine is new and increasing every day, it will help all healthcare deliverers to understand sleep and manipulate its hygiene, whenever possible, by natural means.

Behavioral and Cognitive Therapy

Instead of reaching for a prescription pad, the informed and wise caretaker should consider behavioral and cognitive intervention at least for insomnia. Most clients who consider themselves to be sufferers from sleep disorders are the myriad walking-wounded of hospital outpatient departments everywhere. They are a mixed bag of anxiety disorders, character pathology, and poor health habits. Medication is not always, or even often, indicated for these unfortunate patients. Rather, a vigorous behaviorally and sophisticated educational program is in order. This is the natural task of the health educator, the clinical psychologist, and the nurse practitioner. It is for those people, not the sleep disorders specialist, that this article is written.

See also: Alcoholism; Animal Tests for Anxiety; Behavior Adaptation and Selection; Bioenergetics of Sleep; Brain

Evolution in Vertebrates; Brain Imaging; Cholinergic Systems in Aging and Alzheimer's Disease; Neurotrophic Molecular Analysis; Circadian and Ultradian Clocks/Rhythms; Comorbidity – Depression; Conscious and the Unconscious; Control of Food Intake; Cytokine Effects on Neuronal Processes and on Behavior; Depression; Evolutionary and Developmental Issues in Cognitive Neuroscience; Measuring Stress; Neuropsychology of Sleep; Protein Synthesis and Memory; Psychiatric and Substance Use Disorder Comorbidity; Sleep: Learning and Memory; Sleeping, Waking, and Dreaming; Short-Term Memory: Psychological and Neural Aspects; Thermo- and Mechanosensation via Transient Receptor Potential Ion Channels; Thermoregulation; Voluntary Movement: Control, Learning and Memory.

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Amnesia

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Glossary

Anterograde amnesia – The inability to recall or recognize events and facts that were encountered after the onset of amnesia (postmorbidity).

Confabulation – A false production of memory provided by a patient who is unaware of its falsehood. Confabulations range from plausible responses given in response to questions, apparently to fill a gap in memory, to fantastic imaginary events produced spontaneously and held with great conviction.

Consolidation – The processes by which memories are assimilated and stabilized. Consolidation includes both fast processes operating at a cellular level and slow processes operating at a systems level. At the cellular level, consolidation entails synaptic remodeling and growth that lead to the formation of a stable and long-lasting memory. At the systems level, consolidation involves reorganization, such that memories that were initially dependent for encoding and retrieval on the hippocampus and related medial temporal lobe (MTL) structures can be retained and retrieved neocortically, without reliance on MTL structures.

Declarative memory – A form of memory characterized by the ability to consciously bring to mind (recollect) information. Both episodic memory and semantic memory are forms of declarative memory. Declarative memory depends on the integrity of brain structures and connections in the MTLs and diencephalon – regions that are implicated in amnesia.

Episodic memory – Memory for personally experienced events. Episodic memory enables the mental reliving of an experience in the past. It is a form of declarative memory.

Nondeclarative memory – A variety of forms of memory in which learning is expressed through performance rather than recollection. These include simple classical conditioning, skill learning, and repetition priming. Nondeclarative memory is intact in amnesia.

Repetition priming – An unconscious expression of memory that takes the form of an improvement or bias in processing a stimulus that results from previous exposure to that stimulus. It is a form of nondeclarative memory.

Retrograde amnesia – Inability to recall or recognize experiences and information acquired before the onset of amnesia (premorbidity).

Semantic memory – Memory for facts and concepts. Semantic memory enables the retrieval of information in the form of impersonal knowledge. It is a form of declarative memory.

The Amnesic Syndrome

Global amnesia refers to a dense and a circumscribed disorder of memory in the context of preserved general intelligence and other cognitive abilities. Patients with amnesia have a severe impairment in anterograde memory – the ability to recall and recognize events and facts encountered after the onset of illness. The memory impairment is global, in that memory is affected regardless of the nature of the material (i.e., verbal or visuospatial) or the modality of presentation (i.e., visual or auditory). Typically, the ability to bring to mind experiences or information acquired before the onset of illness (retrograde memory) is also affected, although the extent is more variable. Some cases of isolated or focal retrograde amnesia have been described, but the existence of this syndrome is less well-established, as the absence of anterograde memory loss is not always clearly documented and in some instances, the selective nature of the retrograde amnesia may reflect faster recovery of initially impaired anterograde memory.

Much of our current clinical and theoretical understanding of global amnesia finds its origin in the study of patient HM, who in 1953, at the age of 27, underwent resection of the medial temporal lobes (MTLs) bilaterally for the relief of intractable seizures. As a result, HM was left with a profound inability to consciously learn and remember new information. In daily life, he was not able to recall the gist of a conversation he had with a visitor 30 min ago, nor did he remember whether someone actually visited. In the laboratory, he exhibited severe impairments in word and picture recall, in cued recall of paired associates, and in recognition memory. He also evidenced impaired recall of memories predating the surgery, and most of his personal memories were from the age of 16 or earlier.

Many other patients with amnesia have since been described, and although it is now clear that the amnesic syndrome is functionally heterogeneous, some general

characteristics can be identified. In particular, despite the pervasive nature of the memory impairment exhibited by amnesic patients, it is striking that some aspects of memory remain intact. Patients show normal performance on many tasks of immediate memory and working memory, such as the ability to repeat a string of digits or spell a word backwards. The ability to hold and manipulate information online is essential for the performance of many cognitive tasks, ranging from language comprehension to simple arithmetic. Although the integrity of working memory has long been taken as a given, there is mounting evidence that the ability to hold information in mind may at times be impaired, especially for nonverbal information. Whether this reflects a true impairment in working memory or the fact that maintenance of nonverbal information depends on long-term memory processes is still unclear. Patients with amnesia are also able to retrieve overlearned semantic memories, as evidenced by their knowledge of word meanings and their good fund of general knowledge. Finally, even within the domain of new learning, there is evidence for preservation of skill learning, classical conditioning, and repetition priming – forms of learning often collectively referred to as nondeclarative memory. The distinction between preserved and impaired forms of learning in amnesia has guided much research into the cognitive and neural organization of various components of memory.

Etiologies of Amnesia

Although amnesia can have a psychogenic origin, the focus here is on organic amnesia due to structural brain damage. Amnesia can result from lesions of the MTL (the hippocampal formation and adjacent entorhinal, perirhinal, and parahippocampal cortices), the diencephalon, and the basal forebrain, as well as from disruption of some of their connections such as the fornix. A range of vascular, infectious, and traumatic disease processes can cause such lesions. The most common ones are discussed in the following sections.

Herpes Simplex Encephalitis

Patients with herpes simplex encephalitis (HSE) may initially present with profound confusion and disorientation, amnesia, agnosia, and aphasia, but over time these symptoms may resolve so that the patient is left with a selective, dense amnesia. HSE typically affects MTL regions, including the hippocampus and adjacent entorhinal, perirhinal, and parahippocampal cortices. Damage may also extend anteriorly into the insular cortex and basal forebrain. Damage to the anterolateral and inferior temporal lobes may be responsible for persistent aphasia and agnosia in some patients. Lesions may be

asymmetrical: greater damage to right temporal regions has a more pronounced effect on nonverbal/visual memory, whereas greater damage to left temporal regions has a more pronounced effect on verbal memory. Extensive retrograde amnesia is typically seen in association with lesions extending into lateral temporal regions.

Anoxia

Reduced oxygen to the brain can result from cardiac arrest, respiratory distress, or carbon monoxide poisoning. The MTL region is particularly sensitive to oxygen deprivation, and as such, anoxia can result in selective memory impairment. The severity of memory impairment, both in the anterograde and retrograde domains, can vary from mild to very severe, depending on the extent of MTL damage. Anoxic brain damage is not always limited to the MTL region, but can involve the basal ganglia, thalamus, and diffuse cortical areas. In such cases, other cognitive and perceptual abilities may be affected. Anoxic episodes shortly after birth can lead to a relatively selective form of developmental amnesia.

Wernicke–Korsakoff Syndrome

Patients with Wernicke–Korsakoff syndrome (WKS) develop amnesia as a result of the combined effects of alcohol abuse and thiamine deficiency. In the acute phase, patients exhibit a confusional state, oculomotor problems, and ataxia. The chronic phase is characterized by dense amnesia, which is thought to be due to damage to the anterior and dorsomedial nuclei of the thalamus and the mammillothalamic tract. Additional frontal dysfunction may account for some of the unique characteristics of WKS amnesia, including superficial encoding strategies, confabulation, and source memory errors. Retrograde memory loss is common, but its interpretation is complicated by the fact that (1) patients' premorbid lifestyle may have interfered with the establishment of salient remote memories that serve as anchors for retrieval; and (2) frontal dysfunction may interfere with the strategic search of memories from the past.

Cerebrovascular Accidents

Bilateral posterior cerebral artery (PCA) infarction is a well-known cause of amnesia, and is due to lesions in the posterior parahippocampus or collateral isthmus (a pathway connecting the posterior parahippocampus to association cortex). Reading and visual naming problems may complicate the clinical presentation, and these are thought to be due to disconnection of posterior occipital regions from the left hemisphere language areas. Memory problems also can occur following unilateral (primarily

left) PCA lesions. The extent of retrograde amnesia is variable.

Thalamic strokes, due to infarction of the tuberothalamic and paramedian arteries, also can lead to significant memory loss. Severe amnesia is usually associated with lesions of the anterior thalamic nuclei or the mammillothalamic tract, whose fibers project to the anterior nucleus. Lesions of the dorsomedial thalamic nuclei typically lead to milder memory disturbances. As in WKS, executive dysfunction frequently accompanies the memory impairment. Unilateral lesions lead to material-specific deficits. There is variability in the persistence and extent of retrograde amnesia.

Anterior Communicating Artery Aneurysms

Memory impairments following anterior communicating artery (ACoA) aneurysm result from damage to the basal forebrain, the striatum, and frontal lobes. Several basal forebrain nuclei are rich in cholinergic neurons that innervate the hippocampus. Thus, amnesia may be due in part to disruption of hippocampal functioning, but additional frontal damage will also impact the nature of the impairments. In the acute phase of the disorder, patients often present with severe confusion; once this clears, significant impairment in new learning becomes apparent. Because of the variability in the locus and extent of lesion, there is considerable heterogeneity in the nature and severity of impairment, but a common feature is much worse performance on tasks of recall than of recognition. This may reflect impairments in the strategic search of memory secondary to frontal lesions. Confabulation, lack of insight, and severe retrograde amnesia also tend to be associated with frontal lesions.

While the etiologies reviewed above typically lead to permanent amnesia, other conditions can cause transient selective memory impairment. For instance, severe memory problems are often associated with electroconvulsive therapy, a procedure used for the treatment of severe depression, but good recovery of memory occurs with time. Another condition, termed transient global amnesia, refers to a circumscribed memory deficit of limited duration (<24 h) that typically resolves in the weeks or months after the episode. Common causes include seizure activity, migraine, or temporary disruption of the vascular supply.

Features of Anterograde Amnesia

Amnesic patients are severely impaired at intentionally remembering information to which they were exposed after the onset of their illness. This deficit encompasses both personally experienced events (episodic memory) and impersonal facts and concepts (semantic memory).

These forms of memory are referred to collectively as declarative memory.

The ability to bring to mind previous experiences or facts depends on a series of processes, including the initial processing and representation of information, its storage in long-term memory, and its subsequent retrieval. Amnesic patients are able to process and register information, as evidenced by their normal intelligence and their ability to repeat information immediately after presentation, and thus, their memory impairments are unlikely to reflect deficient intake of information. There is also little evidence for a generalized retrieval deficit, as patients typically are able to retrieve highly overlearned information that was acquired many years prior to the onset of amnesia. Further, a retrieval deficit would predict that in patients with transient amnesia, resolution of the amnestic episode would lead to recovery of the memories that were lost, but such is not the case. Rather, there is a permanent gap in memory. Consequently, it is generally assumed that the amnestic deficit reflects a problem with the consolidation of information into memory. The MTL, through interactions with neocortical regions, plays a central role in consolidation. It is not the site of permanent storage, but is thought to bind together into a coherent representation the different aspects of an event that are neocortically represented.

Depending on the nature and extent of the MTL lesion, different aspects of declarative memory may be differentially impaired. A critical consideration is whether the MTL lesion is limited to the hippocampal formation, or extends into surrounding cortical regions. Some patients with lesions limited to the hippocampus have been described as those who have impaired performance on recall tasks, but intact performance on item-recognition tasks. Such a dissociation has been interpreted with reference to two qualitatively distinct processes (i.e., recollection and familiarity) that can support declarative memory. Recollection refers to an intentional, effortful process by which aspects of an episode are recovered. Familiarity, in contrast, refers to a subjective feeling that arises when information is processed fluently and comes to mind easily. While performance on recall tasks depends on recollection of contextually appropriate information, performance on item-recognition tasks can be supported by either recollection or familiarity. Thus, the pattern of performance of these patients with limited hippocampal lesions has been interpreted as reflecting impaired recollection, but preserved familiarity. Consistent with this notion, when given recognition memory tests that assess memory for contextual information (where or when) or for the relationship among items, a form of recognition that cannot be supported by familiarity of individual cues, these patients again perform poorly. This pattern of impaired recollection and preserved familiarity in patients with limited hippocampal

lesions accords well with findings from neurophysiological and neuroimaging studies that suggest that the hippocampus is critical for recollection, whereas familiarity is supported by perirhinal cortex. It should be noted, however, that this pattern has not been obtained consistently, and some patients with limited hippocampal damage have been reported to have comparable deficits in recollection and familiarity. However, in the absence of histological evidence, it is always possible that the familiarity deficit is due to undetected damage outside the hippocampus proper.

Patients with more extensive MTL lesions show more severe impairments in declarative memory that are evident in recall and in recognition tasks. Their performance reflects impairments in both recollection and familiarity, although occasional reports that these patients can perform simple recognition tasks relatively well suggest that the impairment in recollection may be more severe than the impairment in familiarity. From a clinical perspective, any effort to provide new information to the patients with extensive MTL lesions should capitalize on their remaining ability to use familiarity to support performance.

Differences in performance depending on the extent of MTL lesion are also evident in the domain of new semantic learning. Patients with extensive MTL lesions show minimal ability to acquire new facts or concepts. For instance, HM was unable to acquire the meaning of any new words that entered the vocabulary since the onset of his amnesia. In contrast, patients with limited hippocampal lesions are able to acquire some degree of new semantic knowledge including vocabulary words, and may perform quite well when tested with recognition tasks. Even more extensive semantic learning has been documented in patients with developmental amnesia who, secondary to an anoxic event in early childhood, have damage limited to the hippocampus. Despite severe episodic memory problems, these patients have been shown to acquire language normally and to perform well on scholastic tests, demonstrating a level of learning well beyond that shown by adult amnesic patients. This may reflect the greater opportunity for functional reorganization or compensation associated with early hippocampal damage.

While the above evidence points to differences in the nature of the anterograde memory impairment depending on the extent of MTL lesion, even more striking qualitative differences are seen when nonobligatory frontal deficits are superimposed on the core amnesia. In amnesic patients with frontal lesions, impairment in executive processes that contribute to memory may lead to additional encoding deficits, reflecting an inability to mentally manipulate and organize incoming information. Similarly, additional retrieval deficits are often seen, reflecting impaired initiation and evaluation of memory search. These deficits can lead to impairments in source memory

or temporal memory, and are responsible for unusually high levels of intrusions in recall or false alarms in recognition, a phenomenon known as enhanced susceptibility to false memory.

As noted above, anterograde amnesia involves declarative memory, but leaves unaffected several forms of nondeclarative memory. For instance, amnesic patients show intact procedural memory: they are able to acquire new skills and habits (e.g., operating a cell phone or learning to type), even though their declarative memory (i.e., their knowledge of having been exposed to the information) is severely impaired. Similarly, they show evidence of normal eyeblink conditioning, provided that the conditioned stimulus and unconditioned stimulus are temporally overlapping. These two forms of nondeclarative memory are thought to depend on the basal ganglia and the cerebellum, respectively. A third form of nondeclarative memory is repetition priming. In contrast to procedural memory and conditioning, which are acquired gradually across many presentations, repetition priming requires only a single study exposure. In a typical repetition-priming task, patients are exposed to a series of words or pictures (e.g., the word tulip). During a later test phase, they are asked to perform a seemingly unrelated task, such as reading briefly flashed words or generating as many words as possible when cued with the semantic category flowers. Priming is measured as the enhancement in task performance for stimuli presented in the study phase, compared to stimuli that were not presented. Repetition priming can be due to facilitation of perceptual processes that are reinstated at test (i.e., enhanced accuracy in identifying the word tulip), or to reinstatement of conceptual processes (i.e., enhanced generation of tulip as an exemplar of flowers). In either case, patients with global amnesia show intact priming in such tasks. Such priming does not depend on MTL or diencephalic regions, but is rather mediated neocortically, by those very regions involved in visual or semantic processing.

Features of Retrograde Amnesia

Retrograde amnesia can involve impaired memory for events (episodic memory) as well as for facts and concepts (semantic memory) to which patients were exposed prior to the onset of their illness. A further distinction concerns memory for personally relevant information compared to general, public information. Personal memory for specific events, often referred to as autobiographical memory, entails re-experiencing of personal episodic detail, and thus forms the prototype of episodic memory. It is often tested by asking patients to describe in as much detail as possible an event in response to a cue word provided by the examiner. On the other end of the spectrum, memory

for general information such as famous faces or of vocabulary provides the prototype of semantic memory.

Although many patients show impairment in both forms of memory, retrograde deficits in episodic and semantic memory can be dissociated, as some patients have severe autobiographical memory impairments but relatively preserved memory for facts and concepts, whereas other patients show the opposite pattern. Dissociations between autobiographical and semantic retrograde memory are clear cut when memories reflect exclusively autobiographical or semantic memory, but practically such dissociations are harder to observe because memories often reflect a combination of autobiographical and semantic information: memory for a public event may entail both the fact of the event and information regarding one's personal circumstances at the time of the event. In a similar vein, some personally relevant memories, such as names of schoolteachers or addresses of previous places of residence, are not tied to singular events and likely reflect a combination of autobiographical and semantic information. Such memories are referred to as personal semantics.

A classic finding in retrograde amnesia is the fact that remote memories are better preserved than memories for events or facts to which the patient was exposed shortly before the time of brain injury. The presence of such a temporal gradient is very common, but the extent of retrograde amnesia can vary greatly, from a relatively restricted impairment encompassing only a few years predating the illness (with a steep gradient) to a very extensive impairment encompassing 30 years or more (with a relative flat gradient). The nature of the gradient is multidetermined, with the primary determinants being the location and extent of brain damage and the type of memory test administered.

Many studies of patients with MTL lesions have documented a limited temporal gradient, with the gradient becoming progressively more extensive with larger MTL lesions. This pattern of memory loss has been interpreted in the context of standard consolidation theory, according to which the hippocampus and surrounding MTL areas play a time-limited role in memory: the hippocampus initially serves as an index to the various neocortical sites in which information is processed, but over time, direct interconnections among neocortical sites are forged and the hippocampus is no longer needed for memory retrieval. Thus, older memories can be retrieved directly through reactivation of neocortical traces even in the face of hippocampal lesion, whereas newer ones that are not yet consolidated cannot.

While a limited temporal gradient adequately characterizes the semantic memory loss in MTL patients, it is now apparent that earlier studies may have underestimated the extent of impairment in autobiographical memory because they did not sufficiently probe for

event details. Recent studies evaluating both the number and quality of event details have revealed that patients with MTL lesions have extensive autobiographical memory loss that may cover 30 years or more. Such an extensive temporal gradient is problematic for a consolidation view, as it suggests that the loss of autobiographical memory extends well beyond a time frame during which biological consolidation is likely to operate. In light of these findings, an alternative view, referred to as multiple trace theory, has been proposed, which postulates that retrieval of personal event-specific details (which are critical for autobiographical memory) continues to depend on the hippocampus, irrespective of the age of the memory. According to this view, every time a memory is retrieved, it is re-encoded and a new hippocampal trace is formed. The relative preservation of older memories compared to more recent ones reflects the fact that older memories, which presumably have been re-activated more frequently, are represented by more hippocampal traces, and are therefore more resistant to partial MTL damage. Complete MTL damage, however, can lead to a complete inability to recollect autobiographical details of one's past. Unlike autobiographical memories, semantic memories lose their dependence on specific details as they are gradually formed through extraction of the commonalities among episodes. Thus, although they may initially be supported by MTL-mediated recovery of specific details, they lose their dependence on MTL structures over time so that they can be retrieved directly through neocortical connections and are therefore resistant to MTL lesions. A similar distinction between retrieval of semantic/generic representations and specific detail also applies to the domain of spatial memory: when evaluating memory for an environment known long ago, intact memory for general spatial layouts, but impaired access to highly detailed spatial representations, has been found in some amnesic patients.

In patients whose lesion extends beyond the MTL to involve the anterolateral temporal lobes, very extensive retrograde amnesia with a flat gradient is typically seen, and this may encompass not only autobiographical, but also semantic memory. This is thought to be due to damage to the temporal neocortex, which directly disrupts neocortical storage sites or disconnects these sites from the hippocampi. Impairments in semantic memory appear to be more prominent following left temporal lesions, a finding that is likely tied to the role of the left hemisphere in verbal lexical–semantic processing. Impairments in autobiographical memory appear to be more prominent following right temporal lesions, and may reflect the fact that autobiographical memory relies heavily on the ability to conjure up visual images.

Finally, extensive retrograde impairments can also be seen in patients with frontal lesions. These impairments may encompass both semantic and episodic memory, but

they are particularly evident when the demands on self-initiated retrieval are high, as is the case for autobiographical memory. In these patients, retrograde amnesia is closely linked to frontal executive impairments. Confabulatory responses may also be prominent, and these are thought to reflect impaired monitoring of retrieved information.

Clinical and Theoretical Implications

Amnesic syndromes are functionally heterogeneous, but they share the fundamental characteristic of a relatively isolated impairment in memory, with both anterograde and retrograde components. Clinically, the impact of such memory impairment on functional activities that require retrieval of recent information or maintenance of information in the face of distraction is self-evident, as is the wider psychosocial impact of the inability to reminisce about the past. Equally important and perhaps less well-recognized, however, is the impact of severe memory impairment on the ability to plan and envision future scenarios. Recent studies suggest that there is extensive overlap in the brain regions involved in remembering the past and those involved in thinking about the future, and evidence is now emerging that amnesic patients have great difficulty imagining new experiences. It is possible that richly envisioning future scenarios requires flexible retrieval and recombination of details from the past, drawing on the same processes of (re-)construction as does remote memory. The impact of this impairment on a range of abilities from problem solving to creativity remains to be explored. In a similar vein, much remains to be learned about the effect of amnesia on the ability to maintain an updated sense of self and to develop goals that are appropriate to one's personal history.

On a theoretical level, neuropsychological studies of patients with amnesia have contributed greatly to our understanding of memory processes and the brain regions that mediate them. Within the domain of new learning, striking dissociations in amnesia between declarative memory and different forms of nondeclarative memory have reinforced the notion that memory is not a unitary phenomenon, but rather, that there are multiple memory systems and processes that are mediated by distinct neural systems. It has long been held that the MTL-diencephalic memory system that is lesioned in patients with amnesia is involved only in long-term memory, but recent findings raise the possibility of a similar role in the maintenance of some forms of short-term memory. Within the domain of retrograde amnesia, much continues to be learned from patients with selective impairments in autobiographical or semantic memory, and the existence of distinct gradients for these two forms of remote memory suggests that

the storage and retrieval of autobiographical memories and of semantic information are functionally and neuroanatomically distinct. These theoretical advances in turn make possible the development of more sophisticated methods of memory assessment of patients with amnesia, and may lead to the development of memory rehabilitation approaches that capitalize on preserved memory processes.

See also: Animal Models of Learning and Memory; Brain Mapping of Language and Memory in Epilepsy; Declarative Memory; Episodic and Autobiographical Memory: Psychological and Neural Aspects; Implicit Learning and Memory: Psychological and Neural Aspects; Korsakoff's Syndrome; Neural Basis of Recognition Memory in Nonhuman Primates; Transient Global Amnesia: Neuropsychology, Psychopathology, and Neuroimaging.

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Agnosia

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Glossary

Apperceptive agnosia – A form of visual agnosia in which a person cannot reliably name, match, or discriminate visually presented objects, despite adequate elementary visual function (visual fields, acuity, and color vision).

Associative agnosia – A form of visual agnosia in which a person cannot use the derived perceptual representation to access stored knowledge of the object's functions and associations but is able to copy and match the drawing even though unable to identify it.

Balint's syndrome – Agnosic syndrome that results from large bilateral parietal lesions and is composed of three deficits: (1) paralysis of eye fixation with inability to look voluntarily into the peripheral visual field, (2) optic ataxia, and (3) disturbance of visual attention such that there is neglect of the peripheral field.

Dorsal simultanagnosia – An inability to detect more than one object at a time, with difficulty shifting attention from one object to another.

Dorsal stream – The stream of cortical visual projections from primary visual cortex to posterior parietal cortex, concerned primarily with the visual control of action.

Inferotemporal cortex – Inferior surface of the temporal lobe that is particularly important for object recognition.

Integrative agnosia – A form of visual agnosia in which one retains the ability to recognize elements of objects but is unable to integrate these elements together into comprehensible percept.

Optic aphasia – A condition in which a person cannot name a visually presented object, despite being able to indicate the identity of the object through gesture and sort the visual stimuli into categories.

Prosopagnosia – A form of visual agnosia in which a person cannot recognize familiar faces, despite adequate elementary visual function (visual fields, acuity, and color vision).

Ventral simultanagnosia – A reduction in the ability to rapidly recognize multiple visual stimuli, such that recognition proceeds in a part-by-part fashion.

Ventral stream – The stream of cortical visual projections from primary visual cortex to the inferotemporal cortex, concerned primarily with representing the identity of stimuli by characteristics such as shape and color.

Introduction

Visual agnosia can be broadly conceptualized as an impairment in the higher visual processes necessary for object recognition, with relative preservation of elementary visual functions. This impairment occurs in the absence of dementia or a general loss of knowledge about objects as patients are typically able to recognize objects through other modalities such as through touch, audition, or verbal descriptions. Historically, Lissauer in 1890 suggested that there were two general categories of object recognition deficits following brain damage, apperceptive and associative agnosia. Patients with apperceptive agnosia were described as having early perceptual processing deficits that caused an inability to form a complete conscious percept of the stimulus. Associative agnosics, on the other hand, were characterized by an inability to recognize objects, despite having intact early level representations. Typically, these impairments were assessed by having the patients copy simple pictures of objects. Individuals with apperceptive agnosia were unable to copy or identify the picture while individuals with associative agnosia could produce a copy of the presented object but were unable to recognize or name it (Figure 1).

Although Lissauer's classifications of agnosia remain in current neuropsychological theories of object recognition today, we are now aware that the simple dichotomy of associative versus apperceptive agnosia cannot account for all forms of object recognition deficits reported in the literature. The neural mechanisms supporting different aspects of perception have been well explored, and depending on the specific area of damage, diverse agnosic characteristics have been shown. Although this article limits the topics to object recognition agnosias, tactile, auditory, face (prosopagnosia), and color agnosia have been reported in the literature.

Neuroanatomy

Diverse methodology, including animal, lesion, functional magnetic resonance imaging (fMRI), and behavioral studies, have been employed to identify the underlying visual mechanisms involved in object perception and recognition. Indeed, two distinct, but interconnected, cortical visual pathways have been shown to process the visual information needed for visual perception and visually guided action. The dorsal stream, projecting

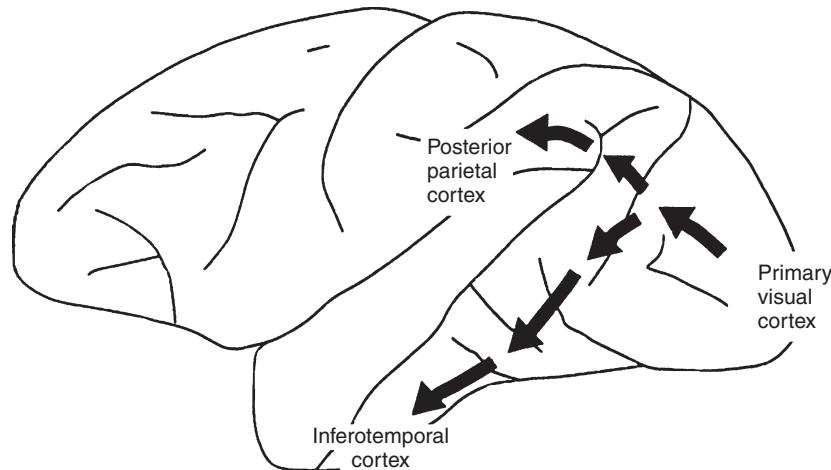


Figure 1 Two streams of visual processing in the primate cerebral cortex. The brain illustrated is that of a macaque monkey. Reproduced from Milner DA and Goodale MA (2006) *The Visual Brain in Action*, 2nd edn. Figure 1.9 (pg. 23), with permission from Oxford University Press.

from the primary visual cortex (V1) to the posterior parietal lobe, deals with moment-to-moment information about the location of objects and is primarily involved in the visual control of skilled movements directed at those objects. The ventral stream, on the other hand, projects from V1 to the temporal lobe, provides us with our visual perceptions of objects and events in the world and codes this information for storage and for use in cognitive processes such as imagining, planning, and recognition (**Figure 2**).

Ventral stream function is associated with object recognition and form representation. Investigations with nonhuman primates have shown that this system has strong connections to the medial temporal lobe (which is associated with long-term memories) and the limbic system (which is involved in emotional processes). Thus, the role of the ventral stream seems to be to form our

perceptual and cognitive representations of the world, providing both the characteristics of objects and their significance to us.

The primary pathway of ventral information from V1 is through visual association areas V2 (involved in the processing of simple properties such as orientation, color, and spatial frequency), V4 (tuned for properties such as orientation, spatial frequency, color, and simpler geometric shapes), and finally to the inferior temporal lobe. fMRI studies in normal individuals have shown activations in the ventral stream during identification of form, texture, and color, as well as during recognition of objects and faces.

As information moves from the visual association areas to the temporal lobe, the cells show remarkable specificity in their responses to visual stimuli. For example, the lateral occipital complex (LOC), which is a region located

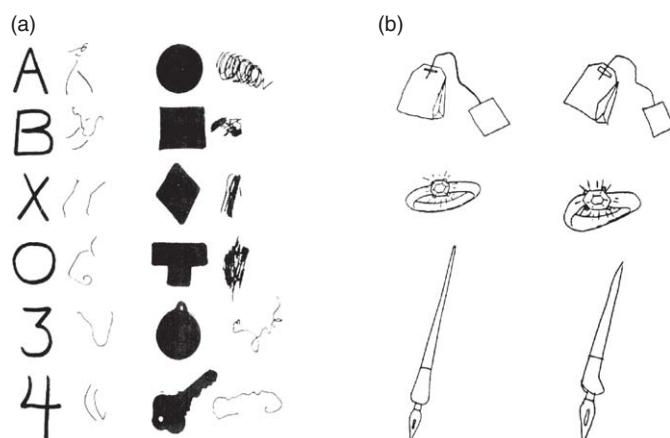


Figure 2 The copying abilities of individuals with (a) apperceptive agnosia and (b) associative agnosia. Reproduced from Farah, MJ. Visual Agnosia, 2nd edn., Figures 2.1 (p. 14) and 6.2 (p. 73), ©2004 Massachusetts Institute of Technology, with permission from the MIT Press.

bilaterally on the occipitotemporal cortex, is more activated when viewing objects when compared to viewing textures or scrambled objects. Within the temporal lobe, many of the cells have large receptive fields, which allow for generalization across the visual field and the coding of intrinsic object features, independent of object location. Lesion studies in monkeys provide further evidence for the role of the ventral stream in object perception. For example, when monkeys have lesions to the temporal cortex, performance on shape discrimination tasks is compromised.

This article examines some of the more profound and, in our opinion, more interesting forms of agnosia that have been reported. While our discussion is primarily limited to agnosias within the visual domain, it is important to note that cases have been reported in other sensory modalities as well (such as auditory and tactile). A broad approach has been undertaken, highlighting key findings in all disciplines of behavioral neuroscience, including case studies, animal models, lesion studies, and systems theory. It is expected that by offering such a broad treatment, the reader will develop an accurate depiction of the current state of knowledge about agnosia, and what it has taught us about the human visual system.

Apperceptive Agnosia

Individuals with apperceptive agnosia are characterized by a difficulty forming a complete visual percept. In extreme cases, termed visual form agnosia, even simple shape discriminations cannot be made as these patients lack the ability to group local visual elements into contours, surfaces, and objects. This type of agnosia is associated with diffuse bilateral damage to the lateral occipital lobes, critical structures involved in visual perception. Individuals with such diffuse damage typically have preserved visual acuity, brightness discrimination, and color vision, and are able to maintain fixations on objects. However, despite the preservation of these elementary visual functions, their ability to point to objects in the environment and match, copy, or discriminate simple and complex geometric shapes is often compromised. Impairments in object identification do not, however, reflect a general impairment in object recognition as patients are still able to accurately identify objects upon tactile presentation and verbal descriptions. In addition, object identification improves when these patients are looking at real objects where cues such as color and texture can aid in recognition.

Despite general impairments in object perception, patients with apperceptive agnosia have been reported to recognize actions depicted in line drawings, such as someone pouring water into a glass, objects whose functions are pantomimed by an experimenter and objects either in motion or drawn in front of them. The

dissociation between impaired perceptual representations of static objects and a preserved ability to perceive motion in these individuals is likely the result of intact parietal and parietofrontal visual networks. As previously described, visual processing in the dorsal stream deals with information about the location of objects and is primarily involved in the visual control of skilled movements and motion perception. In addition, this network forms connections with frontal areas critical for understanding actions performed by others. Despite obvious perceptual deficits, individuals with apperceptive agnosia retain the ability to accurately interact with objects around them and maneuver through their environment. For example, DF, one of the most extensively tested patients with visual form agnosia, clearly demonstrates intact visuomotor control for manual actions on the basis of object size, orientation, and shape, despite her inability to consciously perceive these same details. Indeed, DF can use these intrinsic object properties to accurately scale her grasp when picking up objects with accuracy equal to that of normal controls. Since DF's ventral stream is severely compromised, it is likely that her preserved visuomotor abilities are mediated by intact mechanisms in the dorsal stream, thus allowing accurate visuomotor abilities in the absence of conscious perceptual detail.

In addition to apperceptive patients with severe perceptual deficits, such as those with visual form agnosia, there are individuals that present with higher-order apperceptive impairments known as perceptual categorization deficits. While these patients can typically recognize most objects, their perceptual deficits become apparent when trying to recognize or match objects seen from unusual orientations or under poor lighting conditions. In the past, this impairment has been attributed to the breakdown of the mechanisms involved in object shape constancy. Such mechanisms allow us to recognize objects presented at any orientation or in degraded lighting conditions. However, others have disputed this interpretation. Since patients with this deficit do not have problems with everyday object recognition, and only object recognition at some, rather than all, perspectives is impaired, the deficit may reflect a general loss of visual problem-solving abilities rather than impairment of shape constancy mechanisms.

Simultanagnosia

Simultanagnosia is characterized by an inability to appreciate the overall meaning of a complex picture or stimulus, with preserved perception of isolated elements or details within the stimulus. Patients with simultanagnosia present some visual deficits similar to those seen in apperceptive agnosia, such as an inability to identify an array of stimuli or a tendency to look at parts of an object to guess the object's identity on the basis of local features.

However, unlike apperceptive agnosics, patients with simultanagnosia can use shape information to aid in identification. Two broad classes of simultanagnosia (dorsal and ventral) have been established by Farah in 1990 based on both the presentation of the disorder as well as the corresponding lesion sites to the dorsal and ventral cortical visual pathways.

Despite being able to recognize most objects, patients with dorsal simultanagnosia cannot detect more than one object at a time and have difficulty shifting attention from object to object. For example, when presented with a series of overlapping line drawings, such patients may only report seeing one of the objects. These patients are often confused with blind individuals as they frequently walk into objects and grope for things as if they were in the dark. Attentional impairments, resulting from impaired spatial attention systems, have been shown to be the root underlying cause of dorsal simultanagnosia. Such impairments result in an inability to disengage attention from a specific object or a region of space; thus, these patients can only attend to one stimulus at a time. However, if individual objects are placed close enough together, dorsal simultanagnosics can combine the individual elements to recognize the larger 'global' structure and, in rare cases, perceive multiple objects. This perceptual impairment is typically resultant from bilateral lesions to the posterior parietal cortex and occipital regions and frequently occurs in the context of Balent's syndrome where impaired visuomotor control (optic ataxia), eye movements, and spatial coding deficits are observed.

Ventral simultanagnosia, on the other hand, is characterized by the reduced ability to recognize multiple visual stimuli rapidly. Although these patients are able to see multiple objects at once, like individuals with dorsal simultanagnosia they are only able to recognize one object at a time and often do this on a part-by-part basis. That is, while patients with dorsal simultanagnosia have mainly deficits in perceiving more than one stimulus, patients with ventral simultanagnosia have deficits in recognizing more than one stimulus, even though other objects are seen. Indeed, patients with this visual disorder are most noticeably impaired while reading as they must identify each word letter by letter. The underlying cortical damage for this disorder has typically been associated with left posterior temporal or temporo-occipital cortical lesions.

Associative Agnosia

In comparison to apperceptive agnosia, an associative agnosic can adequately perform figure-copying tasks, even though they may be unable to identify the very picture they just copied. Further, associative agnosics usually perform significantly above chance at matching

tasks. A third similarity, both apperceptive agnosics and associative agnosics are affected by the quality of the stimulus they are trying to identify, with performance on three-dimensional objects superior to two-dimensional photographs, and line drawings being the poorest performance. Unlike apperceptive agnosics, a person with associative agnosia can use shape cues to try to identify the presented object (as opposed to color and texture). Therefore, an associative agnosic is likely to mistake an object for one that is similar in shape. Patient FZ is known to have misidentified a drawing of a baseball bat as a paddle, knife, and thermometer (all objects that have a shape similar to a baseball bat). Since form information seems to be, at least partially, intact, there must be a failure of the structured perception to appropriately activate the network of knowledge about the functional, contextual, and categorical properties of objects that aid in their correct identification.

Of course, one of the highlighted symptoms of associative agnosia is that perception remains intact, that is, it becomes inaccessible. However, recent research has demonstrated that, at least some, associative agnosics do indeed have at least some form of visual impairment. For instance, patient CK's figure copying was quite adept in terms of final product; however, the process employed to reach that point was substantially different from what would normally be observed. CK was slow, and often lost his place if he had to take his pen off the paper. Levine and Calvanio reported further perceptual difficulties observed in patient LH, who was significantly slower in matching tasks and visual search tasks. While in comparison to other agnosics it may seem that those suffering from associative agnosia are displaying 'perception without meaning,' as coined by Teuber few would wholly subscribe to the notion that these patients have fully intact perceptual abilities.

An interesting subset of associative agnosia may manifest in some which recognition is not equally impaired for all classes or categories of object. Category-specific visual agnosia (CSVA) usually distinguishes between biological and nonbiological objects, with patients showing preserved recognition for all categories and a deficit for the biological objects. For example, someone with CSVA may have correct recognition of tools, but demonstrate marked difficulties in recognizing fruit and vegetables. Similar to associative agnosia, it is thought that the deficit is rooted in a dissociation between the mechanisms underlying visual perception and those that provide access to the semantic information associated with objects. However, the exact mechanisms are at present not fully understood. Some have theorized that the split in performance between biological and nonbiological items may be a result of biological items depending on specialized neural mechanisms that are unused (or underused) when recognizing nonliving. Neurological evidence has for the most

part substantiated this line of theory, with deficits in identifying biological objects following inferior-temporal lobe damage.

Integrative Agnosia

While Lissauer's pioneering work dichotomizing forms of recognition disorder (apperceptive vs. associative) is still often cited, we are now fully aware that object recognition is a far more complicated process than Lissauer could have intuited. Rather than simply matching stimuli coded in terms of primitives (like line orientation) to previously acquired knowledge, the visual system must code the spatial relations between lines and features, the object must be parsed from the background, and individual parts of the object need to be related to one another. Problems with this intermediate level of vision can result in poor overall perceptual integration of form information. Perhaps the best-studied patient was described in detail by Riddoch and Humphreys, H.J.A., who had bilateral occipitotemporal damage following an infarct of the posterior cerebral artery. Following the lesion, H.J.A. demonstrated profound impairments in a number of higher-level visual tasks, including face recognition, word recognition, and visual navigation. When attempting to identify objects, H.J.A.'s descriptions generally consisted of fragmented reports of various form-based features, with correct identification of an object seemingly hinged on either an elongated process of deduction or the presence of a highly diagnostic feature. For example, when asked to identify a line drawing of a pig, H.J.A. began identifying features such as having four short legs and a powerful body. Once he realized the mystery animal possessed a small curly tail, he was quick to correctly identify the stimulus. Typically, integrative agnosics over-segment stimuli, with parts of the same object being classified as separate. For example, while describing a paintbrush, H.J.A. suspected there may be two objects close together, rather than the single paintbrush presented. These patients typically perform best when

identifying real-world objects than still photographs, and have the poorest performance when required to identify line drawings. Such findings suggest that the addition of surface information and depth information appears to increase performance via benefiting the processes involved in integrating elements of the stimuli into a perceptual whole. Interestingly, when H.J.A. incorrectly named an object, his errors were visually related to the target objects and never seemed to be a result of semantic association, suggesting a deficit before access of item-specific stored knowledge has been retrieved. This notion is furthered by findings that integrative agnosics are unable to perform matching tasks that require semantic information or, to gesture, the correct use of misidentified objects. In sum, the deficit truly is one of object identification, rather than object naming. Although the disruption in processing is undoubtedly before higher visual processes, such as the attachment of meaning, most integrative agnosics perform quite well on standardized test of early perceptual processing. For example, H.J.A was able to accurately reproduce etchings presented to him, as can be seen in **Figure 3** (although his order of drawing lines was abnormal).

In addition, H.J.A. performed well on a task requiring the perceptual discrimination of objects that are matched for overall area and brightness, which patients suffering from impairments in the encoding of basic visual properties could not. In order to account for all features of performance found in integrative agnosics, basic, local visual elements are thought to be processed in a normal way, but the integration of these elements into unitary wholes is impaired. In more concrete terms, integrative agnosia appears to be a deficit located between the stage of visual processing concerned with shape processing and the visual access to memory representations. Since the human visual system is limited recognizing one object at a time, any deficiencies in the ability to properly segment/group the visual field will lead to the deficits observed in integrative agnosia by way of perception breaking down into a parts-based analysis of objects.



Figure 3 Copy of an etching of St. Paul's cathedral by patient H.J.A. Reproduced from Humphreys GW (2001) *Case Studies in the Neuropsychology of Vision*, Figure 3.1 (pg. 45), with permission from Psychology Press.

Optic Aphasia

Optic aphasia is typically described as having an inability to name a visually presented object, despite being able to identify the object through alternate means such as gesturing the appropriate use. With associative agnosias and optic aphasias often displaying similarities in their deficits, some have argued a classification of a single syndrome, perhaps allowing for varying degrees of severity. Both of these patient populations have profound difficulty in naming visually presented objects, with intact recognition persevering within the other senses, such as touch or sound. Additionally, both 'types' of patients have relatively intact low-level visual processing and an ability to copy presented line drawings. When examining optic aphasics, however, a number of important distinctions are readily apparent. First, and perhaps most importantly, optic aphasics are able to nonverbally identify an object through gesturing the intended use, a skill associative agnosics lack. Patient Jules, who was extensively studied after suffering a posterior left hemisphere stroke, would often pantomime the use of an object correctly, even if his verbal identification was incorrect. For instance, when shown a picture of a boot Jules correctly mimed putting the boot on his foot, but incorrectly identified the object as a hat. The nature of the disorder is further elucidated when one examines the types of errors seen in optic aphasia in comparison to associative agnosia. An associative agnosic commonly makes errors related to visual dimensions of the target object, whereas an optic aphasic is likely to make errors with a semantic underpinning. As is evident from the example above, Jules chose a response from a similar semantic category (clothing, for example), even though a hat and a boot share few visual features in common. Another common finding associated with optic aphasia, although much less frequently observed in associative agnosias, is an insensitivity to the visual quality or nature of the stimulus. That is, optic aphasics perform equally well (or equally poorly) on identifying photographs, three-dimensional pictures, and line drawings of objects. Finally, optic aphasics are far more likely to perseverate once an answer is produced, repeating error responses from previous trials. It is important to close with a caveat regarding the dichotomy created between optic aphasia and associative agnosia: there have been a number of reported cases where both conditions seem to be present in a single patient; indeed, even Lissauer's seminal patient had features of both apperceptive and associative agnosias preventing his categorization.

Summary

This article has served as a brief introduction to the collection of disorders termed visual agnosia. Despite relatively normal visual perception, attentional mechanisms,

intelligence, and language, these patients have a marked deficit in the recognition of previously known visual stimuli. The condition is modality specific, with the other senses (touch, hearing, etc.) providing a route to relatively unimpaired recognition performance. Despite the pedagogical role these interesting cases can fulfill, there is another, arguably more useful role for the study of visual agnosia within neuropsychology. As should be apparent from the provided discussion of neuroanatomy, the visual system as a whole is far more complicated than one would intuit. Indeed, one of the primary impediments to understanding how object recognition is achieved is the contradiction between how easily we accomplish it and the underlying complexity. By studying individuals who demonstrate impairment, we have been able to not only identify major distinctions between stages of object recognition, but also verify the modular organization of the visual system. The dissociations seen within the various subtypes of agnosia discussed here are compelling cases of how distinctions between 'early,' 'intermediate,' and 'late' stages of object recognition can have explanatory power in accounting for the many, seemingly distinct, forms of agnosia one may encounter. Of course, this relationship is two directional, with the study of patients with visual agnosia guiding our investigation of the visual system as a whole.

See also: Brain Imaging; Disorders of Face Processing; From Sensation to Perception; Temporal Lobe and Object Recognition; Vision.

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Current Models and Assessment of Limb Apraxia

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Glossary

Action semantic system – Component of the gesture-processing architecture that contains the conceptual attributes of a gesture, including conceptual attributes related to the knowledge of functions and actions in relation with tools and objects, to the knowledge of actions independently of tools and objects (e.g., arbitrary communicative gestures) but in which objects and tools can be incorporated, and to knowledge related to the gathering of simple actions in a sequence.

Conceptual apraxia – Formerly known as ideational apraxia, it is now proposed to use the term ‘conceptual apraxia’ for alterations that directly affect semantic knowledge about functions and actions related to tools and objects, and about arbitrary actions performed independently.

Ideational apraxia – The term was formerly applied to all disturbances in the action semantic system. It is now proposed to keep the term ‘ideational apraxia’ for disorders of the sequential organization of movements.

Melokinetic apraxia – A form of motor apraxia characterized by difficulties in carrying out successive small digital movements.

Motor apraxia – A form of limb apraxia that induces disorganization of the elementary motor activities that constitute a movement.

Praxicons – Recipients where visuo-kinaesthetic engrams are stored.

Visuo-kinaesthetic engrams – Abstract codes about the physical attributes of a to-be-performed or a to-be-perceived action.

variety of apraxic patterns. Principles and guidelines are provided for the assessment of limb apraxia, still too often overlooked in standard clinical examination.

A Brief History of Limb Apraxia

Conceptual bases for the study of apraxia originate with Hugo-Karl Liepmann (1863–1925) who described the seminal case of MT, an Imperial Counselor hospitalized with a diagnosis of aphasia and dementia following a syphilis attack. Intentional motor activity of the right hand was severely disturbed in MT, as well as global head, face, and tongue movements. Notwithstanding, Liepmann observed that after immobilization of the right hand, which was interfering with contralateral movement production, MT was actually flawlessly able to imitate and execute on verbal command simple movements with his left hand, including pantomiming the use of several objects. Because performance was so strikingly different between the two hands, Liepmann logically deduced that left-hand motor difficulties were not attributable to a deficit in object recognition or understanding, because these deficiencies should have equally affected performance on both hands. He therefore proposed to name this pattern motor apraxia to establish a firm distinction between apraxia and asymbolia, the disorder of symbolic representations that was deemed at that time to encompass aphasic, apraxic, and agnosia symptoms.

Liepmann must also be credited for conducting one of the first group studies in neuropsychology. He investigated apraxic symptoms using movements’ imitation and execution on verbal command in 89 patients with unilateral right or left hemisphere lesions. The outcome was that most left-brain damaged patients were exhibiting apraxia of the left upper limb (the right limb most often being paretic), whereas right-hemisphere lesions were not associated with movement disorders. Given that a cerebral hemisphere controls for motor activity in the contralateral part of the body, Liepmann reasoned that the left cerebral hemisphere should play a predominant role in programming and controlling voluntary movements, as it plays in language, and that movement information processed in the left sensorimotor area is transferred to the right sensorimotor area via the corpus callosum for controlling the left hand. He also proposed a first anatomo-functional model of praxis processing, with the assumption that gestures production passes through a

Outline

Limb apraxia is a cerebral disorder that affects the processing of voluntary movements in the absence of elementary sensorimotor, psychological, or cognitive deficiencies able to explain the observed deficit. Current models of limb apraxia have provided a theoretical framework that globally conceives gesture processing as the result of neural activity in dedicated modules for the treatment and storage of movement-related information. These cognitive and neuropsychological models offer the advantage of a unified explanatory framework for a large

conceptual stage, that is, the development of the idea of the movement, located in the occipito-temporal cortex, followed by a production stage where the appropriate motor programs (also called visuo-kinesthetic engrams) are selected in the sensorimotorium, which includes the sensory parietal area and Brodmann's motor areas 6 and 8. Liepmann's constructs have strongly influenced the clinical practice, to the point that his classification of limb apraxia (ideational, ideomotor, and motor) is still found nowadays in many textbooks, in a barely modified phrasing.

Current Understanding of Apraxia

Nowadays, apraxia is still considered one of the major classical neuropsychological syndromes, along with aphasia, agnosia, and amnesia. It is generally defined as a cerebral disorder that affects the processing of voluntary movements, after having excluded perceptual and motor elementary deficits, paralysis, agnosia, and intellectual or linguistic comprehension disabilities that may explain the observed disturbances. Exclusion criteria are particularly important for the diagnosis of apraxia because disorders of high-order cognitive functions are established only after having ensured that other deficits cannot be responsible for a similar symptomatic. This can be especially difficult in the case of apraxia because apraxic problems are diagnosed throughout a continuum ranging from motor to conceptual disturbances in the processing of gestures. For instance, observing a patient who uses a spoon for combing his hairs should evoke alterations in an action semantic system, because the physical features of the object have been erroneously associated with the functions normally devoted to another object. However, this error can be deemed genuinely apraxic only if conceptual misattributions are proven specific to intentional gestures, and are not observed in many other domains of cognition, in which case a global intellectual deterioration should rather be diagnosed. At the other extreme of the continuum, manual dexterity impairments in buttoning a shirt or holding a pencil may lead the clinician to evoke the possibility of a melokinetic apraxia (see later on), unless a neurological examination evidences a digital sensitivity deficit, which actually entails coarse handling of objects due to the impoverishment of the afferent information necessary to create the appropriate motor programs. On the other hand, it should be kept in mind that the mere presence of an exclusion criterion does not automatically exclude the diagnosis of apraxia. Indeed, the crucial argument must be that the observed deficit should be capable of explaining both the extent and the qualitative features of the apraxic symptomatic. Referring to the example above, it is obvious that a digital sensitivity deficit can harm fine manipulation of small objects, but cannot

explain an incorrect positioning of the whole arm in space, as for instance, positioning its hand behind the head instead of touching the nose.

Although many patients may suffer from apraxia at various levels, its evaluation remains too often overlooked in standard neuropsychological examination. This means that apraxia diagnosis is too often taken into consideration only when symptoms are really obvious during the testing, or whenever the patient spontaneously complains about movement difficulties in everyday life. This relative disinterest comes from various reasons. First, many clinicians still believe that a definite feature of apraxia is the automatic–voluntary dissociation proposed by Jackson (1878), by which apraxic disorders are laboratory symptoms revealed in the course of a specific and effortful examination, but do not impact on routine and automated activities of everyday life. Still popular nowadays, this misconception is challenged by more subtle studies having demonstrated that apraxic patients actually experience concrete difficulties even when performing highly familiar activities such as slicing bread, dialing a phone number or grasping tiny objects. Second, although apraxia and aphasia are separate entities, a mutual predominance of the left hemisphere for language and gesture functions in most right-handed individuals entails a relatively high co-occurrence of the two disorders, from 27% to 80% according to studies, in which case the aphasic symptoms are likely to obscure the presence of an associated apraxia. Third, our understanding of the cerebral and cognitive mechanisms underlying the representation and programming of movements in man is still fragmentary. Despite several proposals for cognitive and neuroanatomical models of limb apraxia in the past 20 years, empiricism still prevails in clinical assessment, which makes conclusions rather uninformative. Finally, clinical evaluation justifies itself by the fact that it is a first step toward therapeutic intervention. Unfortunately, published revalidation studies still remain scarce and have not always been conclusive, which does not encourage neuropsychologists and neurologists to invest much time in a rigorous and exhaustive evaluation of apraxia. This creates a vicious circle that must be broken, as there is convincing evidence that everyday activities can be rehabilitated with a certain success in apraxic patients, for instance, when using error-free training procedures.

Cognitive and Neuropsychological Modeling of Limb Apraxia

Current neuropsychological and cognitive approaches of limb apraxia have provided a theoretical framework that globally conceives gesture processing as the result of neural activity in dedicated modules for treatment and storage of specific information. From this perspective,

limb apraxia is viewed as the behavioral outcome of dysfunctions in the cerebral structures that underlie these various levels of processing. Here, we briefly describe such a cognitive neuropsychological model of limb apraxia, partially adapted after the popular model proposed by Rothi and colleagues to account for the complex processes involved in the production and reception of gestures under various modalities.

In this model illustrated by **Figure 1**, the visuo-gestural analysis stage corresponds to the high-level perceptual visual analysis of human gestures, that is, the elaboration of a structural description of the visually perceived gesture, which is segregated from the visual analysis of objects. At the following step, visuo-gestural information is forwarded to the input praxicon stage, in which visual descriptions are compared with canonical representations of well-known and meaningful gestures stored in memory. This intermediary stage is mandatory for discriminating between familiar and novel gestures, and for meaningful gesture recognition and naming upon access to the action semantic system, where conceptual attributes of the gesture are retrieved. These conceptual attributes relate to the knowledge of functions and actions in relation with tools and objects, to the knowledge of actions independently of tools and objects (e.g., arbitrary communicative gestures) but in which objects and tools can be incorporated, and to knowledge related to the gathering of simple actions in a sequence. Besides the

input praxicon, the output praxicon is proposed to contain complementary codes about the physical attributes of a to-be performed action, at variance with the input praxicon that contains the codes about the physical attributes of a to-be perceived action. Access to the output praxicon stage and its visuo-kinaesthetic engrams or codes for to-be-performed actions is mandatory in order to pantomime gestures upon verbal command, a task in which the movement can only be evoked from memory. In collaboration with the input praxicon, it is also assumed that the output praxicon provides a processing advantage for imitation of familiar gestures, in that it avoids the cost to compute on-the-fly all the parameters needed to analyze and implement the spatial and temporal features of the skilled movement at each imitation attempt. The relatively abstract information provided by the output praxicon is then implemented in its motor form at the innervatory pattern stage for effective gesture production. Disturbances in one or more of these cognitive components will thus result in specific deficits in imitating, performing upon verbal command or presentation of an associated object, and discriminating or naming familiar gestures. On the other hand, imitation of meaningless, novel gestures is a particular case in that no corresponding representation is previously available in memory.

It has been long believed that imitation of novel, unfamiliar gestures is processed through direct transposition

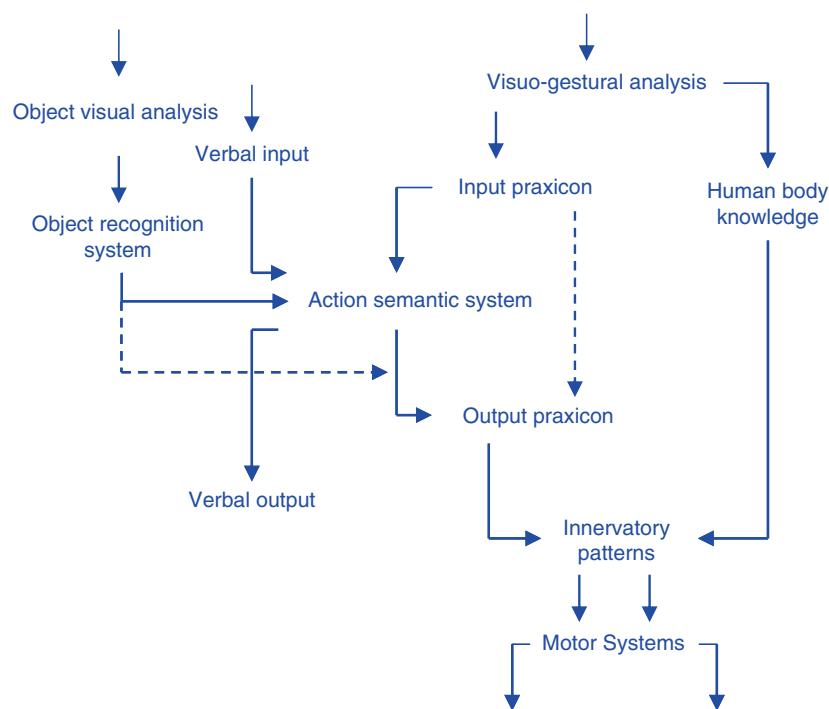


Figure 1 Cognitive neuropsychological model of limb praxis processing. Adapted from Peigneux P, Van der Linden M, Andres-Benito P, et al. (2000) Exploration neuropsychologique et par imagerie fonctionnelle cérébrale d'une apraxie visuo-imitative. *Revue Neurologique* 156(5): 459–472.

between visual analysis and motor implementation. However, neuropsychological evidence indicate that after demonstration of a nonfamiliar gesture, an intermediate representation is build after the visuo-gestural analysis stage into the human body knowledge system, which specifies the possible relationships between the main body components (for instance, the relative position of the upper arm, the arm, and the hand). This operating step creates a schematic representation which is more easily held in working memory and manipulated, and is subsequently conveyed toward the innervatory pattern stage for motor implementation. Thus, human body knowledge coding facilitates the transition between visual analysis and motor execution in that it reduces the complexity of the visuo-motor transformations needed to imitate a previously unknown gesture.

As compared to classical taxonomic approaches of limb apraxia, this type of modeling offers the advantage of a unified explanatory framework for a large variety of limb apraxic patterns. Indeed, if one or more specific processing modules are involved in the completion of each given task according to specific modalities (e.g., imitation, verbal command, recognition, etc.) and to the nature of the movement to process (familiar, novel, etc.), each apraxic pattern should be associated with alteration in one or more cognitive components. Identification of altered and preserved components may therefore be achieved by (1) making a comparison of the performance patterns under different conditions using comparable stimuli (e.g., imitation versus execution on verbal command of familiar gestures), (2) making comparison of performance patterns for specific categories of stimuli with identical task settings (e.g., imitation of familiar versus novel gestures), and (3) through the qualitative analysis of errors made during gesture processing.

Assessment of Limb Apraxia

Assessment of limb apraxia should take into account the diversity and specificity of apraxic patterns highlighted above. We will provide here some principles to guide the examination of limb apraxia for the interested reader. It should be stressed, however, that a detailed investigation of apraxia may take up to several hours and should be carried out by a trained neuropsychologist familiar with these specific assessment methods. Notwithstanding, it is also important for other professionals to apprehend apraxia examination and apply similar principles in standard investigations aiming at evidencing possible apraxic disorders. In addition, it must be reminded that the diagnosis of apraxia can be established only after having excluded the possibility that the observed symptoms are caused by other factors. Therefore, thorough neurological and neuropsychological examinations should be

performed beforehand to leave out other psychological, cognitive, or sensorimotor deficiencies that could account for an probable apraxic deficit.

Examination Conditions

In the assessment of limb apraxia, the variety in testing conditions aims at highlighting dissociations of performance that may point toward specific deficits. In the evaluation of gesture production, the examiner should always present the patient with at least three pantomime conditions: (a) executing familiar gestures on verbal command, (b) imitating familiar gestures, and (c) imitating novel, unfamiliar movement patterns. Those conditions are best suited to highlight the most frequently observed dissociations in performance. Whenever possible, however, the examination will be complemented with pantomimes performed upon (d) visual and (e) tactile presentation of man-use objects associated with specific actions (e.g., hammer, comb, screwdriver, etc.), and (f) concrete manipulation of the same objects. Gesture reception should also be carefully assessed using at least (g) denomination of visually presented familiar gestures, and (h) reasoning tests in which functional knowledge about tools and associated actions is probed. In the latter case, this can be achieved, for instance, by asking the patient to decide whether a hammer and a nail works together (i.e., probing functional associations between two objects), or whether a hat may be a replacement for a bucket to carry some water on a short distance (the answer here being 'Yes' since both objects are potential containers even if the hat will not hold water for very long).

Referring to the cognitive neuropsychological model described above, each of these tasks usually involves direct access to more than one processing module. Therefore, any hypothesis about the functional localization of the observed deficit will be made based on differential patterns of performance across the different tasks. For instance, a common deficiency in the imitation and execution on verbal command of familiar gestures would be potentially attributable to deficits affecting the action semantic system, the output praxicon, or the innervatory patterns. However, if it can be established that gestures denomination is preserved, this positive result will demonstrate the integrity of the action semantic system, which is mandatory for attribution of the correct conceptual label to the proposed action. Having excluded an action semantic system deficit, it remains to differentiate further whether the deficit is located at the output praxicon or the innervatory patterns levels. In this case, flawless performance on novel gestures imitation will be sufficient to certify the integrity of innervatory patterns, knowing that successful imitation always requires access to the innervatory pattern stage. In such a case, it may be

concluded that it is an output praxicon deficit which is responsible for this disorder predominantly affecting the production of familiar gestures. When confronted with a patient exhibiting deficits in novel gesture imitation, an additional test can be proposed, which is the reproduction of the same unfamiliar limb postures on a manikin or a collaborator. The rationale behind this test is to highlight disorders of the human body knowledge component. Indeed, if similar errors are produced when imitating a novel gesture or having to reproduce the same pattern on another body structure, despite strikingly different motor demands, then this will indicate that the imitation deficit lies in the ability to create a coherent representation of the perceived posture with reference to the topographical organization of the human body.

Examination Procedures

Before and during the course of apraxia assessment, it is essential that the instructions provided by the examiner strongly emphasize the need to produce and analyze gestures in a most precise as possible manner. Similarly, it must be carefully ensured that the patient has an adequate understanding of the performance levels that

should be targeted, systematically using training items and providing examiner's feedback. Most importantly, at least a second trial should be systematically proposed after failure to process a gesture, in order to ensure the consistency of the observed deficit. Indeed, many examiners overlook the fact that apparently apraxic errors may be produced due to the influence of various nonspecific cognitive factors, for instance following attentional and working memory deficits. Such nonspecific errors are usually corrected by the patient if allowed a second attempt, demonstrating preserved access to the underlying praxis system.

This is why a difference should be made between simple and consistent errors. Simple errors are observed at the first attempt in performing a gesture, but corrected in a subsequent attempt, even if this correction is sometimes associated with the production of a qualitatively different error. These simple errors may suggest a limitation in processing capabilities, but cannot ensure the genuine apraxic characteristic of the deficit. On the other hand, consistent errors are errors repeated at consecutive attempts, despite the fact that the patient's attention has been drawn towards the previously committed error (**Figure 2**). Consistent errors are thus more



Figure 2 Consistent spatial error produced by patient R.M. during imitation of a meaningless gesture (i.e., to put the back of the right hand on the left ear). At first attempt (top left picture), a spatial configuration error is observed as R.M. put the palm of her right hand on the ipsilateral cheek instead of the contralateral one. The examiner then attracted her attention on the incorrectness of the gesture, and a second attempt was allowed. After marked hesitations (illustrated on middle picture), R.M. produced a second spatial configuration error (bottom picture) in putting the back of the right hand was on the ipsilateral cheek. Thus, spatial configuration errors are consistently produced across trials, suggesting genuine apraxic difficulties to elaborate an appropriate representation of the gesture to imitate (see main text). Left inferior drawing represents the posture demonstrated by the examiner. Reproduced from Peigneux P, Van der Linden M, Andres-Benito P, et al. (2000) Exploration neuropsychologique et par imagerie fonctionnelle cérébrale d'une apraxie visuo-imitative. *Revue Neurologique* 156(5): 459–472.

likely to be genuinely apraxic, because reproduction of the same error despite reinstruction provides more confidence that this error reflects a specific alteration in the movement-processing system. All these precautions are especially important in apraxia assessment because there is a large, natural variability in the way movements are produced among different individuals. Owing to this fact, relatively vague attempts to produce gestures may be erroneously classified as correct or flawed when these exigency levels are not made perfectly clear to the patient. Further, to prevent facilitation of performance by an order effect between the various conditions of gesture production (e.g., production of same or similar gestures on imitation vs. verbal command), presentation order of the testing items should be randomized across these conditions, unless other cognitive problems (e.g., an executive dysfunction characterized by altered flexibility) make it difficult for the patient to switch from one condition to another. On another note, apraxia should be as much as possible systematically investigated at both limbs, taking in account the normal asymmetry of performance between the right and left hands in interpreting the results. Finally, bimanual gestures may also be administered to probe limb-coordination difficulties.

Qualitative Error Analysis in Gesture Production

The most accurate manner to assess gesture production is to record the patient's performance on videotape, allowing delayed in-depth analysis by one or more independent evaluators. Although not always attainable in clinical settings, this procedure largely facilitates the qualitative analysis of gesture-production errors, which is an essential complement to the quantitative analysis of performance dissociations presented above. Indeed, the mere usage of quantitative scores computed based on a number of successfully performed items, or on the number of trials needed to achieve the correct execution of gestures, provides only crude information about the mechanisms underlying disturbed performance. Such measures completely obscure the fact that individuals with qualitatively different deficits can obtain identical quantitative scores. For instance, it is very different to perform a gesture on verbal command in a seemingly recognizable way, but inadequately positioned in space (e.g., combing on the shoulder instead of on the head) than producing a perfectly performed gesture, but which is not the one required by the examiner (e.g., playing piano instead of playing violin). In the first case, the error suggests that either the visuo-kinesthetic representations of gestures in the output praxicon or their motor transcription in the innervatory patterns are altered, whereas the action semantic system is preserved. In the latter case, flawless motor production of a gesture with an inadequate meaning suggests the opposite profile, that is, a deficit in

the action semantic system coupled with preserved functioning of the output praxicon and innervatory patterns. These conclusions will complement congruent findings of performance dissociations between tests performed under various conditions and using different stimulus type. For instance, an action semantic deficit should lead to marked differences in performance between defective gesture production on verbal command and preserved imitation of these same gestures, the latter being made possible by a direct link between the input and output praxicons that bypasses the action semantic system, or by accessing the alternative imitation route and processing familiar gesture to imitate in the same manner as novel gestures, through access to the human body knowledge system.

A Brief Taxonomy of Limb Apraxic Disorders

A recurrent problem with taxonomies of limb apraxia is their lack of theoretical foundations. In addition, similar labels have been applied to different apraxic patterns all the way through the history of clinical practice and publications, and these concurrent labels still prevail nowadays. Still, these taxonomies remain largely in use in the clinical domain, which makes it important for the reader to be briefly informed about the main limb apraxia categories and their possible variations.

Ideational Apraxia

Ideational apraxia is a term that defines two distinct apraxic patterns that may be deemed related to the action semantic system. In its first acceptance, inherited from the French author Morlaas, ideational apraxia is a selective disturbance in the use of current objects, in which the patient cannot use any more a real object and/or produces errors in using tools, even for simple tasks that imply only one object. In most severe forms, the patient may try writing with scissors or brushing teeth using a spoon. In its second acceptance, ideational apraxia defines the inability to order the set of elementary movements that makes a complex action into their correct sequence. For instance, the patient will omit, add, or transpose elements in the sequence of movements used in pantomiming eating soup with a spoon. To avoid terminological disputes, it has been proposed to keep the term ideational apraxia for disorders of movement sequence organization, while defining under the term 'conceptual apraxia' the other alterations that directly affect semantic knowledge about functions and actions related to tools and objects, and about arbitrary actions performed independently.

Ideomotor Apraxia

Ideomotor apraxia is probably the most common form of limb apraxia, which affects a gesture production system including sensorimotor knowledge of actions and the perceptivo-motor processes required for their organization and execution. Complementary to the two main definitions of ideational apraxia, ideomotor apraxia knows at least two concurrent definitions. According to its first acceptance, again inherited from Morlaas (1928), ideomotor apraxia relates to the gesture execution component in itself, independently of the object associated to the action. The disorder will therefore be especially visible during the execution of arbitrary or symbolic movements (e.g., military salute, praying, etc.) that cannot be improved by the presence of an object. Still, deficits will be also observed during pantomime of object-use gestures, carried out in the absence of the associated object. According to the second acceptance, based on the bottom-up, hierarchical distinction between movement, action and sequence, ideomotor apraxia defines a deficit that affects action execution by itself, characterized by difficulties in performing isolated gestures or fragments of a movement sequence upon imitation or verbal command. Beyond these differences, these two forms of ideomotor apraxia have in common the description of the incorrect production of an action appropriately selected at the conceptual level. Besides supra-modal ideomotor apraxia that affects movements in all production forms, ideomotor apraxia can also be restricted to specific modalities of gesture production (imitation, verbal command, upon visual or tactile object presentation, etc.), stimulus types (object-use, symbolic, arbitrary unfamiliar, etc.), or effectors (upper or lower limb, unilateral or bilateral, etc.).

Motor Apraxia

Motor apraxia defines a disorganization of the movement that closely resembles elementary motor disorders. Motor apraxia is almost always unilateral and expressed across all gesture production modalities as manual dexterity disorder. Its best-known form is melokinetic apraxia in which small and rapid, alternated or serial movements are disturbed and carried out awkwardly. For instance, the patient may not be able to tap fingers in alternation or tinkling fingers smoothly on the edge of the table any more. Melokinetic apraxia can be distinguished from afferent or kinesthetic apraxia, characterized by a loss of selectivity (rather than by loss of dexterity) of digital movements with major difficulties in the reproduction of elementary fingers configurations.

See also: Contribution of Split-Brain Studies to the Evolution of the Concept of Hemispheric Specialization.

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Physical and Emotional Pain

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Glossary

Active emotional coping – Integrated psychological, behavioural and physiological responses engaged to control and reduce the impact of any stress threat or pain; these responses may be either ‘proactive’ or ‘reactive’ strategies depending on the specific behavioral patterns engaged.

Anterior cingulate cortex – The cingulate cortex is found in the medial part of the cerebral cortex; it lies above and around the corpus callosum, and its anterior part is found beneath the prefrontal and frontal cortices. It is also known as Brodmanns area 24.

Conservation-withdrawal – Integrated psychological, behavioral, and physiological response engaged in response to stress threat or pain, for which ‘proactive’ or ‘reactive’ active emotional coping strategies have either failed or are inappropriate.

Midbrain periaqueductal gray – The cell dense region surrounding the canal that joins the third and fourth ventricles of the brain. It is organized into longitudinal columns of cells, each of which plays an important role in the mediation of integrated psychological, behavioral, and physiological responses to deal with stress threat or pain.

Passive emotional coping – Another term for conservation-withdrawal responses; however, it is sometimes, and somewhat confusingly, used to describe ‘reactive’ active emotional coping by some scientists.

c-fos – An immediate early gene, whose expression has been used quite extensively by neuroscientists, to identify recently active neurons in post-mortem histological sections of nervous system tissues. The gene is identified from either (1) the presence of its protein product Fos, using immunohistochemical techniques, or (2) the expression of c-fos mRNA, using *in situ* hybridization techniques.

To expand on this idea, what is being stated is that should we be able to describe precisely, in both structural and functional terms, the pathway from the peripheral receptor (nociceptor) via the sensory ganglia, and along through the spinal, thalamic, and cortical pathways, our understanding of the pain experience would be both incomplete and inadequate to explain the clinical presentation of the pain patient.

A similar fundamental view permeates some of the writings of Patrick Wall, another scientist at the vanguard of modern pain research. In the John J. Bonica lecture of 1979, he challenged the view of many pain neurobiologists by placing the definition of ‘pain’ in a new context. He states that “... pain is better classified as an awareness of a need state than as a sensation: that it has more in common with the phenomena of hunger and thirst than it does with seeing and hearing; and that it serves more to promote healing, than to avoid injury.” In identifying an immediate, and acute and a chronic stage following pain eliciting events (i.e., tissue injury), Wall recognized that “... in each stage pain has only a weak connection to the eliciting stimulus, but a strong connection to the body state...” That is, as time passes pain may resolve or persist, and thus the drives for behavioral change will alter; however, these drives do not necessarily correlate with the presence of pain as a sensation.

What each of these authors were highlighting was that pain as a sensation is only part of an organism’s experience and that pain represents a fundamental drive for the need for behavioral change. That is, pain is better viewed as a motivational state, rather than simply sensation, and further that this need state changes over time, relative to the occurrence of the injury. On balance, each of these views arrives at the same conclusions, albeit on the bases of slightly different arguments, that to understand pain we must understand more than just the sensory pathways and that any definition of the central neural representations of pain must include the motivational or emotional state changes that it generates.

This viewpoint underpins the definition of pain offered by the International Association for the Study of Pain in 1979, “that pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.” However, despite the acknowledgement of the inextricable link between sensation and emotional state change, the design and execution of most experimental studies in the neurobiology of pain remained unaltered, until recently.

Concepts of Pain

In the posthumously published works of William K. Livingstone, a pioneer of contemporary pain research, the point is repeatedly made that “... pain is not strictly a physical sensation that can be defined simply by its anatomical and physiological substrates....”

It has been appreciated for many years that pain of different origins is associated with emotional state changes with distinct and different qualities. Almost 70 years ago, it was noted by Thomas Lewis, a clinician and physician in his book *Pain*, in which he presents findings based on many years of clinical observations, that pain arising from different body structures triggers distinctive emotional behaviors. He notes specifically that pain arising from the human skin is associated with "brisk movements, with a rise in pulse rate and a sense of invigoration," whereas pain derived from deeper body structures is associated usually with "quiescence, slowing of the pulse, falling of the blood pressure, sweating and nausea." He went on to comment that the nausea is responsible for the description of deep pain being sickening, an emotional state most usually applied to pain of deep origin but seldom to that from cutaneous pain. Indeed, this sickening feeling or vaso-vagal response (which Lewis first termed vaso-vagal syncope) occurs often with pain arising from joints, muscle, periosteum, and the vasculature; it also occurs during angina, gall stones and colic, and is a major feature of testicular and bladder pain.

The clearly opposing emotional state changes triggered by pain arising from different body regions (tissues) led Lewis to state that given the differences in the emotional responses to pain of different origins, it may in fact be "unsafe to classify both states under the one unqualified term pain." Thus, it is clear that Lewis recognized the critical position of emotional state in our definition of pain.

If then, it is essential that we formulate our understanding of the neurobiology of the representation of pain in the nervous system by including an understanding of the location of brain regions mediating the emotional state changes evoked by pain. The observations made by Lewis, almost 70 years ago; the clinical reasoning presented in the writings of Livingstone; and the challenges of Wall's provocative lecture must all be integrated fully into the conceptual framework of the modern pain neurobiologist. Lewis' observations that two distinct emotional state changes are evoked by superficial and deep pain have direct implications in this regard.

Lewis was clear about the significance of his findings, when he stated that the qualities of superficial versus deep pain are so distinct, that from a clinical perspective, they challenge a unitary conceptualisation of pain. That is, the experience of superficial pain and that of deep pain share few common properties. He recognized that the distinct differences in both sensory and emotional experience between superficial and deep pain could only have its basis in differential representations within the central nervous system. He states... "we need to consider whether or not the system of fibres subserving pain derived more deeply connect basically to another distinct

part of the sensorium..." compared with those subserving superficial pain. "Although both follow the path of the anterolateral tract as shown by the abolition of both in cases in which this tract has been divided surgically, we should bear in mind the possible serious fallacy of regarding both types as being represented in a common centre." This suggests that Lewis expected distinct and separate neural representations of pain of superficial versus deep origin.

A Dilemma for the Pain Neurobiologist?

A longstanding difficulty for experimental research into the neurobiology of pain is revealed after consideration of the approaches of Livingstone, Wall, and Lewis. It becomes clear that there are two major strategies to approach the study of pain, which are well illustrated by considering how pain of cutaneous and deep origin has been investigated most recently. The most widespread and extensively published approach has been that derived from the classical paradigms of the sensory physiologist, which have been fuelled by the clinical significance and diagnostic importance of the phenomenon of referred pain. Not surprisingly from this vantage point, anatomical and (electro-)physiological experiments have focused almost exclusively on viscero-somatic convergence. That is the common representations of pain of superficial and deep origin with the central nervous system.

In contrast, the approach suggested by the writings of Livingstone, Wall, and Lewis, with their emphasis on the emotional and behavioral significance of the pain signal, suggests that consideration of the different central representations of the distinct emotional states evoked by pain of cutaneous and deep origins will reveal a deeper understanding of the brain mechanisms underlying the array of sensory and emotional state changes commonly referred to as pain.

Emotional Pain in Animals

In humans, it is clear from the findings discussed above that there are two ways in which an individual attempts to cope with pain. These emotional state changes, or emotional coping strategies, are adopted in situations other than pain states, and broadly represent the strategies engaged to deal with any major stressor challenging the integrity of the individual. The strategy engaged in response to superficial pain has been defined as an 'active emotional coping response,' and is identical to the classically defined 'fight-flight' response. This response strategy leads an individual to engage with its environment, and in particular, with the source or the location of the painful event. The response is characterized by

increased vigilance, hyper-reactivity, increased somatic activity, and increased sympathetic tone (which shows regional specificity, depending on the specific behaviors engaged in). This particular emotional state is triggered usually by painful events that can be controlled or escaped from, as well as other stressors that are perceived as escapable and/or controllable.

The second class of response, engaged during pain arising from deeper structures, has been referred to as a ‘passive emotional coping response’; it is in fact the complete opposite of the active emotional strategy. Rather than engagement with the painful stimulus and/or the environment, there is disengagement with the environment, a response termed conservation-withdrawal, in earlier studies, which focused on the coping styles evoked by other classes of stress or threat. The passive coping strategy is characterized by decreased vigilance, hypo-reactivity, reduced interest in the environment, and often a withdrawal of sympathetic tone resulting in falls in arterial pressure and heart rate. This strategy is usually engaged in response to stimuli that are, or are perceived as, inescapable. Thus, with regard to pain arising from distinct body structures, acute cutaneous pain triggers active emotional coping behaviors, whereas persistent cutaneous pain, or pain of deep somatic or visceral origin, will trigger primarily passive emotional coping behaviors. In addition, there is a transition point for pain of cutaneous origin, insofar as acute pain from superficial structures triggers initially an active emotional coping response. If this pain persists, the emotional coping response will switch over time to a passive style of response, in exactly the way Wall described some 30 years ago.

It is thus possible now to re-address the issue of physical and psychological pain, in terms of the distinct and different emotional behaviors evoked by different classes of pain. The important neurobiological questions raised by this conceptual shift are: (1) are there different neural circuits mediating active and passive emotional coping behaviors; and (2) are there different and distinct neural pathways accessing these circuits following noxious activation of cutaneous and deep body structures?

Brain Representations of Active and Passive Emotional Coping Responses: Animal Studies

In unravelling the distinct neural circuits in which active and passive emotional coping responses in experimental animals, investigations of one brain region, the periaqueductal gray (PAG) region of the midbrain, has provided an invaluable entrée point. The PAG is the cell dense structure that surrounds the midbrain aqueduct, the conduit between the third and fourth ventricles of the brain.

Studies by a number of investigators in a number of laboratories revealed that the PAG is divisible into a series of longitudinally oriented functional columns. Active emotional coping responses (fight or flight; increased arterial pressure and heart rate; and a non-opioid mediated analgesia) are evoked when neurons lying either dorsolateral (dIPAG) or lateral (IPAG) to the midbrain aqueduct are activated.

Neurons of the dIPAG are distinguished from neurons of the IPAG by the presence of a number of transmitter substances, including nitric oxide synthase, cholecystokinin, acetylcholine and Met-enkephalin, and also by the absence of cytochrome oxidase and the glycine transporter (Gly-2). The major inputs to neurons of this region arise from the medial prefrontal cortex and specific nuclei of the medial hypothalamus; however, these cells do not receive inputs from the brainstem or the spinal cord, regions which themselves receive dense inputs from nociceptive primary afferent fibres carried in: spinal nerves, the trigeminal nerve (cranial nerve V), the vagus (cranial nerve X), or the glossopharyngeal nerve (cranial nerve IX) (**Figure 1**). The predominance of cortical inputs into the dIPAG has led to the suggestion that when evoked by psychological stimuli (i.e., signals arising primarily from the cortex), active emotional strategies are mediated by neurons in this PAG column. Data from a number of animal studies support this view, for example, the threat, either potential or immediate, signaled by the odor or sight of a cat (critically though, not the physical contact) evokes an active emotional coping response and a selective activation of neurons in the dIPAG as indicated by the neuronal expression of the immediate early gene, c-fos (**Figure 4**). Thus, it may be that the psychological/emotional states, which people describe as having painful qualities, are mediated in part by neural circuitry, which may be defined by its afferent and efferent connections with dIPAG neurons.

Although a clear boundary can be defined between the dIPAG and the ventrally adjacent IPAG on neurochemical grounds, the boundary between the IPAG and the ventrally adjacent ventrolateral PAG (vIPAG) column rests primarily on functional criteria. The lateral column of the PAG receives comparatively little cortical input, comprising, for the main part, a small projection from the dorsolateral convexity of the medial prefrontal cortex; similarly, its hypothalamic projections are also modest, receiving again a small projection from nuclei of the dorsal hypothalamus. By far the largest projection to the IPAG arises from the spinal cord and the spinal trigeminal nucleus (Sp5) (**Figure 2**). These projections terminate somatotopically within the IPAG column, and arise from spinal and Sp5 laminae (Rexed’s laminae I, II, IV, and V), which contain noci-responsive neurons. These anatomical observations of a significant input from noci-responsive brainstem and spinal cord regions suggest that neurons of the IPAG play

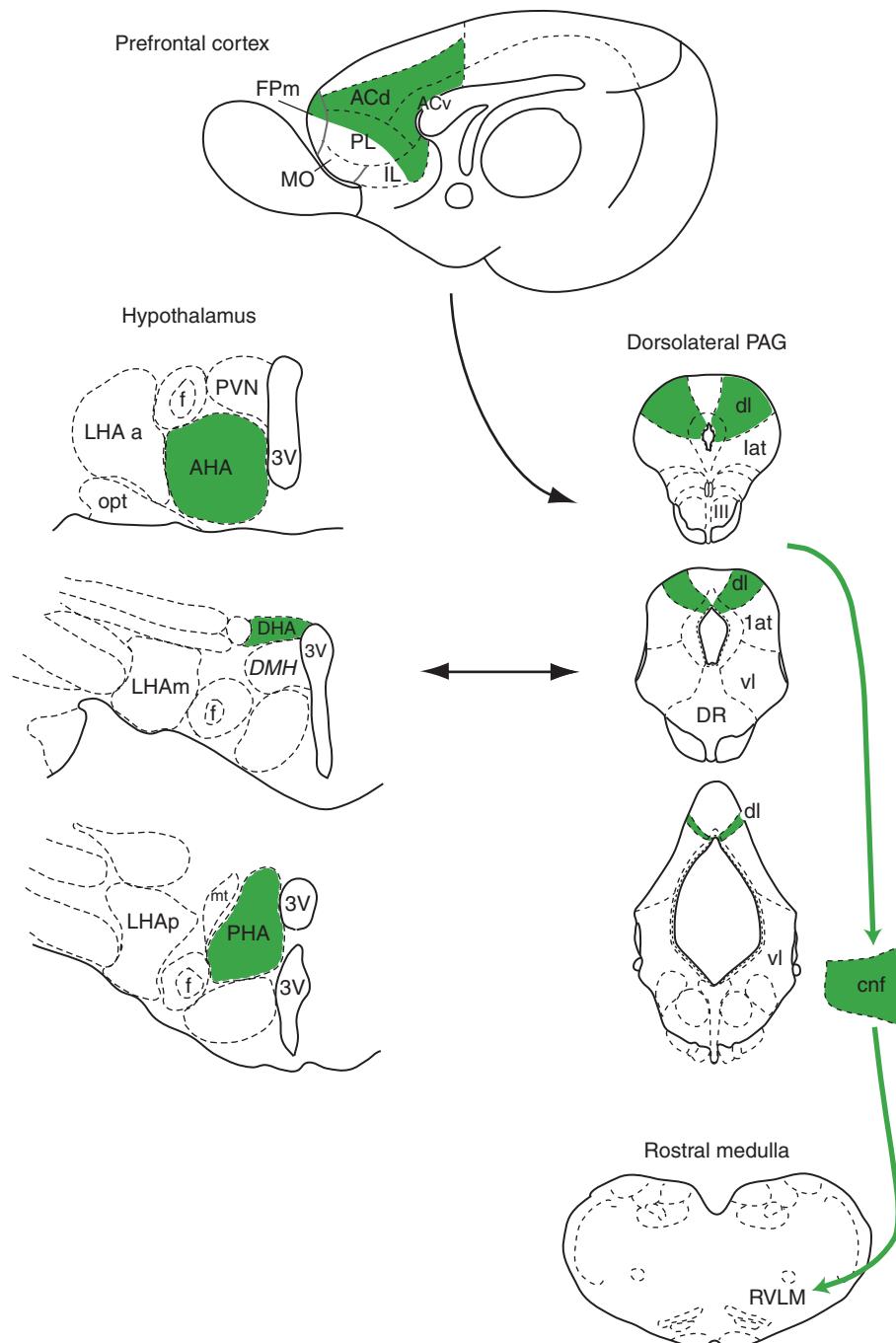


Figure 1 Schematic illustration of the major inputs to the dPAG from the forebrain and hypothalamus (dorsal, medial prefrontal cortical fields, and medial hypothalamic regions). The dPAG does not project directly to the medulla, but can influence the rostral ventrolateral medulla via the cuneiform nucleus. Modified from Keay KA and Bandler R (2009) Emotional and behavioural significance of the pain signal and the role of the midbrain periaqueductal gray (PAG). In: Basbaum AI and Bushnell CM (eds.) *Science of Pain*, pp. 627–634. San Diego, CA: Academic Press.

a critical role in integrating active emotional coping responses in response to physical (rather than psychological) stimuli, for example, acute cutaneous pain. Once more, data in support of this idea are found in experiments using expression of the *c-fos* gene as a marker of neuronal activity, which have shown that brief applications of noxious

thermal stimuli: selectively increase *c-fos* expression in neurons of the IPAG; trigger increases in arterial pressure and heart rate and evoke active emotional coping responses (Figure 4). The active emotional coping responses evoked from the IPAG have directional qualities, which are related to the precise location of the stimulus, accentuating the

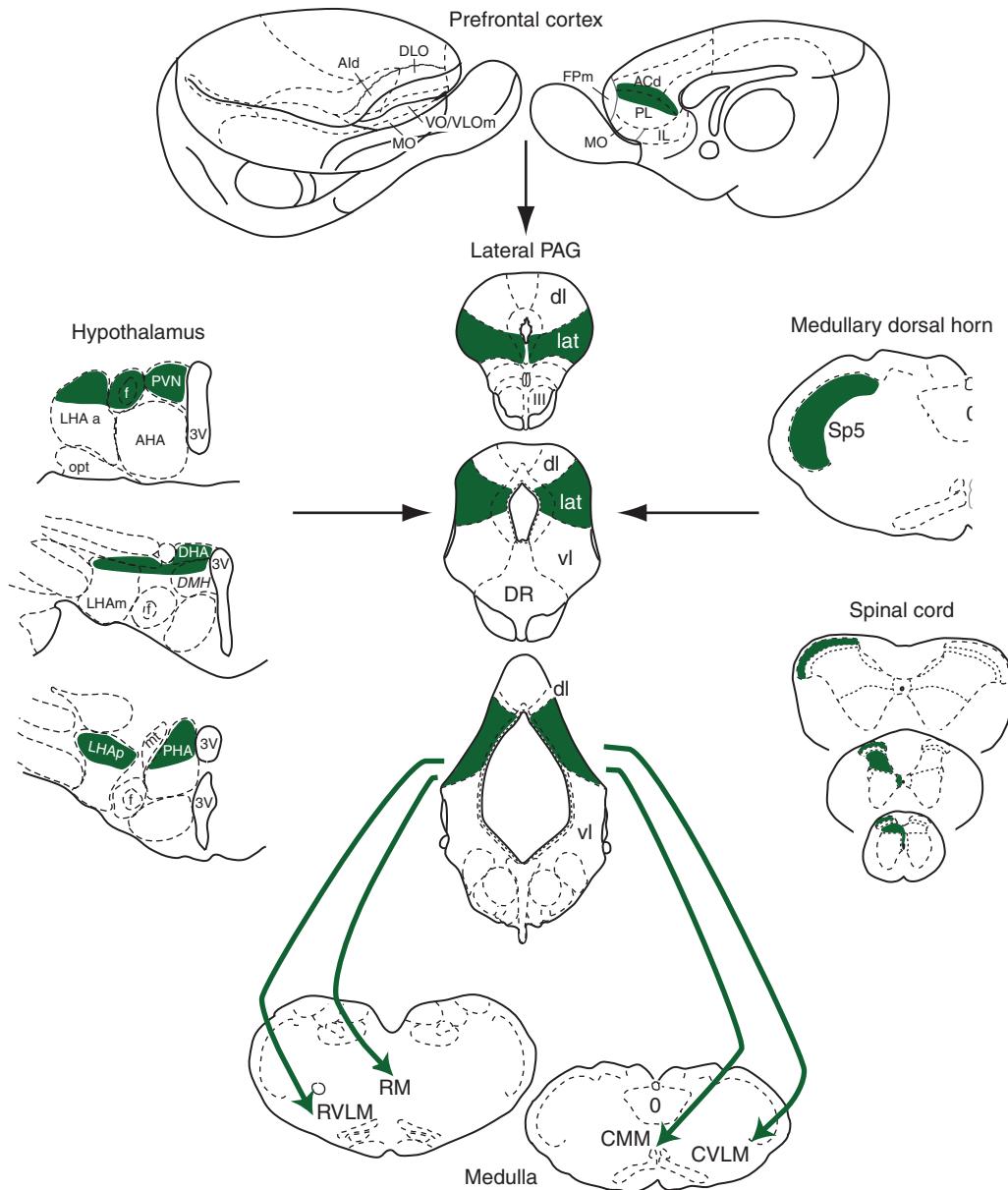


Figure 2 Schematic illustration of the inputs to lateral PAG. Major ascending inputs arise from spinal cord and the medullary dorsal horn (Sp5). In addition, restricted parts of medial prefrontal cortex and the dorsal subnuclei of the hypothalamus provide smaller inputs to this region. The IPAG projects directly to the rostral and caudal ventrolateral medulla (RVLM, CVLM) as well as the rostral and caudal ventromedial medulla (RM, CMM). Modified from Keay KA and Bandler R (2009) Emotional and behavioural significance of the pain signal and the role of the midbrain periaqueductal gray (PAG). In: Basbaum AI and Bushnell CM (eds.) *Science of Pain*, pp. 627–634. San Diego, CA: Academic Press.

likely functional significance of the somatotopically organized spinal and Sp5 projections defined in anatomical studies (Figure 2). On balance, therefore, it appears that neurons of the dorsal PAG trigger active emotional coping responses to psychological stimuli (dIPAG) and to physical stimuli (IPAG). The neural circuits within which each of these PAG columns (dl vs. IPAG) is embedded form discrete networks critical in integrating the affective/motivational/drive states, which characterize distinct classes of escapable pain.

In contrast to the dorsal PAG (dIPAG and IPAG), neurons within the vIPAG have been shown to mediate a coordinated response that resembles the conservation-withdrawal response defined in earlier literature investigating stress responses. The activation of vIPAG neurons triggers reduced responsiveness to external events, behavioral quiescence, falls in arterial pressure and heart rate, as well as an opioids-mediated analgesia. The behavioral reaction triggered by the vIPAG comprises the passive emotional coping response. In common with the IPAG,

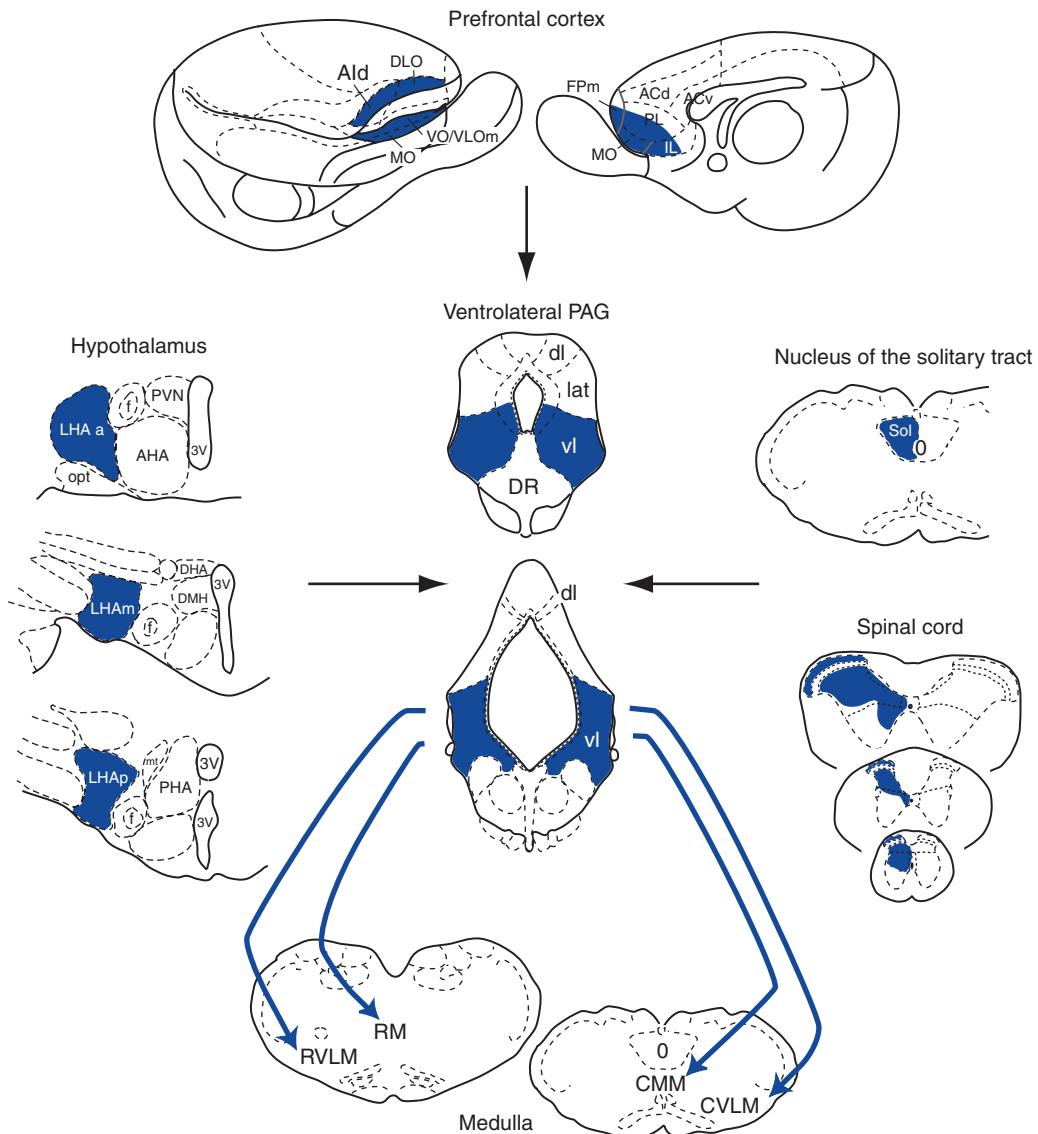


Figure 3 Major ascending projections to the vlPAG arise from the spinal cord and the nucleus of the solitary tract. Significant descending inputs arise from ventromedial prefrontal cortex, orbital/insular cortices, and the lateral hypothalamus. The vlPAG projects directly to the rostral and caudal ventrolateral medulla (RVLM, CVLM) as well as to the rostral and caudal ventromedial medulla (RM, CMM). Modified from Keay KA and Bandler R (2009) Emotional and behavioural significance of the pain signal and the role of the midbrain periaqueductal gray (PAG). In: Basbaum AI and Bushnell CM (eds.) *Science of Pain*, pp. 627–634. San Diego, CA: Academic Press.

the vlPAG receives substantial inputs from the spinal cord and SpV; these inputs arise from the same laminar divisions (Rexed's laminae I, II, IV, and V) (Figure 3); however, the projections to IPAG and vlPAG columns arise from different neuronal populations. That is to say, the IPAG and the vlPAG each receive unique signals from spinal cord and SpV regions. Furthermore, in contrast to the somatotopy seen in spino- and SpV projections to the IPAG, the projections to the vlPAG terminate throughout the neuronal cell column. In addition to its spinal/SpV input, the vlPAG also receives substantial inputs from nuclei of the solitary tract (NTS); these inputs again terminate throughout the cell

column showing little topographic specificity (Figure 3). Besides being the recipient of substantial ascending projections, the vlPAG also receives major descending inputs from cortical and hypothalamic nuclei. Specifically, the vlPAG receives input from orbital and insular cortical regions, and from the lateral hypothalamus. Thus, the vlPAG receives convergent forebrain, medullary, and spinal/SpV afferents, which led to the suggestion that passive coping, irrespective of being triggered by physical or psychological triggers, is mediated by the vlPAG. Strong support for this view has once again been derived from studies using c-fos as a marker of neuronal activation. It has been shown that when passive coping is

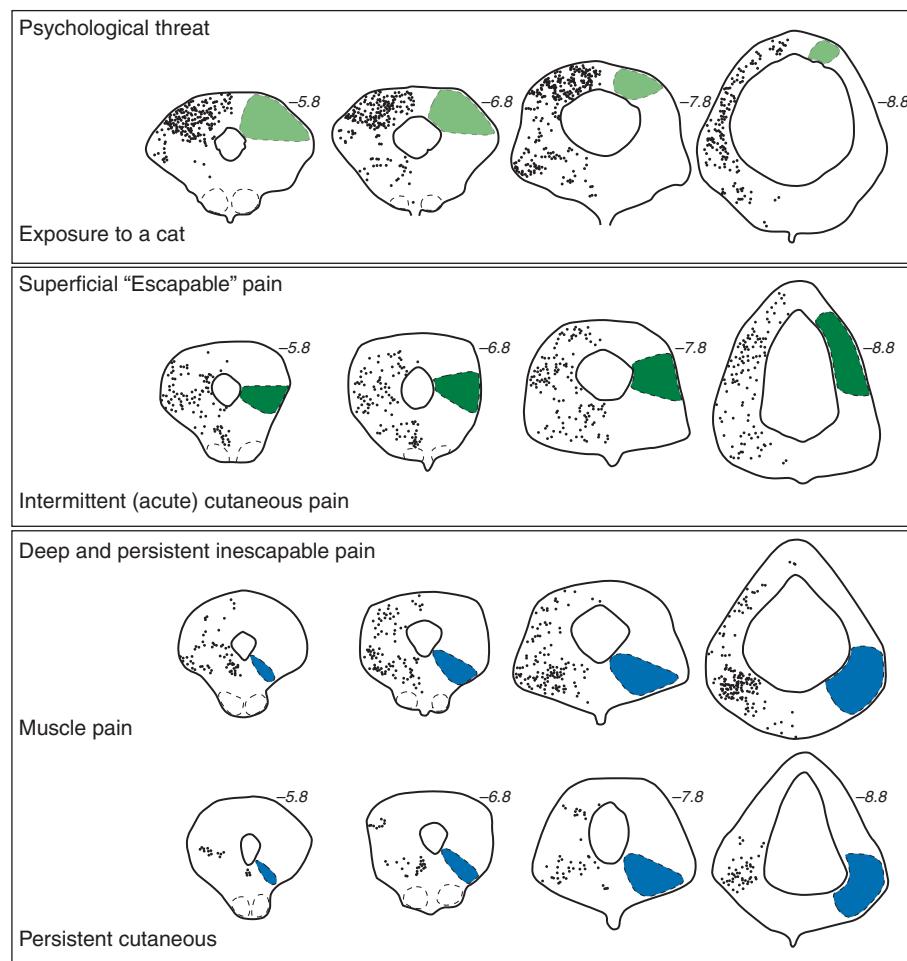


Figure 4 The upper panel illustrates the pattern of neural activation in the midbrain PAG of rats exposed to a psychological threat (exposure, without physical contact, of a rat to a cat), which evokes active emotional coping. The majority of active neurons are located within the dorsolateral PAG column, indicated by the shaded area. The center panel illustrates the location of activated neurons evoked by superficial escapable pain, evoked by intermittent (acute) cutaneous pain, which evokes active emotional coping. Majority of active neurons are located within the lateral PAG column, indicated by the shaded area. The lower panel shows patterns of neural activation evoked by deep and persistent inescapable pain. Muscle pain and persistent cutaneous pain, each of which evokes passive emotional coping, both activate neurons selectively in the ventrolateral PAG as indicated by the shaded area. Modified from Keay KA and Bandler R (2009) Emotional and behavioural significance of the pain signal and the role of the midbrain periaqueductal gray (PAG). In: Basbaum AI and Bushnell CM (eds.) *Science of Pain*, pp. 627–634. San Diego, CA: Academic Press.

triggered as the initial response to pain of deep origin, or when a painful state becomes persistent, or in order to promote recovery and healing as a delayed response to acute injury, vIPAG neurons demonstrate significantly increased c-fos expression (**Figure 4**).

Although the lateral and ventrolateral columns of the PAG mediate distinct and different emotional coping strategies, and receive inputs from distinct and different forebrain, medullary, and spinal/SpV inputs, their descending output projections appear identical. Neurons in the lateral and ventrolateral PAG project onto the same ventrolateral and ventromedial medullary regions (**Figures 2 and 3**). However, consistent with their opposing functional roles, these projections mediate different effects on their target sites; for example, the IPAG projection to the rostral ventrolateral medulla, which

contains neurons whose activity increases arterial pressure, is primarily excitatory, whereas consistent with the hypotensive actions of the vIPAG, projections from the vIPAG to the rostral ventrolateral medulla are primarily inhibitory. In contrast to common descending output targets, the lateral and vIPAG provide distinct ascending projections to different hypothalamic and midline and intralaminar thalamic nuclei. Although the dIPAG mediates active emotional coping responses, unlike the IPAG, it has few direct medullary outputs. Its ability to influence the ventrolateral medulla is due most likely to its projections to the region of the cuneiform nucleus, which has substantial inputs to this region (**Figure 1**). In common with both the IPAG and vIPAG, the dIPAG also projects to a distinct set of thalamic and hypothalamic subregions.

It is clear, therefore, that animals have the capacity to respond to escapable (acute/superficial) or inescapable (chronic/deep) pain with different emotional coping strategies, in ways similar to that described in the observations of Livingstone, Wall, and Lewis. The PAG region of the midbrain is divisible into distinct longitudinal neuronal columns, which mediate distinct coping strategies (dIPAG/EPAG: active emotional coping; vIPAG: passive emotional coping), again reflecting the response profiles described by Livingstone, Wall, and Lewis. Furthermore, each of the PAG columns comprises critical nodes within parallel and distinct circuits. These discrete neural circuits are defined by: inputs from specific cortical (i.e., prefrontal cortex and insular regions), hypothalamic, brainstem, and spinal cord regions; outputs, either direct or indirect, to ventrolateral and ventromedial medullary regions in which somatic and autonomic ‘premotor’ neurons, and neurons regulating sensory responsiveness are located.

An important question raised by the data derived from experimental observations in animals is whether, as predicted by Livingstone, Wall, and Lewis, pain of superficial or deep origin is represented by more than just coding of stimulus properties (i.e., the pain) in the brains of humans. The advent of brain functional imaging techniques has allowed us to begin to describe the forebrain and brainstem representations of both the sensory and emotional dimensions of pain of different origins.

Brain Representations of Active and Passive Emotional Coping Responses: Human Studies

Although human brain imaging, using functional magnetic resonance imaging (fMRI), has the potential to investigate the brain representations of a wide range of noxious events, both superficial and deep, and acute and chronic, the vast majority of studies have focused on acute noxious cutaneous (thermal) stimuli, because such stimuli are reliable, reproducible, and easily administered within the restricted experimental environment. Data from these investigations have shown that acute cutaneous pain triggers signal changes in a consistent set of neural structures including somatosensory, anterior cingulate, and insular cortices. Such findings revived the idea that pain activates an invariant set of neural structures, the so-called neuromatrix for pain. Despite compelling experimental, clinical, and anecdotal evidence that pain arising from different tissues evokes different sensory, emotional, and motivational qualities, surprisingly few human brain imaging studies that investigate acute pain processing have addressed the possibility that acute deep and superficial pain may be differentially represented.

The limited spatial resolution of human functional brain imaging techniques has not allowed the activation

patterns within small structures such as the PAG to be determined with any reliability; however, the cortical regions defined by their connectivity to the PAG in the animal studies discussed above have been specifically explored in human imaging studies. In particular, during the last few years, fMRI investigations have been used to explore the activation of these forebrain sites during acute superficial and deep noxious stimuli.

Consistent with the clinical observations of Lewis, human subjects subjected to experimentally induced superficial and deep pain in the leg report differences in the spread of pain and use distinct and different sensory descriptors to define the pain. Superficial pain evoked by subcutaneous injections of hypertonic saline evokes: sharp; hot; burning sensations restricted to the region immediately surrounding the injection site. Deep pain evoked by intramuscular injection of hypertonic saline evokes deep throbbing and cramping sensations that spread distally from the injection site to the dorsum of the ankle and foot. Although both superficial and deep noxious stimuli recruit signal changes in structures broadly within the pain neuromatrix, the striking finding is that the regional patterns of change often are different.

The largest difference in regional signal intensity change – a large decrease during deep pain and no change during superficial pain – occurs in the contralateral perigenual anterior cingulate cortex (**Figure 5**). The perigenual cingulate cortex constitutes a component of the affective subdivision of the cingulate cortex and lesions encompassing this region decrease social interactions in monkeys and trigger emotional lability in humans. Given that deep pain often evokes behavioral changes characterized by conservation withdrawal, social disengagement, and negative emotion (e.g., depression), there may be a causal link between the decrease in signal intensity in the perigenual cingulate and the negative emotional feelings commonly triggered by deep pain. Further, imaging studies of emotion have revealed that negative emotional states such as sustained sadness consistently evoke signal intensity changes in the perigenual cingulate region. In patients with chronic negative emotional states, such as unipolar depression, the perigenual cingulate cortex is one of few brain sites that display significant baseline hypoperfusion and hypometabolism.

A second cortical region, the anterior insula cortex, is also differentially activated with deep pain evoking a large signal increase, and in superficial pain no change in signal intensity. Detailed analyses of insula activation patterns during deep and superficial pain have revealed both a somatotopic organisation and a tissue-based segregation of activity. Thus, it appears that within this cortical region, multiple representations of body location and pain type exist. The insular cortex in the rat projects strongly to the vIPAG. If there is any commonality of organization within mammals, then it is possible that the insula may be

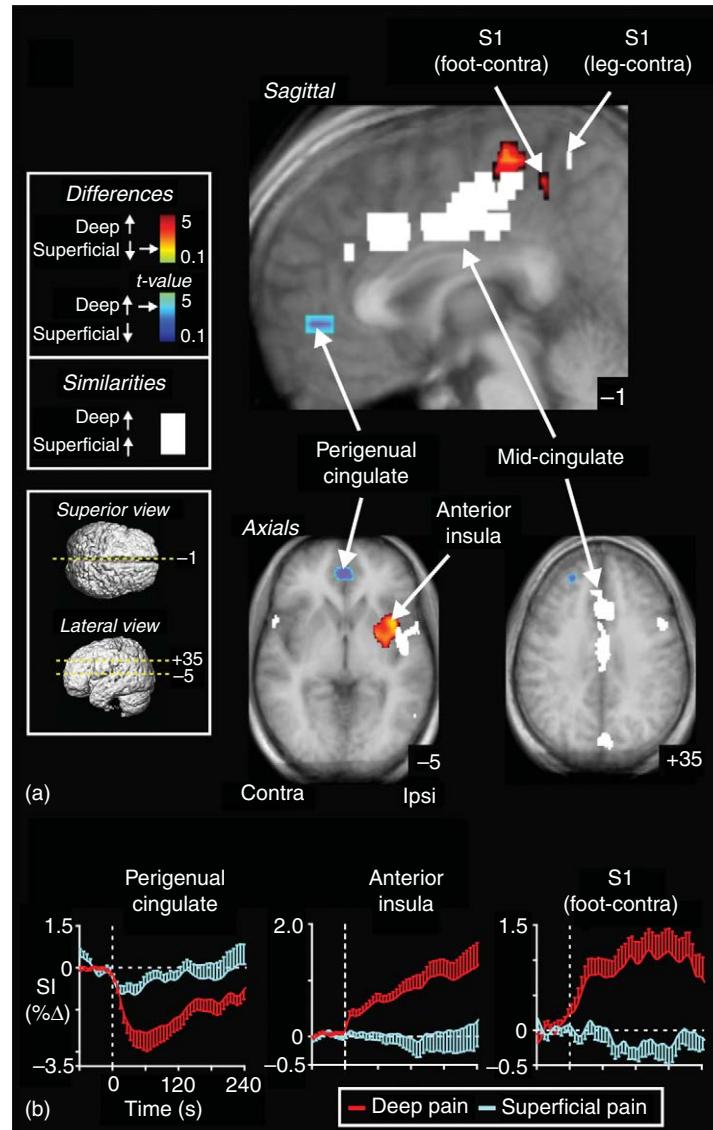


Figure 5 Brain activation patterns during acute cutaneous and muscle pain stimuli. (a) Significant fMRI signal intensity differences and similarities overlaid onto an average anatomical MRI scan. The hot color scale (coded for t-value) indicates regional signal increases during deep pain and either decreases or does not change during superficial pain. The cool color scale indicates regional signal decreases during deep pain and either increases or does not change during superficial pain. The white shading indicates regions in which signal increased during both pain stimuli. Slice positions are indicated in the right inset and by Montreal Neurological Institute coordinates at the bottom right of each image. (b) Percent changes in signal intensity. Modified from Henderson LA, Bandler R, Gandevia SC, and Macefield VG (2006) Distinct forebrain activity patterns during deep versus superficial pain. *Pain* 120: 286–296.

one cortical region from which signals relevant to deep pain and chronic cutaneous pain may arise to modulate the activity of vIPAG neurons. In humans, it has been shown that the anterior insula encodes not only the unpleasantness of painful stimuli, but also the unpleasantness evoked during challenges that trigger either olfactory or visual disgust. Further, different internally generated emotions activate different subregions of the right anterior insula. It is interesting to note that the region of the right anterior insula activated by deep pain is the same as the region activated by internally generated states of

sadness and anger. Thus, the role of the right anterior insula may be to monitor the internal state of the body, replicating the function and organization of the interoceptive cortex of the dorsal posterior insula. A pivotal role for the right insula cortex in the integration of the response and experience of both superficial and deep pain is well supported by brain imaging data. The importance of this role is confirmed further by clinical observations of individuals with lesions encompassing large areas of the insula. In humans, insula damage can result in asymbolia for pain – a condition in which

patients can distinguish between superficial and deep painful stimuli and describe their quality (sharp, dull, etc.) but fail to display appropriate emotional responses, including motor withdrawal, or usual facial signs (i.e., grimacing).

The somatic marker hypothesis suggests that the anterior insula integrates somatic and external cues of emotional relevance. The right anterior insula, thus, may integrate somatotopically organized information from nociceptors with the personal relevance of these emotionally charged stimuli helping to decide on an appropriate course of action. As is the case for the PAG, information regarding the stimulus location would be advantageous in deciding on the appropriate behavioral response to a noxious stimulus. In addition, given that the anterior insula is organized both somatotopically and, according to pain modality, it may also code the different sensory qualities associated with pain originating in different body tissues, that is, activation of the anterior insula may be necessary for both the location and quality of pain to be appreciated. Indeed, in animal studies, signals from noxious stimuli are known to be transmitted from the dorsal horn of the spinal cord to the posterior division of the ventral medial thalamic nucleus (VMpo). Although the existence of the VMpo in humans is still a matter for debate, it is proposed that somatotopically organized noxious information is transmitted from the VMpo to neurons in the ipsilateral anterior insular cortex via a relay in the contralateral posterior insula. The simultaneous activation of the primary somatosensory and insula cortices, by VMpo neurons, could then provide the brain with representations of both stimulus location and the sensory qualities associated with a painful stimulus. A further striking difference in the central representations of superficial and deep pain is their perceived spread. Whereas superficial pain is restricted to the region surrounding the injection site, deep pain almost always radiates distally to the ankle and foot. This pattern of perceived spread is highly correlated to the spread of fMRI signal intensity change within the primary somatosensory and insular cortices. That is, the greater the perceived spread of pain, the greater the extent of primary somatosensory and insular cortex activity, further supporting the notion that the primary somatosensory and insular cortices code the perceived stimulus location.

Evidence from human experimental studies support the notion that the opposing emotional state changes triggered by acute pain arising from different body tissues are underpinned by differential brain-activation patterns. Although pain originating in muscle and skin evoke similar activity changes in a number of brain regions, differential activation patterns are revealed in cortical regions that process perceptual and emotional state changes. As the spatial resolution of brain imaging techniques improves, it will

be possible to explore the activation patterns within brain-stem sites such as the midbrain PAG, to determine if the predictions of the animal studies are borne out in the human brain also.

Endnote

Although our understanding of the central representation of pain has in the past been constrained by the viewpoint of the sensory physiologist, the recognition that pain has both sensory and emotional qualities has triggered new approaches to the study of pain. Understanding that merely describing the anatomical pathways and physiological processing of the sensory dimensions of a noxious stimulus results in an incomplete picture of the neural representations of any pain state has resulted in the incorporation of neural circuits that mediate emotional behaviors into our neural representations of both superficial and deep, and acute and chronic pain states. To divide pain into physical and emotional defies our experience of any pain state: Pain is simultaneously a physical and emotional experience.

See also: Brain Imaging; Depression; Emotions; Motivation; Pain and Addiction; Stress and Emotionality.

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Active Avoidance and Escape Learning

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Glossary

Fear – A hypothetical brain state that results from the activation of brain structures, such as the amygdala, involved in processing aversive or threatening stimuli.

Negative reinforcement – The relationship between an instrumental response and an aversive stimulus arranged in a way that the response decreases the probability of the stimulus delivery.

Obsessive-compulsive disorder – A mental disorder characterized by repetitive thoughts or actions in an effort to reduce the level of fear and anxiety.

Phobia – Excessive fear of certain objects or situations, often expressed as extreme tendency to avoid the fear subject.

Punishment – The relationship between an instrumental response and an aversive stimulus arranged in a way that the response produces the stimulus.

Activation of the fear system initially triggers emotional reactions such as freezing behavior, autonomic nervous system activity, and hormonal release. It also mobilizes brain and body resources and prepares the organism to perform active responses that cope with the danger (i.e., flight). The former is usually referred to as fear, and the latter as escape or avoidance. Fear-elicited reactions prevent threat escalation and fear-motivated actions function to distance organisms from threat. Note that fear responses are adaptive in the sense that they protect individual organisms from life-threatening circumstances and, ultimately, improve evolutionary fitness. However, in the context of human pathology, fear responses can be maladaptive if their occurrence is inappropriate, excessive, or prolonged.

In the present article, we provide an overview of the psychological and neural mechanisms of active avoidance (AA) and escape ‘learning,’ rather than innately programmed responses. Note, however, that the responses themselves are usually not learned, but instead come under the control of previously innocuous stimuli through experience. Note also that although there are many procedural variations on avoidance conditioning, such as unsignaled (Sidman) and passive avoidance, for simplicity we focus on signaled AA. Finally, for our discussions of animal studies the term ‘fear’ refers to internal brain and body states that alter sensory processing and control specific defensive responses, not necessarily the subjective state of conscious fear experienced by the human.

Active avoidance and escape are forms of aversive, or defensive, instrumental learning. In a typical AA experiment, rats are placed in a chamber with two compartments separated by a small door. Training trials consist of presenting the rat with a conditioned stimulus (CS) that signals the upcoming delivery of an aversive event. Indeed, CSs in AA experiments are sometimes referred to as ‘warning signals.’ Brief visual or auditory stimuli usually

Overview of Avoidance and Escape Learning

Stimuli associated with predators or other dangers elicit defensive responses that function to keep organisms safe. Each species has certain stimuli that are innately wired to activate the fear system. Emotionally neutral stimuli – when associated with innate stimuli through experience – acquire the capacity to activate the system. By examining how the brain processes information concerning innate or learned fear stimuli, and how it generates specific defensive responses on the basis of this processing, the defense circuitry is being mapped. In this sense, emotion can be thought of as the process by which the brain computes the value of a stimulus for the purpose of responding adaptively.

serve as the CS, although stimuli of any sensory modality are effective. The aversive event, or unconditioned stimulus (US), is typically a mild electric footshock delivered through the grid floors in the chamber. Note that other aversive events such as very loud noises, hot/bright lights, and cold water can also be effective USs in AA experiments. Early in training, rats lack the knowledge that the CS predicts the US and make few, if any, avoidance responses. This results in CS-US pairings that rapidly create fear of the CS. As training progresses, rats learn that a shuttling response during the CS to the opposite chamber side terminates the CS presentation and prevents the delivery of the aversive US. Thus, AA learning is indicated by decreases in AA-response latencies during CS presentations, as well as increases the number of AA responses and the percentage of USs successfully avoided.

Escape learning is a component of most AA protocols but can also be studied independently. In the context of AA, escape learning typically precedes AA learning. On early trials, rats lack the knowledge that the CS predicts danger and thus fail to shuttle resulting in delivery of the aversive US. In most AA studies, the US persists until the rat emits the shuttling response and ‘escapes’ to the opposite chamber side. As training progresses, escape latencies become shorter and shorter until the rat ultimately begins shuttling before the US and actually avoids its delivery altogether. Although AA responses can be acquired if the escape and AA responses are different, AA learning progresses much more rapidly if they are the same. Note that animals will learn to escape most innately aversive stimuli but can also learn to escape fear-eliciting stimuli that are not inherently aversive.

AA and escape are forms of instrumental learning. In instrumental learning paradigms, responses under the animal’s control determine whether or not a US will be delivered. For instance, in an appetitive experiment a rat can press a lever to get food. Without pressing, no food is delivered. Thus, the rat’s response is ‘instrumental’ in the attainment of food. Similarly, in AA, the shuttling response is instrumental in preventing the delivery of footshock. This is in contrast to Pavlovian conditioning, where delivery of CSs and USs occurs irrespective of whether the rat makes or does not make any particular response.

Before moving on, it is important to point out two practical considerations related to designing successful AA experiments. The first is the choice of the AA response. AA learning is much slower than Pavlovian conditioning and typically requires multiple sessions consisting of many trials. However, some AA responses are learned more rapidly than others. Shuttling, for instance, is learned relatively rapidly, whereas lever pressing is learned extremely slowly. Robert Bolles and others suggest that AA responses compatible with an animal’s innate defensive responses (species-specific defense responses (SSDRs)) are much more likely to be acquired than

responses incompatible with innate defensive responses. Thus, shuttling is more easily acquired because it resembles the ‘flight’ SSDR. Lever pressing is difficult to train because rats rarely make such responses in dangerous situations (probably because they were unsuccessful in preventing danger throughout evolution).

A second factor important for AA experiments relates to ‘feedback stimuli.’ Although animals can learn AA without explicit feedback, training is usually faster if there is a brief stimulus marking the successful completion of the AA response (and prevention of the US). For instance, in an AA experiment where a tone predicts footshock, the experimenter may blink the houselight when the rat successfully shuttles and avoids the footshock. Feedback stimuli are especially useful when subtle response variations define the AA response, or when using unsignaled (Sidman) AA. Note that unintentional feedback stimuli are probably components of all AA protocols (i.e., proprioceptive sensation and CS termination), but salient intentional feedback signals enhance AA acquisition even further.

Psychological Mechanisms and Theoretical Considerations

Soon after Ivan Pavlov published his seminal work on associative conditioning, many researchers attempted to study conditioned fear, and its neural basis, with AA protocols. However, AA proved to be a difficult paradigm for the analysis and explanation of learning mechanisms. First, learning occurs on trials where the animal successfully avoids shock, and it was difficult to explain how the absence of a US could reinforce a response. Second, researchers realized that avoidance was actually a complex learning process where animals first learned that the tone predicted shock, and then learned to escape the tone and prevent shock delivery. Two-factor theory was initially proposed by O. H. Mowrer and N. E. Miller hypothesizing that avoidance conditioning involves both Pavlovian fear conditioning and instrumental response conditioning. In the almost 80 years since the emergence of two-factor theory, researchers have heatedly debated the contents (i.e., S-S, S-R, or R-S), conditions (Pavlovian operations, instrumental operations, or both) and mechanisms (drives, perceptions, etc.) of AA conditioning. However, one idea was proposed early and remains a viable possibility today: Escape from fear (EFF).

EFF learning was proposed to explain how Pavlovian conditioning and instrumental conditioning could interact to mediate AA. The basic idea is that Pavlovian conditioning first establishes fear of the CS. Then on later trials, active responding is reinforced by fear reduction associated with CS-termination. Another way to say this is that the response is instrumental in leading to the

reinforcement: fear reduction. Formally, this sort of learning is called conditioned negative reinforcement of a stimulus–response association. Importantly, expression of EFF/AA learning is also believed to be motivated by fear. As training progresses, escape responding is motivated by fear of the CS and reinforced by CS-termination.

Despite the promise and simplicity of EFF as a mechanism of AA, it has been the subject of considerable controversy over the years. A major reason is that some researchers have had trouble reliably reproducing EFF learning (independent of avoidance conditioning) in the laboratory. Many have reported successful EFF learning, but failures to obtain EFF learning are also common. In addition, some argue that EFF theory predicts that CSs eliciting greater fear should produce stronger instrumental learning, since the hypothetical reinforcement, fear reduction, should be greater when these CSs terminate. However, this relationship between the degree of CS fear and strength of instrumental response acquisition does not appear to be supported by the available data. These and other issues have hindered the widespread acceptance of EFF as an important mechanism of AA learning.

We recently conducted an extensive behavioral examination of EFF, using rearing as the escape response, in order to address reasons for the controversy and to suggest procedural improvements. For EFF training, rats were presented with fear-eliciting CSs in extinction (no footshocks). Rearing up on the hind legs during the CS caused its immediate termination. We found that EFF training led to a twofold increase in CS-evoked rearing relative to yoked control animals (**Figures 1(a)** and **1(b)**). This learning was long-lasting (24 h) and response specific (no increase in other nonreinforced behaviors). Interestingly, successful EFF learning also resulted in a transition from passive Pavlovian freezing reactions to active instrumental escaping; rats that learned the EFF response showed no spontaneous recovery of freezing following the extinguishing CS presentations used for EFF training (**Figure 1(c)** and **1(d)**). Importantly, expression of EFF learning was also motivated by fear of the CS; animals that went through EFF training did not rear differently than yoked controls until the CS was presented. Thus, our data suggest that instrumental escape responses can be reinforced by CS termination and be motivated by fear.

Although EFF learning likely contributes to AA acquisition in a significant way, it is also likely that other psychological factors play important roles. As mentioned earlier, feedback stimuli can play a powerful role in facilitating AA learning. Any stimulus that marks the successful completion of the AA response, and successful avoidance of the US, can serve as a feedback stimulus. Feedback can be an explicit stimulus delivered by the experimental protocol or can simply be proprioceptive or spatial stimuli associated with the response sequence.

Since feedback stimuli are negatively correlated with both the fear-eliciting CS and the painful US, they are likely to become conditioned inhibitors (safety signals) during AA conditioning. Thus, AA responses may also be approach responses to safety, where the AA response is reinforced by conditioned positive reinforcement (presentation of the feedback stimulus).

Others have also argued that the US-avoidance contingency plays an important role in AA acquisition; that is, independent of EFF or approach to safety, nondelivery of the shock US is registered by the animal and serves to reinforce AA responding. Although it is admittedly difficult to disentangle these separate influences on AA learning, it is important to point out that each may play a role in supporting AA learning and expression; the involvement of one of these mechanisms does not negate the involvement of another. However, the jury is still out on the relative importance of these psychological processes.

Brain Mechanisms

The neural circuits mediating AA learning and expression are far from understood. This is not surprising given the controversies over the psychological mechanisms involved, the variations in AA protocols studied and the different experimental tools employed. The following section summarizes the available data and suggests new directions for future research of AA neural mechanisms.

Pavlovian Conditioning

Pavlovian fear conditioning likely plays a fundamental role in AA learning and expression. The neural mechanisms of Pavlovian fear conditioning are covered in detail elsewhere in this encyclopedia, but we give a brief overview here given its importance. The amygdala – a temporal lobe structure comprised of a dozen or so distinct nuclei – is critical for the learning and expression of fear conditioning (**Figure 2**). The lateral amygdala (LA) is believed to receive sensory inputs relaying CS and US information. Prior to conditioning, CS inputs are not strong enough to drive LA activity and downstream regions controlling fear reactions. However, plasticity in LA CS pathways during conditioning strengthens these inputs so that subsequent CS presentations can drive LA activity. Direct and indirect connections then drive central amygdala (CE) activity. CE outputs to the hypothalamus and midbrain – regions mediating fear reactions – then drive defensive responding. LA is believed to be critical for the learning and storage of the Pavlovian CS–US memory, and CE for the generation of fear reactions. Another major output nucleus of LA, the

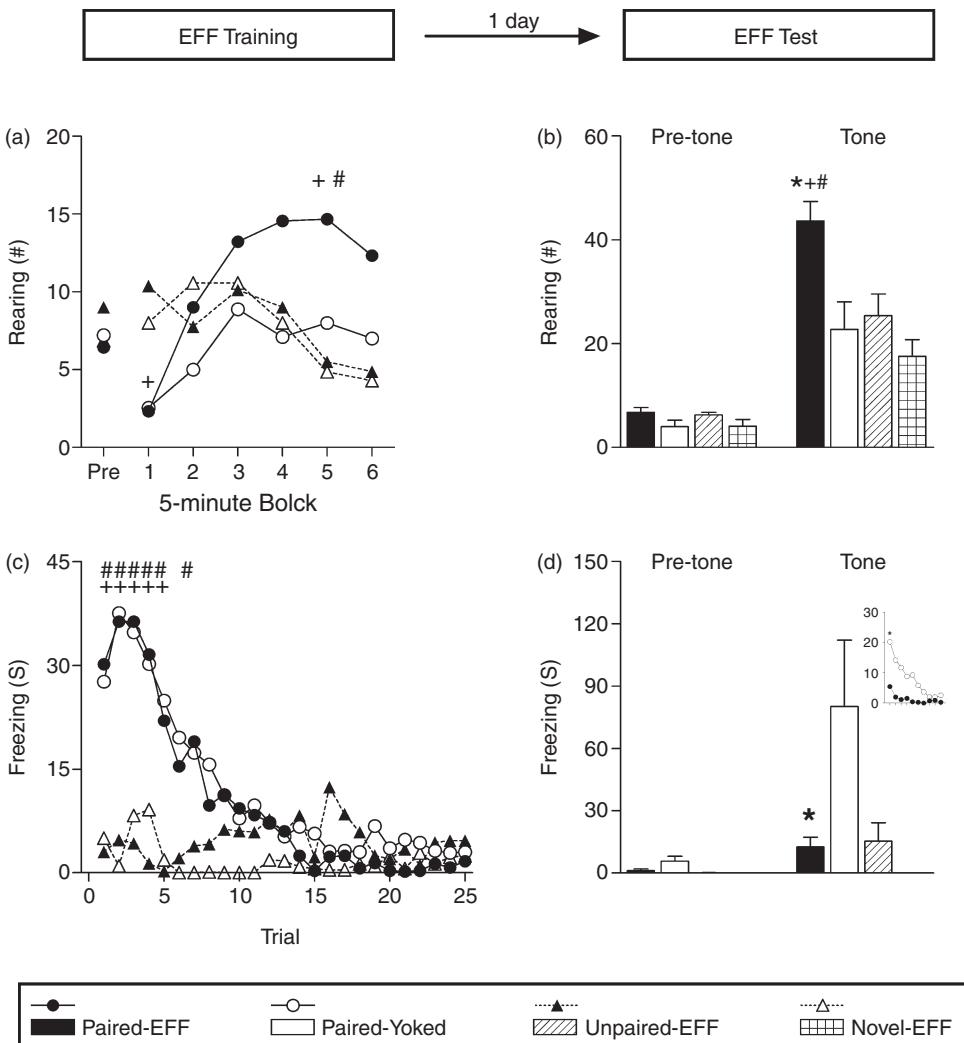


Figure 1 Escape from fear learning represents instrumental learning that is motivated by fear and reinforced by fear reduction. One day after Pavlovian tone–shock pairings, rats were presented with 25 tone-alone presentations in a novel context (EFF training, left). For Paired-EFF rats, rearing during a tone presentation led to its immediate termination (response-reinforcement pairing). Paired-Yoked rats received identical tone presentations independent of their behavior. One day after EFF training, rats were presented with a single, continuous 10-min tone presentation to assess long-term EFF memory (EFF test, right). Rearing and freezing were assessed during both phases. Paired-EFF rats showed a twofold increase in the EFF escape response (rearing) during the training and testing session compared to Paired-Yoked rats (a) and (b); note change in scales between training and testing). Unpaired-EFF rats had no fear of the CS and did not acquire the EFF response (enhanced rearing). Although freezing extinguished in both Paired-EFF and Paired-Yoked groups during EFF training (c), successful acquisition of the active escape response (Paired-EFF only) was associated with less spontaneous fear recovery (d). The inset in (d) shows minute-by-minute freezing during the final EFF tone test for Paired-EFF and Paired-Yoked groups. Further analysis demonstrated that EFF learning was response-specific and performance was motivated by fear (no difference in rearing in the absence of the CS; data not shown). Adapted from Cain CK and LeDoux JE (2007) Escape from fear: A detailed behavioral analysis of two atypical responses reinforced by CS-termination. *Journal of Experimental Psychology: Animal Behavior Processes* 33(4): 451–463.

basal amygdala (B), is not required for Pavlovian fear conditioning or expression.

Signaled Active Avoidance

Investigations into the brain mechanisms of signaled AA peaked midway through the last century and then tapered off. This may be partly due to the emergence of Pavlovian conditioning as an ideal model for relating behavioral

learning to brain activity and partly to the theoretical controversies with regard to instrumental conditioning. Many of these studies focused on the amygdala as a region important for AA. The techniques most commonly employed to investigate amygdalar involvement in avoidance were lesions, local drug infusions, and sub-seizure electrical stimulation. However, it is important to note that many, if not most, of these studies were conducted before researchers appreciated the importance of

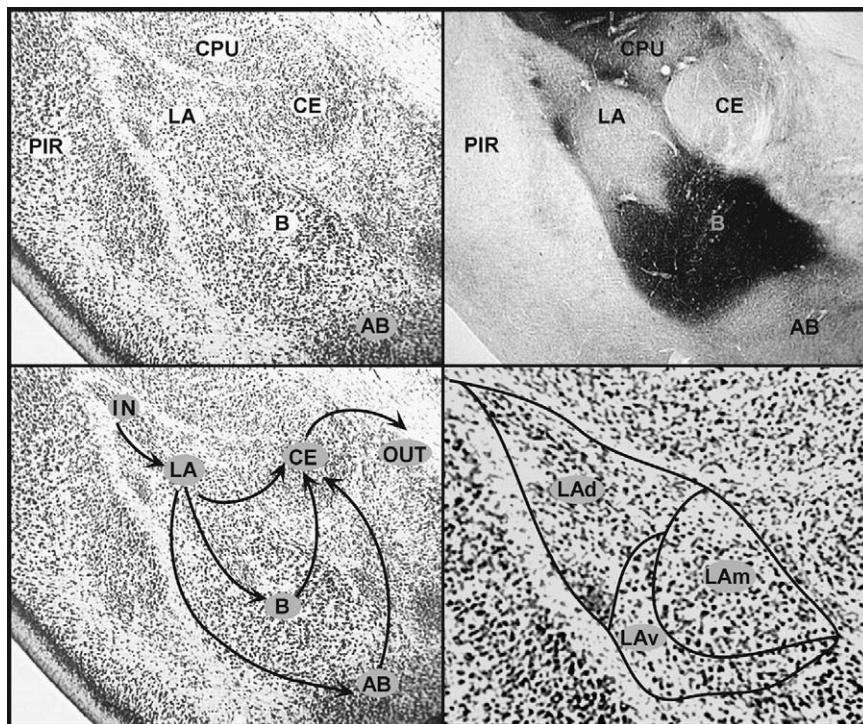


Figure 2 At least 12 nuclei comprise the mammalian amygdala. Particularly important for fear conditioning are the lateral nucleus (LA), the basal nucleus (B), and the central nucleus (CE). Top panels show coronal slices taken from rat brain (left: Nissl's stain, right: Acetylcholinesterase stain) including the LA, B, and CE. The basic amygdalar circuit mediating auditory fear conditioning is shown in the lower-left panel. Sensory inputs enter through the LA, and the LA connects to CE both directly and indirectly by way of the B. The CE is a major output nucleus mediating expression of Pavlovian conditioned reactions. Direct connections from the LA to the B appear to be critical for active avoidance and escape from fear learning. Bottom-right: higher magnification image of the LA demonstrating that amygdala nuclei can also be divided into functional subregions called subnuclei. Abbreviations: Pir = piriform cortex, AB = accessory basal nucleus, CPU = caudate putamen, LAd = dorsal subdivision of the LA, LAm = medial subdivision of the LA, LAv = ventral subdivision of the LA. Reprinted from LeDoux JE (2000) Emotion circuits in the brain. *Annual Reviews of Neuroscience* 23: 155–184.

investigating the function of amygdalar subnuclei separately, and thus the results are mixed and bit difficult to interpret. In addition, the majority of studies involved prelearning manipulations that can potentially affect acquisition, consolidation, and storage functions.

The most common finding from early studies was that impairing amygdalar function disrupts the acquisition and recall of AA. For instance, pretraining lesions that included the entire amygdala, or the basolateral complex alone, resulted in slower rates of within-session acquisition that persisted across days. However, a number of studies employing complete or partial amygdalar lesions resulted in no impairment of AA, or rather even facilitated AA conditioning.

Michael Gabriel and colleagues have identified a number of brain regions and neural mechanisms important for signaled AA. In their preparation, rabbits are trained to discriminate between a CS+ and CS- in an AA paradigm that requires the animals to walk on a rotating wheel to avoid shock delivery during CS+ presentations. Using an elegant combination of lesion, pharmacology, and multisite single-unit recording techniques, this group has implicated

the amygdala (LA, B, and CE), cingulate cortex (anterior and posterior), thalamus (medial geniculate and anterior nuclei), and auditory cortex in the acquisition and storage of signaled AA. The amygdala appears to be particularly important in this task and is required for behavioral learning and the development of training-induced neuronal activity changes in all of the other regions. These authors also demonstrate that discriminative changes in neuronal firing in the LA develop with the initial AA training session, consistent with previous reports in Pavlovian conditioning. The work from this laboratory emphasizes the interactivity of multiple brain regions in coordinating the learning and performance of complex AA.

Recent work in our own laboratory has focused on the dissecting the contributions of specific amygdala subnuclei to signaled AA learning (Figure 3). Rats were trained in a two-way AA paradigm where the animal was required to perform a shuttle response to the opposite side of the chamber in order to avoid shock delivery at the end of a tone CS presentation. Posttraining lesions of LA or B severely impaired performance of this task. CE lesions had no effect on performance in animals that

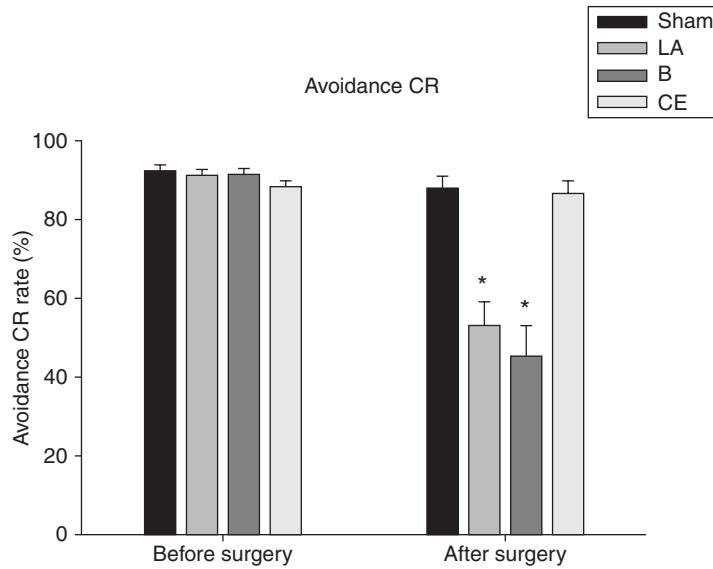


Figure 3 Posttraining lesions of the lateral or basal amygdala impair expression of signaled active avoidance. Rats were trained daily to criterion (>80% successful avoidance) on a two-way active avoidance task where tone presentations signaled footshock. After this moderate training protocol, rats received sham or bilateral electrolytic lesions of the lateral (LA), basal (B), or central (CE) amygdala. After recovery, all rats were given a single test session where the AA-conditioned response (CR) rate was assessed. Lesions of the LA or B severely impaired AA performance whereas CE lesions had no effect. Reprinted from *Learning and Memory* (Choi et al., (2010) The role of amygdala nuclei in the expression of auditory signaled two-way active avoidance in rats. *Learning and Memory* 17: 139–147).

showed high levels of initial AA conditioning. Interestingly, CE lesions rescued the ability to learn AA in poorly avoiding rats. Unoperated rats that failed to acquire the AA response with three training sessions (<20% avoidance) were subsequently given sham or CE lesions. Following recovery from surgery, poor learners in the sham group remained unable to acquire AA with five additional training sessions. CE-lesioned rats, on the other hand, were now able to acquire the response reaching an asymptote of 70% successful avoidance by the fifth post-lesion session (data not shown). These data are consistent with the notion that LA is important for the acquisition of CS-US associations important for both Pavlovian fear reactions and instrumental fear-action learning. B may be important for using this conditioned incentive information to guide goal-directed action learning and CE for mediating Pavlovian reactions. Given that the primary rodent fear reaction to a conditioned CS is freezing, it seems likely that some reciprocal inhibitory mechanism between B and CE outputs exists that determines the likelihood of reaction versus action responding, although it is currently unknown how this might occur. The present findings may also help explain apparent inconsistencies present in early studies of signaled-avoidance brain mechanisms, where some manipulations impaired avoidance and others facilitated avoidance. These contradictory effects may be due to the degree the manipulations affected LA/B versus CE.

Escape from Fear

Research on EFF learning has been sporadic over the last half-century and only one study to date has investigated brain mechanisms of EFF learning. Amorapanth et al., in 2000, created selective electrolytic lesions of amygdaloid subnuclei to investigate their potential involvement in EFF learning (Figure 4). Lesions of the LA, CE, or B were made prior to fear conditioning and EFF training, and both conditioned freezing and escape responding were measured. In this case, chamber crossing served as the escape response. LA lesions disrupted both conditioned freezing and EFF learning. CE lesions disrupted freezing, but not escape responding. In addition, B lesions disrupted escape responding but not conditioned freezing. Thus, consistent with the model proposed above, LA damage prevented the acquisition of CS-elicited fear, which is necessary both for fear reactions (freezing) and fear actions (escaping). The double dissociation between the CE and the B on freezing and EFF suggests that the CE mediates fear reactions and the B participates in the motivation/reinforcement of fear actions like instrumental escape learning. Interestingly, Simon Killcross and colleagues found a similar profile with conditioned positive reinforcement; lesions of LA + B, but not the CE, impaired conditioned punishment supporting the notion that the LA→B pathway may be critical for learning about aversive instrumental actions.

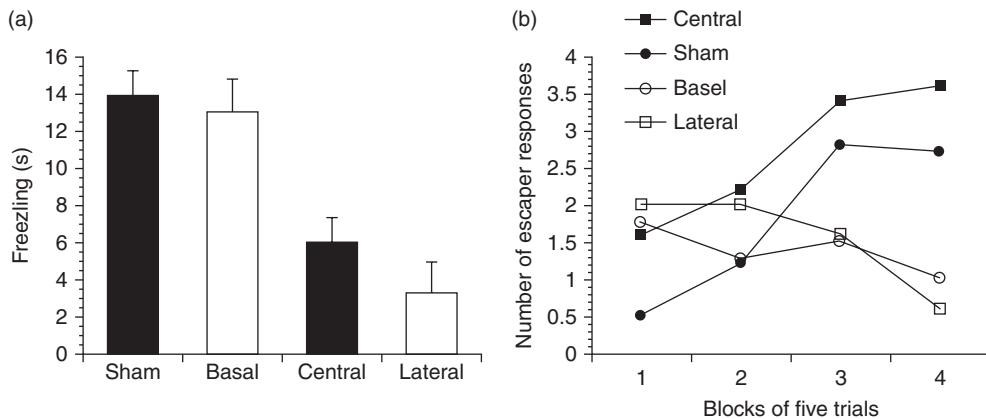


Figure 4 Escape from fear learning depends on the lateral and basal, but not the central, amygdala. Prior to behavioral training, rats received bilateral electrolytic lesions of the LA, CE, or B. Rats were first subjected to Pavlovian fear conditioning and then EFF training using chamber crossing as the escape response. LA and CE lesions disrupted performance of a passive fear reaction to the CS ((a) freezing). LA and B lesions disrupted performance of an active EFF response ((b) chamber crossing). These data suggest that the LA is necessary for establishing the CS as a conditioned incentive. This information is then relayed to the CE to initiate passive Pavlovian reactions and to the B for active escape responding. Reproduced from Amorapanth P, LeDoux JE, and Nader K (2000) Different lateral amygdala outputs mediate reactions and actions elicited by a fear-arousing stimulus. *Nature Neuroscience* 3: 74–79.

Approach to Safety

The learning of safety (conditioned inhibition (CI)), and subsequent learning of approach to safe feedback signals, likely contributes to AA acquisition and expression. The brain mechanisms of safety learning and approach are only beginning to be understood. An elegant recent study by M. T. Rogan and E. R. Kandel implicated both the LA and overlying caudal caudate putamen (CP) in safety learning. Physiological responses to a CI stimulus were recorded during safety training in these two regions and were found to decrease in the LA and increase in the CP. Since the LA is necessary for fear learning and expression, decreases in neuronal responses are consistent with fear reduction/suppression. The authors argue that the caudal region of the CP is involved in reward and euphoria, suggesting that safety signals trigger positive affect. Consistent with this idea, they also demonstrate that mice prefer to spend time in a chamber where the CI is present. Thus, safety signals appear to support approach learning possibly by increasing physiological responses in the caudal CP. This dorsal striatal region has not yet been evaluated in aversive AA but will be an important candidate region for future study.

Other Potentially Important Regions

The LA and B appear to be critical for AA acquisition and expression, but which downstream brain regions are potentially responsible for receiving information from the B and translating it into instrumental escape/avoidance responding? One possibility is the nucleus accumbens (NAcc) which contributes to appetitive motivated action, including action learning about conditioned

reinforcers. Anatomically, NAcc shares direct connections with the B and regions important for motor control. Thus, NAcc is positioned to receive information about aversive CSs and translate this into motivated action. NAcc activity has been shown to correlate with AA learning and NAcc manipulations affect unsignaled AA; however, the role of the NAcc in signaled AA has not been systematically explored.

Note that, earlier we described how animals initially react to AA CSs with Pavlovian fear reactions mediated by the CE. But as training progresses, CE-mediated responses appear to be suppressed allowing for information flow from the LA to the B and AA learning. Two candidate regions may be involved in this suppression of Pavlovian reactions and switch from passive to active coping strategies, although neither have been thoroughly investigated. The medial prefrontal cortex (mPFC) has been shown to be critical for the suppression of Pavlovian fear reactions in studies of extinction. Thus, the mPFC may play a role in suppressing Pavlovian reactions allowing for AA learning and expression. The orbitofrontal cortex (OFC) has been implicated in behaviors involving integration of incentive value with instrumental action, and in flexibly changing behavior as contingencies change. Thus, the OFC may play a role in the switch from passive Pavlovian to active instrumental responding in AA.

Finally, a large body of work in appetitive instrumental conditioning indicates that with continued training, instrumental responses progress from goal-directed (or outcome-dependent) to habitual responses. Thus, changing the value of the US alters responding after moderate training, but not after overtraining.

Interestingly, the neural circuit mediating instrumental responding changes as well. Bernard Balleine's group has shown that lesions of the dorsomedial striatum impair instrumental performance after moderate training, but not overtraining. Conversely, lesions of the dorsolateral striatum impair instrumental performance after overtraining but not moderate training.

An interesting parallel may be seen with aversive AA conditioning. Although changing the value of the US is difficult in aversive studies, the neural circuit important for AA expression does appear to change with overtraining. After moderate training, lesions of the LA or B severely impair AA performance. However, with overtraining, lesion or inactivations of the LA and B have no effect on AA performance. This begs the question of which brain region(s) mediate AA performance after overtraining? It will be interesting to see if the dorsomedial striatum is initially important, but then gives way to the dorsolateral striatum with overtraining. Future studies will be needed to investigate this possibility.

Summary of Brain Mechanisms

The brain mechanisms of AA learning and expression are poorly understood. However, guided by progress in Pavlovian fear-conditioning studies and appetitive instrumental studies, the complex neural circuit is beginning to be unraveled. Our current model is as follows (see **Figure 5**): Early in training, CS-US pairings produce Pavlovian fear conditioning via plasticity in the LA. This plasticity initially drives fear reactions via connections to the CE and downstream effector regions. As training progresses, the CE is suppressed, possibly by the mPFC or OFC, and information flow is diverted from the LA→CE pathway to the LA→B pathway. Initial conditioned negative reinforcement and conditioned motivation important for AA learning and expression may then depend on interactions between the B and striatum (NAcc and/or dorsomedial striatum). At this stage, safety-signal approach mediated partly by the caudal CP may contribute to instrumental response reinforcement. However, with overtraining, these regions become unnecessary and the dorsolateral striatum may then mediate a more reflexive S-R-avoidance habit.

Relation to Human Psychopathology and Treatment

National Institutes of Mental Health resources indicate that approximately 40 million American adults, or ~18% of the adult population in any given year, suffer from anxiety disorders such as panic disorder, obsessive-compulsive disorder, posttraumatic stress disorder, or phobias. A hallmark of most anxiety disorders is

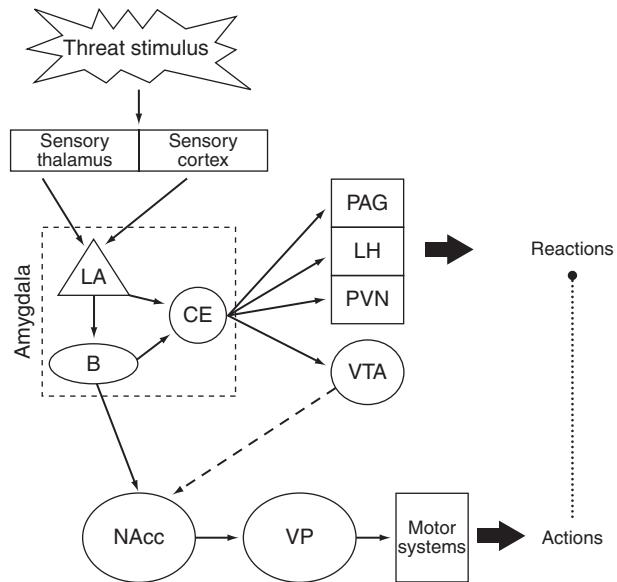


Figure 5 Schematic representation of a hypothetical brain motive circuit mediating active avoidance/EFF learning. Following Pavlovian fear conditioning (CS-US pairings), threatening stimuli – processed in the sensory thalamus and cortex – drive activity in the LA and CE leading to passive fear reactions (like freezing) and also activate arousal centers like the ventral tegmental area (VTA). Incentive information flows also from the LA to the B which projects to the nucleus accumbens (NAcc). The NAcc processing of incentive information invigorates and guides active behavior via projections to the ventral pallidum (VP) and downstream motor systems, with the aid of dopamine arriving from the VTA (dashed line). Note that early portions of this model are derived from work on amygdala-dependent fear conditioning and EFF learning while downstream portions of the model are borrowed from work in appetitive conditioning. Once avoidance or EFF is well-learned, there is a hypothetical inhibition of passive fear actions (dotted line). Additional abbreviations: PAG = periaqueductal gray, LH = lateral hypothalamus, PVN = paraventricular nucleus, LA = lateral amygdala, B = basal amygdala, CE = central amygdala.

pathological avoidance of fear-eliciting stimuli established through trauma. Avoidance is pathological when it interferes with normal day-to-day activities and causes personal distress. Although avoidance responses can take time to learn, once established, they are extremely difficult to extinguish or eliminate. Once the pattern of avoidance is established, the subject rarely withdraws the response to test whether or not the warning signal (CS) still accurately predicts danger. On the contrary, each fear-elicited avoidance response is reinforced when no aversive outcome is encountered. Treating pathological anxiety requires that new behavioral and/or pharmacological strategies be developed to break this vicious cycle. Elucidating the core neural circuits underlying AA behavior will be an essential first step.

One must keep in mind, however, that escape from fear (EFF) and avoidance are common precisely because they are such an effective means of coping with the fear. Prior

to learning an EFF or avoidance response, aversive stimuli trigger distressful physiological reactions and emotions. EFF and avoidance acquisition give the subject control over exposure to the fearful CS resulting in suppression of these reactions. Indeed, animals, including humans exhibiting avoidance responses often show now overt signs of fear other than the avoidance response itself. In addition, unlike passive treatments based on fear extinction (e.g., exposure therapy), return of overt fear is much less likely following avoidance learning. The recognition of this fact has led researchers and clinicians such as B. A. van der Kolk, J. E. LeDoux, and J. M. Gorman to suggest that active coping strategies should be pursued more regularly during treatment of anxiety. The key here is that the active (avoidance) response strategy not interfere with normal living and well-being. Thus, future research into the neural mechanisms of avoidance holds out the hope of not only breaking the vicious cycle of pathological avoidance, but also of improving treatment by harnessing the power of avoidance learning.

See also: Cognition: Learning and Memory: Pavlovian; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Fear, Anxiety, and Defensive Behaviors in Animals; Fear Conditioning; Motivation.

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Behavioral Pathologies in Nonhuman Primates

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Glossary

Affiliative behavior – Acts which promote friendly or peaceful interaction with another individual (e.g., grooming and huddling).

Agonistic behavior – Any act involving aggression (normally producing injury or flight by the target individual, e.g., threat and attack) or fearful behavior (normally reducing injury or aggression, e.g., submissive expression or posture, or flight).

Stereotypy – Behaviors performed excessively repeatedly and without obvious purpose, either involving the whole body (e.g., pacing or somersaulting) or individual body parts (e.g., mouthing or manipulating).

Introduction

Laboratory- and zoo-housed nonhuman primates (hereafter, ‘primates’) commonly display abnormal behaviors. The present review will not deal with unusual behaviors that are species-typical responses to variations in the animals’ habitual environment, such as drastic food shortages or inclement weather, or to changes in physical health, (e.g., disease or injury). Nor will behavioral pathologies induced by invasive treatments (e.g., involving drugs, pain, brain stimulation, or lesions) be addressed. Instead, the focus will be on aberrant behaviors that result from rearing or living in environments that provide the animal with insufficient stimulation. In one survey of over 360 rhesus macaques housed in individual cages in a North American primate research center, almost 90% were found to show at least one aberrant behavior, with individuals typically demonstrating more than one kind. Aberrant primate behaviors may include repetitive whole-body movements such as pacing, swaying, or somersaulting, or smaller-scale acts such as mouthing or manipulation, which may be externally directed (e.g., stereotyped cage manipulation) or self-directed (digit-sucking or hair-pulling). Such stereotypies and other abnormal behaviors may be physically harmless, but they are certainly esthetically undesirable, and, in extreme cases, they can lead to serious health issues (see below). They are almost always a sign of psychological maladjustment.

The Isolation Syndrome

Abnormal and Self-Directed Behaviors

Reports of abnormal behavior can be found in the early primatological literature; a case of self-directed aggression (biting of own hands and feet) by a young adult male rhesus macaque on dismounting a female following an incomplete copulation was described over 80 years ago. However, it was not until publication of studies by Harry Harlow and his colleagues in the late 1950s and the 1960s that primate behavioral abnormalities started to receive widespread attention. Neonatal rhesus monkeys were separated from their mothers and reared in individual cages that permitted only nontactile contact with neighbors (partial isolation), or in enclosed cages that offered no social experience of any kind (total isolation). Isolation-reared infants soon started to show a range of behaviors that are almost never seen in young monkeys reared in more natural social settings. The so-called ‘isolates’ directed toward their own bodies behaviors that would typically be directed toward other individuals (under normal circumstances for neonates this would primarily be the mother). The young isolates spent considerable amounts of time sucking their own digits or fur (‘auto-orality’) and hugging themselves (‘self-clasping’). They might also perform stereotypical movements, including body rocking. The predominance of self-directed acts is interpreted as the isolation-reared infant redirecting onto itself behaviors, probably innately programmed, that would normally function to maintain contact with the biological mother, including grasping (the mother’s fur) and sucking (mother’s nipple). Stereotyped body movements can also be viewed from this perspective as an attempt to replace the vestibular stimulation that would normally arise from being transported by the mother. Correspondingly, long periods of immobility accompanied by staring into space and softly caressing one’s own body might be viewed as an ‘imitation’ of the attitude of an individual receiving grooming.

Another aspect of what is generally called the ‘isolation syndrome’ consists of a range of bizarre postures, including the ‘floating limbs’ phenomenon. For example, one or both legs might be extended behind and above the head so that the feet remain suspended in the air, or an arm might start to rise up and appear to float in the air for no apparent reason. Some infants looked at and then attacked their floating limb, and isolates spent more time overall looking at their own body parts compared to socially

reared controls. Again, in the absence of conspecifics to look at, isolates redirected their visual attention onto their own bodies. The lack of sufficiently varied visual stimulation might be one reason for the development of another common abnormality, known as the ‘salute.’ Here, the monkey presses with its hand against its eyelid or pulls on the skin around the eye. This causes a deformation of the visual image experienced by the monkey, which it may find preferable to its normal, monotonous visual environment.

Social Deficits Following Early Social Deprivation

Affiliative and agonistic behavior

A range of other effects of inadequate early social experience were revealed, some of which clearly compromised isolates’ ability to adapt to environmental events and opportunities. Isolation-reared rhesus monkeys showed excessive fearfulness to unfamiliar objects and situations, abnormal information processing, and impaired learning abilities. However, it was especially in the social domain that the inadequacies of isolation-reared monkeys caught the attention of scientists, philosophers, and the lay public. After their period of isolation from birth onward (typically between 6 and 12 months), when placed with other monkeys, isolates were clearly inept in almost all aspects of social life, including affiliative and agonistic interactions, dominance hierarchy formation, alliance formation, and sexual behavior. They played much less than control monkeys that had extensive early social experience, neither soliciting play bouts nor responding appropriately to solicitations by others. They would often get involved in serious aggression with other individuals, both as perpetrators and recipients. Thus, instead of responding to threats from more powerful individuals by showing appeasement or submissive gestures that might prevent the episode escalating into a physical conflict, socially deprived monkeys might counter-threaten, a clearly inappropriate response that would elicit an attack.

Note that isolation-reared monkeys possess all of the facial expressions and vocalizations of their species. However, they appear to lack the ability of normal individuals to cope with potentially volatile situations through appropriate exchanges of signals. The early social environment is clearly important for learning this ability. For isolates, however, the combination of fearfulness, disorganized behavior, aggressiveness, and self-directed tendencies prevented their full integration within a group. Consequently, they were often rejected by socially competent individuals, kept on the periphery of the group – where they might associate to some extent with similarly reared deviants; experiments showed that isolates preferred to approach other isolates over normal individuals.

As already mentioned, one aspect of the impaired social abilities of isolates was their difficulty in forming alliances with other monkeys. In other words, they showed little reciprocal support to others in situations of contest or conflict. Their behavior was often at odds with respect to the dominance hierarchy, resulting in further intra-group hostility. One mechanism that appears to contribute to the higher levels of aggression in early socially deprived macaques is their compromised post-conflict reconciliatory tendencies. Clearly, if an isolate is less likely than another individual to come together with a previous opponent to make peace, then continued aggression appears more likely. There is evidence that reconciliatory tendencies may be learned especially during infancy and the juvenile period, but this requires social partners, which isolates did not have.

Sexual and maternal behavior

Sexual behavior was found to be seriously affected by early social deprivation. Excessive masturbation in the home-cage was recorded (so-called ‘auto-eroticism’), and attempts at copulation when isolates were eventually placed with other individuals typically failed. Infrequent reproduction by isolates was due to inappropriate attempts at copulation, rather than a lack of motivation. Males appeared generally more vulnerable to the disabling effects of early social deprivation. In the sexual domain, their attempts at mounting females were often disorganized, mistimed, and poorly oriented. Females also showed sexual inadequacies, such as failing to maintain the posture of lordosis required to accept the male’s copulatory mount, or a female might take fright and run away just before intromission was achieved.

Although the sexual behavior of males was more severely impaired than that of females, if the latter did eventually become pregnant (sometimes through artificial means) then another exclusively female aspect of reproductive behavior was found to be grossly inadequate: maternal behavior. Although normal primiparous macaques may be less accomplished than older and more experienced females at caring for their newborn, first-time mothers are generally able to clean, support, feed, and protect their newborn. However, this was not the case for females raised in isolation (‘motherless mothers’). The inadequacies of motherless mothers were expressed in a variety of ways, ranging from indifference (allowing the weakened offspring to fall from her body, and failing to retrieve it), to preventing access to the nipple (pushing the hungry baby away), to serious abuse (biting, or even killing the infant). In some cases, the only way to ensure the newborn’s survival was to remove it from the abusive mother and rear it by hand. Although this was effective, if hand-rearing again meant early and prolonged social deprivation, then the infant could be expected to develop abnormal behaviors. In spite of receiving continuous

maltreatment, however, some infants were able to survive being reared by an abusive, motherless mother. For those female infants that went on to have their own baby, an abusive style of mothering was also noted.

Early deprivation and the brain

Prolonged lack of appropriate social experiences (isolates were typically reared alone for 6–12 months) resulted in the emergence of behavioral pathologies that may always remain in the repertoire. Clearly, aberrant behaviors resulting from an inadequate rearing environment are the expression of central nervous system changes. Studies have examined the neural consequences of isolation rearing in monkeys using a range of techniques including electrophysiology (EEG), neuroanatomy, and neurochemistry. Several studies have reported reductions in stereotyped motor acts following treatment with drugs, such as fluoxetine, a serotonin reuptake inhibitor. There have even been attempts to improve the behavior of isolation-reared monkeys through electrical brain stimulation. However, the literature on the brain mechanisms underlying the isolation syndrome in primates is tiny compared to that on the effects of early environmental and social deprivation on the brain and behavior of rodents. It is sufficient to note that the developing brain is highly sensitive to environmental effects, and that arrested or corrupted cellular development due to early deprivation is likely to have lasting effects at the psychological and behavioral levels.

Self-Aggression and Self-Injury

With increasing locomotory competence and independence from the mother, normal infant monkeys soon start to play with each other, grappling and play-biting. In the absence of social partners, isolation-housed infants will start to play with their own bodies, rolling around and pulling and gently biting their own limbs while displaying the ‘play-face’ facial expression. At around the same time, the first occurrences of another early social-deprivation-induced behavioral pathology may be seen: self-aggression. Self-aggression progressively becomes more prominent in the repertoire. In some cases, self-aggression may result in self-mutilation serious enough to require acute veterinary intervention; self-castration has even been described in a self-aggressive macaque. However, in most cases habitual self-aggressors appear to control the intensity of their actions, so that either only superficial or no tissue damage results.

It is useful to distinguish self-aggression from self-injury that might arise as a consequence of repeated stereotyped motor acts such as cage-biting, or habitually gnawing one’s fingers or tail. What typifies self-aggression is the expression of truly aggressive motor patterns,

including postures, facial expressions, and, sometimes, vocal threats directed toward the individual’s own body. However, some authors do not make the distinction between general ‘self-biting’ and self-aggression; in any case, the greater the degree of early deprivation, the greater the incidence of these pathological behaviors. One study of singly housed, male, pigtailed macaques found that the amount of abnormal behavior shown in adults was related to the proportion of time they had been housed alone during the first 4 years of life. Although it is usually a sign of early inadequate social experience, self-aggression – like some other abnormal behaviors – may also occur in normal individuals as a response to extreme frustration or stress. One study found an increased occurrence of abnormal behaviors in socially housed female baboons after the birth of their infants. This was related to psychological stress, as measured by the incidence of self-directed scratching.

Self-aggression may be expressed idiosyncratically, but a typical bout in a habitual self-aggressor might start by the individual slowly reaching up and tugging at the hair on the top of its head. The ‘offending’ hand might then be brought down in front of the face and threatened, with the threat face appearing identical to one that would normally be directed toward a real victim (although no confirmatory analysis of facial action patterns has yet been done). The bout might end at that point, or the tug-threat sequence might be repeated, and/or the monkey might start to bite its hand. In some cases, when the foot is the target, then biting might be interspersed with the monkey kicking its own face repeatedly, in a sequence somewhat similar to repeatedly scratching the head with the foot, though more violent. Self-fighting monkeys might literally throw themselves around their cage or enclosure in a fit of rage (see **Figure 1**).

Once self-aggression becomes established in the repertoire, it may become the preferred, if not the exclusive, form of aggression, even for socially housed monkeys. This is probably because the self-aggressor learns that it is safer to target itself than to get into real fights; although self-mutilation may occur, in most cases, the intensity of self-biting is controlled, so that any injury sustained is minor. Experimental studies have shown that, much like normal aggression, self-aggression may be increased by factors such as alcohol ingestion and frustration. Individuals who injure themselves due to self-aggression clearly respond to frustrating situations differently to nonself-injurious counterparts, for example, showing extinction deficits following the acquisition of a learned response (lever-pressing) for continuous reinforcement. Self-aggression-induced injury is more often reported in males than in females, probably due to a combination of the formers’ greater strength and canine development.

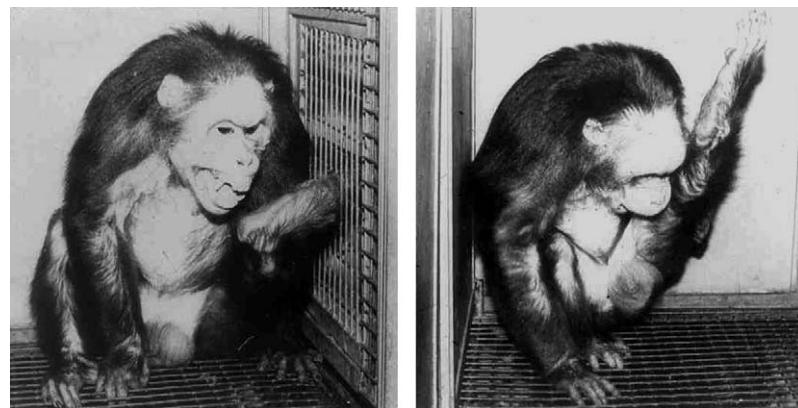


Figure 1 Self-aggression in an adult male stumptail macaque, showing self-directed threat (left), and self-biting (right). Photos by J. R. Anderson.

Some studies have looked at the underlying pathophysiology of self-aggression in monkeys. For example, a hypothesized possible link between propensity for self-injurious behavior and abnormalities in serotonergic function has been studied, although the results are not clear-cut. A reduced cortisol response to mild stressors indicates compromised hypothalamic–pituitary–adrenal axis function, in association with disturbances in central and peripheral opioid systems. Studies of self-aggressive macaques have found that self-injury and other abnormal acts were reduced by cyproterone acetate, which among other effects may decrease testosterone. The serotonin precursor, tryptophan, also reportedly decreased the incidence of self-wounding. Another study, using macaques and a baboon, reported positive effects of guanfacine, an α_{2A} -adrenergic receptor agonist; it was suggested that the treatment might have strengthened inhibitory processes in the prefrontal cortex.

Preventing Abnormal Behaviors in Socially Deprived Monkeys

The pathological behavioral consequences of early prolonged isolation have been shown to be preventable by early intervention, based around providing alternative social experiences. For example, as a result of daily sessions with younger, so-called ‘therapist’ infant, isolation-reared rhesus monkeys gradually showed a recovery in social abilities. Another alternative rearing system consisted of pairing infants with same-aged partners, in the absence of any adults. These so-called ‘together-together’ monkeys grew up to be well adjusted and relatively free of isolation-syndrome behaviors, although their tendency to cling to each other beyond the age at which mother-reared infants would start to show greater independence resulted in a delay of some behaviors, including play. One

conclusion that can be drawn from these studies is that, in captivity at least, access to the mother is not critical for an infant monkey to develop adequate social capabilities; rather it is adequate physical contact with individuals that both solicit and respond to different kinds of social interactions. Thus, younger ‘therapist’ monkeys, and peer-only groups, were able to promote good social adjustment. On the other hand, inanimate mother surrogates, even those that afforded clinging and provided ‘contact comfort’ to the infants, failed to prevent the development of behavioral abnormalities.

Enrichment of the Physical Environment

As indicated above, in the absence of effective therapeutic interventions early isolation-syndrome behaviors may become permanently established in the repertoire, being expressed even after many years of living as a member of a group. However, their incidence can be lowered by appropriate environmental-enrichment techniques, such as provision of increased foraging activities. There can be dramatic decreases in the incidence of stereotypies and self-aggression when appropriate environmental enrichment is provided.

Research into the effects of early social and environmental deprivation in primates has declined in the past few decades, but there are still plenty of primates displaying abnormal behaviors. It is not uncommon for normal (i.e., socially reared) laboratory-housed primates, especially those housed in single cages, to show a range of abnormal acts such as stereotyped pacing or somersaulting, manipulation or biting of cage mesh or other structures, and excessive self-grooming. They may also manipulate, smear, and even eat their feces; coprophagy (and regurgitation) is particularly reported for captive great apes, even some that have been socially reared and that live in groups.

The kinds of behaviors seen in these circumstances tend to be different from those of the isolation syndrome; for example, floating limbs and bizarre postures are rare, as is self-aggression. Moreover, unlike isolation-syndrome behaviors, the aberrant behaviors of normal, individually housed primates are reversible. Removal from individual cages and being housed instead with compatible social partners leads to a marked reduction in abnormal behaviors, as the primates invest time and energy in their social relationship (which allows social grooming, huddling, exchange of signals, etc.). In other words, if animals can be occupied by something that elicits desirable behaviors, they are less likely to engage in undesirable alternatives. Changing from single housing to compatible social housing is viewed as best practice as a means of reducing the incidence of abnormal behavior in captive primates.

In recent years, abnormal behaviors have been increasingly used as a measure of the psychological well-being of captive primates (and other animals) in laboratories and zoos. For example, in one study of Indian zoos, only macaques housed individually in barren cages were found to show behavioral abnormalities (especially stereotypic pacing, but including floating limb and self-aggression as well). In addition, macaques housed in cages or enclosures with a hard substrate (e.g., cement or concrete) were more prone to abnormal behaviors than those in enclosures with a soft substrate (e.g., grass or soil). Studies of several species of primates including marmosets, macaques, baboons, and great apes have shown dramatic reductions in abnormal behaviors following transfer from individual cages to more spacious enclosures, and as mentioned above, housing primates with others is seen as the most effective way of normalizing behaviors.

The studies mentioned above are examples of applied animal behavior science, which addresses problems in husbandry and housing aiming at behavioral enhancement through the provision of more naturalistic and stimulating environments. In some cases, the aim is a behavioral profile that resembles that seen in the wild, and in other cases intervention targets a specific pathology, such as self-aggression, or regurgitation of food. Providing simple toys, sticks, or other manipulable objects can often reduce the incidence of abnormal behavior, as the primates turn their attention toward exploring and manipulating the objects. Similarly, there are many reports of positive effects of environmental enrichment in the form of increasing foraging opportunities. This can involve simply providing a deep woodchip litter on the floor of the enclosure and scattering some small food items to encourage foraging, or presenting specialized devices such as foraging boards and puzzle feeders. Combinations of foraging-based enrichment interventions have been found to decrease behaviors such as coprophagy, feces smearing, and hair pulling in chimpanzees.

Species Differences

The outcomes of interventions aimed at normalizing the behavior of primates vary across studies. At least part of the variation is due to the diversity of species in this taxonomic group. There are around 250 species of primates, ranging from the so-called ‘primitive’ species among the prosimians, to our nearest evolutionary neighbors – the great apes. Given the sheer diversity of behaviors seen across the primate order, it should be expected that species differ in vulnerability to developing behavioral pathologies and in the form these pathologies take. For example, although prosimians may develop stereotyped motor acts, they do not appear to develop the full-blown isolation syndrome described in macaques. Even between closely related species there may be differences: pigtail macaques were found to show a much milder isolation syndrome than rhesus macaques, assessed both in the home-cage and other environments. Rhesus isolates might be more prone to stereotypies, whereas stump-tail macaques might show more self-aggression. These days few, if any, researchers deliberately deprive primates of sensory and social stimulation in order to induce abnormal behaviors. But as long as zoo and laboratory primates continue to exhibit behavioral pathologies, researchers will continue to study them both in order to try to resolve the problem, and also to better understand them.

See also: Animal Models of Learning and Memory; Animal Models of Sexual Function; Animal Tests for Anxiety; Behavioral Development and Socialization; Cognitive Decline in Laboratory Animals: Models, Measures, and Validity; Communication of Emotions in Animals; Depression; Fear, Anxiety, and Defensive Behaviors in Animals; Feeding; Infant Bonding and Attachment; Male Sexual Behavior; Maternal Deprivation; Motivation; Neural and Pharmacological Substrates of Aggression; Neurobiology of Offensive Aggression; Offensive and Defensive Aggression; Parental Behavior; Personality, Temperament, and Behavioral Syndromes; Play Behavior; Primate Origins of Human Behavior; Sexual Motivation; Social Bonding and Attachment; Social Communication; Social Competition and Conflict Resolution.

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Deep Brain Stimulation in Psychiatric Disorders

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Glossary

Basal ganglia – A group of subcortical gray nuclei located in the depth of the cerebral hemispheres, interconnected with the cerebral cortex, thalamus and brainstem, and associated with a variety of functions: motor control, cognition, emotions, and learning.

Limbic system – A set of phylogenetically primitive brain structures located on top of the brainstem and which comprises cortical areas as the cingulate gyrus, the medial orbitofrontal cortex, the olfactory cortex, and the hippocampus as well as subcortical gray nuclei as the amygdala, fornix, hypothalamus, and thalamus. This system is involved in many emotional and motivational aspects of behavior, particularly those that are related to survival.

Obsessive-compulsive disorder – Anxiety disorder characterized by recurrent, unwanted thoughts (obsessions), and/or repetitive behaviors (compulsions). Repetitive behaviors such as handwashing, counting, checking, or cleaning are often performed with the hope of preventing obsessive thoughts or making them go away. Performing these so-called rituals, however, provides only temporary relief and not performing them markedly increases anxiety.

Stereotaxis – Relating to or denoting techniques for surgical treatment or scientific investigation that uses medical imaging to precisely locate in three dimensions an anatomical site to which a surgical instrument or a beam of radiation is directed.

Tourette syndrome – Also called Tourette's syndrome, Tourette's disorder, Gilles de la Tourette syndrome, GTS, or, more commonly, simply Tourette's or TS, it is a neuropsychiatric disorder with onset in childhood, characterized by the presence of multiple physical (motor) tics and at least one vocal (phonic) tic, which characteristically wax and wane.

stimulation (DBS). The technique has been used as a tool for the functional exploration of the brain and as a therapeutic tool, while also being employed in a complementary fashion for lesion surgery. DBS has opened up new opportunities for empirical studies in humans, following up and developing old lines of investigation and initiating new ones.

Neurophysiological investigation by electrical stimulation was massively boosted by the work of the neurosurgeon Wilder Penfield in the early 1930s. With electrical stimulation, every neurosurgical case opened up the opportunity for functional exploration. During neurosurgical interventions for epilepsy, he systematically carried out cortical stimulation in the awake patient with a neurologist continuously noting clinical responses. This method of the experimental human investigation completely transforms the nature and temporality of the observations. Unlike an irreversible lesion, electrical stimulation enables repetitive stimulation of the same human or animal subject. It allows, above all, in the case of neurological disorders, one to observe the clinical signs, record the subjective experience of the patient, and locate the underlying pathological causes within a manageable and reproducible time frame. Penfield therefore succeeded in performing experimental and clinical work within the same individual and fruitfully combining functional neurology, experimentation, and patient therapy in the same operative procedure. He therefore laid the foundations of functional neurosurgery, systematized the methodology, and prepared the ground for DBS which, as a combined therapeutic and research tool, falls into the same category of complementary approaches and techniques (preclinical, clinical, neuropathological, and neurosurgical). It was in this framework of complementary clinical investigation and research, with an ultimate therapeutic goal, that DBS was born. The empirical discovery in the 1960s by several independent research groups that high-frequency electrical stimulation of the thalamus has the same effects on tremor as lesions found its therapeutic application in the 1980s with the invention of DBS by the French neurosurgeon Alim Louis Benabid. He would be the first, as a result of this observation, to propose a chronic electrical stimulation system implanted within brain structures of the patient. Stimulation therefore became chronic and deep and took over from lesion surgery for tremor.

Introduction

Electrical stimulation of the brain has a long history, starting with the classic nineteenth-century animal experiments and then its systematic use in functional neurosurgery by Penfield and now to deep brain

Principles and Uses of Deep Brain Stimulation

DBS consists of stereotactic implantation of electrodes in deep brain structures. The electrodes are connected by a subcutaneous cable to an implantable electric stimulator enabling the application of high-frequency (80–180 Hz) current to the target structure. The effect of the stimulation is reversible and the various stimulation parameters (frequency, pulse width, and voltage) are adjusted in order to obtain the best possible results in the absence of undesirable side effects and low morbidity. Furthermore, bilateral implantation of DBS electrodes is possible, which was up to now impossible with traditional lesioning techniques. For many years now, this surgical intervention has been proposed for several movement disorders, principally Parkinson's disease but also essential tremor with high-frequency stimulation of the ventromedial thalamic nucleus and dystonia with stimulation of the internal globus pallidus showing sometimes spectacular improvement of the disability. Finally, recent studies provide hope that DBS could be an effective treatment for patients suffering from abnormal movements secondary to prolonged treatment with neuroleptics.

Modulation of Associative and Limbic Circuits

The spectacular efficiency of DBS in the amelioration of Parkinsonian motor symptoms is considered to be the result of the modulation of the striato-pallido-subthalamo-thalamo-cortical neuronal loops implicated in motor behavior. Reports of emotional behaviors during subthalamic nucleus (STN) stimulation such as mirth and acute depressive symptoms or symptomatic improvement of such symptoms as depression, anxiety, OCD, and pathological gambling suggest that DBS can also modulate the nonmotor loops within these same pathways. Functional imaging studies, in agreement with these clinical observations, have demonstrated modulation of limbic circuits during DBS. The clearest demonstration of emotional behavior during DBS is seen during stimulation of the substantia nigra pars reticulata (SNr), which triggers extreme manifestations of depression, or mania with functional imaging revealing a concomitant activation of limbic regions. Stimulation of anteromedial STN induces a hypomanic state without any effect on motor activity. Functional imaging data indicate increased regional blood flow concomitant with the hypomanic state in the antero-ventral thalamic nucleus (implied in limbic circuitry) and a decrease in the right prefrontal anteromedial cingulate

gyrus – thereby replicating the map of cerebral activity modifications seen during mood swings. On the basis of our knowledge of STN organization, this observation demonstrates the way in which this nucleus, despite its small size ($10 \times 6 \times 3$ mm), can receive and process a variety of different information. Thus, neural information related to motor control (explaining its efficiency in treating the motor signs of Parkinson's disease), cognition (i.e., the social and intellectual content of our behavior), and the emotional information which necessarily more or less colors all our expressed behavior. The extremely small size of the STN and the minimal separation of the four electrode contacts used in DBS have enabled to demonstrate that this brain region brings together these three aspects of our behavior to generate an output perfectly adapted from the social, affective, and motor points of view (Figure 1). Therefore, when we stimulate the STN, the exact position of the electrode determines which of the cortical–subcortical loops is activated and hence which of the motor, social, or affective components of behavior are modified.

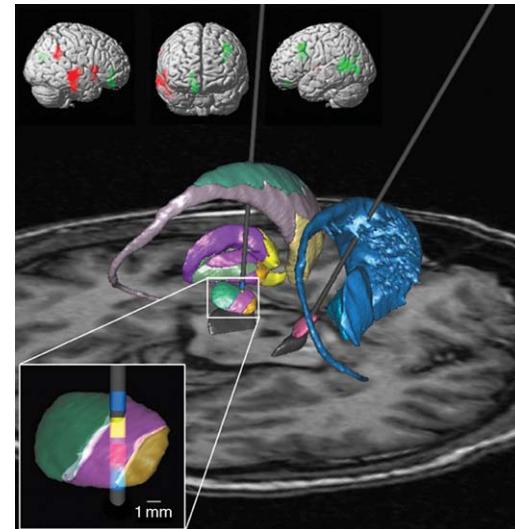


Figure 1 Upper part: Functional imaging of the cerebral regions activated (red) or deactivated (green) during stimulation inducing hypomanic symptoms. Lower part: electrodes implanted in subthalamic nuclei. Motor, associative, and limbic territories of basal ganglia are shown in green, violet, and yellow, respectively. Left inferior corner: subthalamic nucleus with the four contacts of the electrode. Stimulation through the red contact induces hypomanic symptoms. Stimulation through the yellow contact improves motor symptoms as well as the red one, without inducing behavioural changes. Credit: Image Luc Mallet / Jérôme Yelnik/Eric Bardinet (Inserm, CNRS-INRIA, CEA Orsay). From Mallet L, Schupbach M, N'Diaye K, et al. (2007) Stimulation of subterritories of the subthalamic nucleus reveals its role in the integration of the emotional and motor aspects of behavior. *Proceedings of the National Academy of Sciences of the United States of America* 104: 10661–10666.

The Basal Ganglia: The Integration Site for Cognitive, Motor, and Emotional Information

The basal ganglia consist of a series of nuclei found deep in the cerebral hemispheres: the striatum (caudate nucleus and putamen), the pallidum (internal and external), the substantia nigra (reticulata and compacta), and the subthalamic nucleus. Their principal source of information is the cerebral cortex, in other words, a copy of the motor plan constructed by the cortical motor areas but also a copy of all other events which may arise in other nonmotor cortical areas. Two opposing theories have been proposed concerning the organization and analysis of this mass of information. The first postulates the existence of three perfectly segregated parallel circuits within which the information is independently analyzed. The other, based on more recent anatomical data, suggests, on the contrary, that the different kinds of information are integrated within the basal ganglia to produce an output, which carefully balances these different sources of information. The basal ganglia circuitry is in the form of a loop originating over the whole of the cortical mantle and returning to the frontal cortex (Figure 2). It receives,

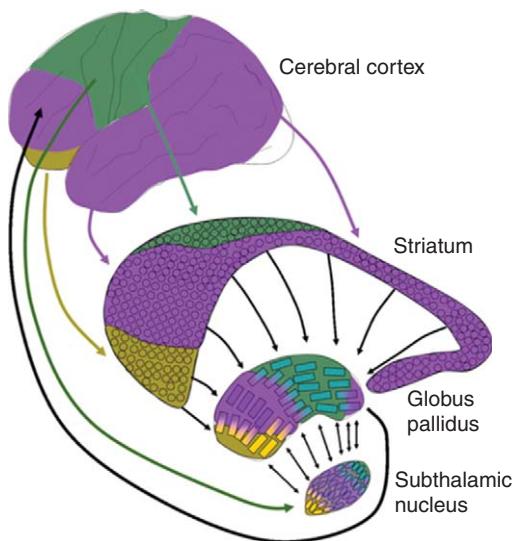


Figure 2 A diagrammatic representation of the basal ganglia organization. The basal ganglia receive information from three functional territories of the cerebral cortex, sensorimotor (green), associative (violet), limbic (yellow), that are transmitted to separate regions of the striatum. Information is received by spiny-striatal neurons, which have small spherical dendritic arborizations (spheres), which preserve functional specificity. In the globus pallidus, neurons are 100 times less numerous and have flattened and large dendritic arborizations (rectangles), which make transmission of striatal information onto pallidal neurons highly converging. In the subthalamic nucleus, information comes not only from the three functional territories of the globus pallidus, but also directly from motor cortices, which confers to this nucleus the role of a nexus in the circuit. From Yelnik J (2008) Modeling the organization of the basal ganglia. *Rev Neurol* 164: 969–976.

therefore, a copy of the motor plan originating in the motor cortical areas as well as the contextual cognitive and emotional information within which the plan is formulated. These sources of information are integrated within the system in order to produce the perfect balance of the three: motor, cognitive, and emotional components. In addition, a circuit exists that is more specifically devoted to the processing of emotional and motivational information and which involves the ventral part of the striatum (the so-called nucleus accumbens) and the ventral globus pallidus. This circuit is likely to be implicated in the pathophysiology of such psychiatric disorders as OCD or the Gilles de la Tourette syndrome. The internal organization of the basal ganglia also enables the learning of effective behavioral sequences arising from the dopaminergic reward signal present in the nigro-striatal pathway, although the reward-prediction error hypothesis can be discussed. The cortical inputs terminate on the heads of the striatal dendritic spines while the dopamine terminals are found at their necks, strategically positioned to enable the selection of the descending cortical information. The striatum is thus in a position to modulate (by excitation or inhibition) the cortical motor plan.

The Basal Ganglia as a Target for the Treatment of Psychiatric Disorders

In the context of therapeutic targeting, the basal ganglia offer the great advantage of a control center both receiving and sending information from and to the whole spectrum of cortical areas functionally subdivided into sensorimotor, oculomotor, associative, and limbic. Dysfunction of the associative loop with its cognitive functions and the limbic loop with its emotional function might therefore be expected to be involved in psychiatric disorders. This notion is in fact supported by neuroimaging data being collected for the development of DBS techniques in relation to both OCD and depression.

Certain advantages of DBS are also linked to the complexity of its action and require that we deepen our understanding of the neural mechanisms at play in target structures. Stimulation has its effect on cerebral function through several distinct but interrelated mechanisms, of which their respective roles are dependent on the stimulation site, the disorder being treated, its neuropathology, and the stimulation parameters employed. The exact mechanism, whether it be excitation or inhibition, and its effects on the chemistry of neurotransmission are all still unclear. Another important factor relates to the spatial features of the electrode/target arrangements and tissue conduction characteristics. Finally, the clinical effects of DBS reflect a complex combination of excitation and inhibition of cell bodies and axons dependent on the orientation of the electrodes. This range of factors

allows one to imagine the restoration of complex information processing such as emotional behavioral disorders by means of DBS and furthermore to extend its application to the field of psychopathology. DBS provides a range of possibilities for adjusting the anatomical specificity of the stimulation. Frequency, intensity, and pulse width are all programmable noninvasively after the implantation procedure in the awake patient. Furthermore, the stimulation can be unipolar or bipolar, redirected to any combination of the four electrode contacts, and can be continuous or intermittent. The patient, within limits determined by the doctor, can also be given the possibility of activating or deactivating the stimulator and even controlling his own stimulation parameters. This wide range of possibilities for parameter adjustment thus provides the opportunity for the optimization of the treatment and controlled therapeutic trials.

As far as the indications for DBS therapy are concerned, this remains reserved for the moment for the traditional cases appropriate for neurosurgery, in other words, disorders resistant to any alternative course of treatment. At the conceptual level, the neuropathological models for psychological disorders (basal ganglia dysfunction) are as yet still insufficiently detailed to propose DBS other than in the structured framework of multidisciplinary clinical research. Thus, even the simple clinical observations of emotional and abnormal behavioral reactions to DBS, carried out for the treatment of motor disorders, require extremely precise anatomical localization in order to establish reliable correlations with the clinical data. Below we have given some examples of the use of DBS in the treatment of psychological disorders: obsessive-compulsive disorder, Tourette syndrome, and depression in cases resistant to all available therapies.

DBS in Obsessive–Compulsive Disorder

OCD is a frequent illness in the general population with a whole life prevalence of between 2% and 3%. It is characterized by a chronic increase in severity and devastating disruption of social, professional, and family life. The classic treatment for OCD is by prescription of antidepressants of the class of selective serotonin uptake inhibitors, either alone or in conjunction with cognitive and behavior therapy. Nevertheless, these therapeutic strategies turn out to be inefficient in 25–30% of cases. Because of this, we can understand the drive to develop various types of psychosurgery based on various neurosurgical techniques, such as bilateral anterior capsulotomy, bilateral anterior cingulotomy, subcaudate tractotomy, and limbic leucotomy. These all had the objective of the destruction of the pathways linking prefrontal cortical areas with the subcortical limbic structures. When applied in the domain of psychiatric

disorders, these techniques were found to be efficient in 50–60% of cases, but they are rarely practiced today because of the irreversibility of the lesions and a number of undesirable cognitive and emotional side effects (euphoria, psychomotor agitation, affective blunting, and lack of motor spontaneity). As we have seen, the development of DBS therapy in the domain of psychological disorders is a new departure and poses the problem of the correct choice of a target whose neuropathology is incompletely understood. Functional imaging data suggest, however, that basal ganglia hyperactivity is affecting both the cingulate and the orbitofrontal cortex in these cases which raises the possibility of stimulating certain key nuclei within these loops.

The application of DBS for the treatment of OCD started in the late 1990s using as target the anterior limb of the internal capsule/nucleus accumbens, aiming initially to reproduce the results of lesioning neurosurgery. Other studies have since explored the effects of stimulation in the region of the ventral striatum with various specific anatomical targets. Certain more recent results point to the efficiency of more ventral and posterior regions than the initial internal capsule target, and would specifically identify the nucleus accumbens. The divergence of results sometimes found in these studies raises two questions: (1) the anatomical question which concerns the exact definition of the target and (2) the methodological question of the limited number of patients included in these studies and the lack of randomized designs; which, given the heterogeneous nature of the disease, would explain the variability of the results obtained.

Recently, STN stimulation (Figure 3), which has been previously shown to improve OCD symptoms in patients



Figure 3 The whole skull with the caudate nuclei and two electrodes implanted in the subthalamic nuclei. Credit: Image Luc Mallet/Jérôme Yelnik/Eric Bardinet (Inserm, CNRS-INRIA).

stimulated for disabling Parkinson's disease, was found to significantly improve OCD and global functioning. Nevertheless, these results necessitate some caveats. Prudence is required in their interpretation, given the behavioral effects induced by the stimulation, which, although reversible, demonstrates the narrow therapeutic margin inherent in the STN target, underlining the considerable functional convergence within this structure. Furthermore, this highlights the need for a high degree of technical expertise and coordination of the medical teams. Finally, regular psychiatric follow-up is indispensable both before and after the operation. The conclusions of this study open up great hope for these severely disabled patients but underline the need for great caution in the use of the technique. It would appear therefore crucial to apply these techniques only within a carefully controlled framework, taking into account the ethical issues and exploring in detail the risk/benefit ratio of potential DBS targets.

Modulation of Limbic Relays in Tourette Syndrome

Tourette syndrome (TS) arises from simultaneous dysfunction of both basal ganglia and limbic areas. The illness is characterized by both motor and vocal tics. The most frequently employed treatment for TS are dopamine antagonists and neuroleptics. These mainly improve motor tics but at the price of numerous side effects, in particular weight gain and tardive dyskinesias and other abnormal movements difficult to treat. Starting in 1960 ablative neurosurgery began to be practiced for severe forms of TS. The thalamus, zona incerta, anterior cingulate, the cerebellar dentate nucleus as well as frontal lobes were all chosen as targets for the treatment of tics and associated pathologies. Although the first case of DBS treatment for this disease was reported 9 years ago, the choice of target for optimal results remains a current issue. Stimulation of the centromedian–parafascicular complex (CM–Pf) of the thalamus, the internal part of the globus pallidus (GPi), and the anterior limb of the internal capsule, have been tested with a positive effect on tics. Bilateral stimulation of the CM–Pf and/or the ventrooralis nucleus of the thalamus has been applied to 18 TS patients with a 65% improvement in tics reported at various postoperative delays through an open follow-up. In line with pathophysiological hypotheses of a dysfunction in the associative-limbic component of the basal ganglia circuitry in TS, the efficacy of modulation of two associative-limbic relays (the CM–Pf of the thalamus and ventromedial part of the GPi) has been evaluated in a double-blind-randomized-cross-over design control study which demonstrated the importance of targeting the limbic networks. The best results were obtained by

stimulation of the anterior GPi, with an improvement of the order of 70% of the symptoms. The long-term follow-up of the patients (7 years of data for the first patient operated upon) demonstrates the maintenance of the effect over time and allowing a return to both professional activity and a normal social life.

The Neurosurgical Approach to Depression

Depression is a frequent, disabling, and recurring illness present in 6% of the general population. Depressive symptoms persist for at least 2 years in 10–20% of cases. Within the patient group showing a chronic worsening of symptoms, 20–30% are resistant to drug therapy and, of these, 50% are resistant to electroconvulsive therapy (ECT). For these patients again, neurosurgery was proposed in the past. Three approaches have been attempted: bilateral anterior cingulotomy, subcaudate tractotomy (destruction of the pathways descending from the orbitofrontal cortex to the ventral striatum), and limbic leucotomy which combines subcaudate tractotomy and anterior cingulotomy. These lesioning psychosurgical interventions, while improving certain depressive symptoms, showed their limitations in relation to poor patient selection, simultaneous presence of other psychological disorders, wide variability in surgical techniques and undesirable side effects. Furthermore, these studies were characterized by the lack of a multidisciplinary approach (grouping psychiatrists, neurologists, and neurosurgeons) and suffered from the irreversibility of the lesions produced.

The DBS approach when applied to depression is founded on neuropathological and functional imaging data, both of which demonstrate a dysfunction with basal ganglia-cortical loops originating within the orbitofrontal, dorsolateral prefrontal, and anterior cingulate cortices. Specifically, the ventral subgenual anterior cingulate cortex (sgCCA) would appear to be a seat of hyperactivity in the depressive state, and is correlated with the severity of the episode as well as its level of resistance. In an attempt to modulate the activity of these regions to reverse the depressive state, DBS of the white matter adjacent to the genu of the cingulate gyrus was applied in six patients suffering from severe drug- and ECT-resistant depression. Chronic stimulation of this area produced a state of remission in four out of the six patients implanted. More recently, another therapeutic option would appear to be the ventral striatum, since analysis of the early lesioning neurosurgical approaches showed that the greatest number of operations leading to remission involved interventions including the region below the head of the caudate nucleus. This was the case for both the subcaudate tractotomies and the limbic leucotomies. In these techniques, the lesions included the

entire ventral striatum, simultaneously touching the nucleus accumbens and the head of the caudate. Now, DBS of the ventral striatum in the region adjacent to the anterior arm of the internal capsule has been shown to improve depressive symptoms. Furthermore, stimulation of the nucleus accumbens in three depressed patients produced an immediate effect on the anhedonia, as has stimulation of the caudate nucleus targeted for treatment of severe OCD.

In summary, OCD and depression represent fairly different pathologies of the psychiatric domain which require different therapeutic approaches. In the domain of deep brain stimulation, two completely different targets have been proposed: the cortical Brodmann area 25 on the basis of functional imagery data and the subthalamic nucleus based on direct fortuitous observations in Parkinsonian patients. Experimental data in the nonhuman primate have revealed the role that the ventral caudate nucleus plays in the development of repetitive behaviors, and application of DBS in this nucleus for OCD patients has shown that both OCD and depressive symptoms were improved. This could suggest that both pathologies have common physiopathological processes. Nevertheless, the caudate nucleus is a very large structure and further work is needed to determine whether both pathologies respond to stimulation of exactly the same territory.

Conclusion

The DBS paradigm for the treatment of psychological disorders enables us to deepen our understanding of these afflictions on the basis of models founded on our improved knowledge of their underlying neuropathology. By specifying the neural bases of emotional behavioral disorders, in particular in relation to the basal ganglia, we have seen substantial improvement in the therapies available. Not only are we able to define the targets for neurosurgery more accurately but we are also able to systematically correlate the DBS results with the clinical observations. Finally, the application of DBS in the field of psychopathology has been a powerful stimulus allowing the integration of experimental scientific results, obtained in carefully controlled studies, with a greatly improved service to patients.

See also: Contribution of Split-Brain Studies to the Evolution of the Concept of Hemispheric Specialization; Mouse Genetic Approaches to Psychiatric Disorders; Transient Global Amnesia: Neuropsychology, Psychopathology, and Neuroimaging.

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Human Fear and Anxiety

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Glossary

Amygdala – Almond-shaped subcortical brain structure located in the medial temporal lobe. It belongs to the limbic system and has been involved in fear learning and expression.

Antidepressants – Drugs that are used to treat several depressive and anxiety disorders. They are effective only after several weeks of daily administration and do not induce a drug dependence.

Anxiolytics – This term refers mainly to benzodiazepine agents that reduce anxiety after a single administration. They are used to treat generalized anxiety disorder for a few weeks, since prolonged use can result in psychological and physiological dependence.

Cognitive behavior therapy (CBT) – A modality of psychotherapy based on learning principles. Its main goal is to promote the extinction or unlearning of fear-inducing associations by gradual exposure to the fear stimulus or context.

Ethoexperimental analysis of behavior – Study of animal behavior with a combination of systematic observation and experimental control of intervening variables.

Ethology – Systematic and comparative study of animal behavior from a biological and evolutionary perspective.

Generalized anxiety disorder (GAD) – Chronic, free-floating anxiety for at least six months, characterized by excessive worry about everyday events, with mild autonomic symptoms.

Hippocampus – Subcortical brain structure shaped like a seahorse and located in the medial temporal lobe that belongs to the limbic system. It has been involved in many functions, such as memory, spatial navigation, and anxiety.

Nuclear magnetic resonance imaging (MRI) – Imaging technique used to visualize the structure and function of bodily organs. It uses a powerful magnetic field to align the nuclear magnetization of atoms in water and provides detailed images in any plane.

Obsessive-compulsive disorder (OCD) – Disorder characterized by recurrent, unwanted thoughts (obsessions) and/or repetitive behaviors (compulsions) that is often accompanied by intense anxiety.

Panic attack – Sudden surge of intense fear, lasting for nearly ten minutes, and accompanied by marked autonomic changes, like sweating, shortness of breath, hyperventilation, choking, palpitation,

dizziness, nausea, stomach ache, trembling or shaking.

Panic disorder (PD) – Anxiety disorder characterized by a recurrence of panic attacks. With time, anticipatory anxiety about having another attack and avoidance of places where having an attack is embarrassing, develop.

Panicogenic agents – Chemical agents used to experimentally induce panic attacks. Examples are lactate and cholecystokinin injected intravenously, and carbon dioxide (CO_2) inhaled at concentrations higher than that in the atmosphere.

Periaqueductal gray matter (PAG) – Gray-matter layer that surrounds the midbrain aqueduct. It has been involved in the control of proximal defense (freezing/fight/flight) and of panic attacks.

Posttraumatic stress disorder (PTSD) – Anxiety disorder that can develop after exposure to terrifying events of physical or psychological nature. Soon, or even a long time after the traumatic event, patients undergo distressful experiences, such as reliving the traumatic event, tend to avoid situations reminding the trauma, as well as show emotional numbing and hyperarousal.

Receptor – Set of protein molecules localized in cell membranes, having sites where an endogenous substance, such as a neurotransmitter, binds with high affinity and selectivity to cause physiological changes.

Selective serotonin reuptake inhibitor (SSRI) – Second-generation antidepressant agent that inhibits 5-HT reuptake carried out by a transporter molecule at the neuronal membrane, with little or no action on the reuptake of other biogenic amines, such as noradrenaline or dopamine. As a result, 5-HT concentration in the synaptic cleft is increased.

Serotonin (5-HT) – Biogenic amine neurotransmitter chemically named 5-hydroxytryptamine.

Social anxiety disorder (SAD) – Intense, persistent fear of being watched and judged by others, what interferes with ordinary activities. Physical symptoms that often accompany the anxiety include blushing, sweating, trembling, nausea, and talking difficulty.

Specific phobia – Abnormally intense, persistent fear of certain objects, animals, activities, or situations.

Tricyclic antidepressant – First-generation antidepressant agent that nonselectively inhibits biogenic amine reuptake.

Anxiety, fear, and panic: do these emotions belong to a continuum of increasing intensity or differ qualitatively from each other? These opposed views have been sustained by respected scholars, but it is unlikely that the dispute will be settled on phenomenological grounds alone. Other levels of analysis are clearly necessary and, in this regard, the evolutionary approach, taken from biology, provides important material for this discussion.

From a biological perspective, it is important to remark that these three emotions are triggered by the perception of threat and mobilize defensive reactions to cope with the perceived challenge. Therefore, a way of understanding their function and neural substrate is to study the expression of defensive reactions in animals, especially in those phylogenetically related to our own species. This approach has been introduced by Charles Darwin, who first organized his observations and theoretical views in the book *The Expression of Emotions in Man and Animals* published in 1872. Basically, Darwin extended to the basic emotions, the notion of natural selection, through which certain characteristics of living organisms are selected and preserved along generations because of adaptive advantage, essentially the ability to breed more offspring capable of reaching reproductive age. In this way, the evolutionary perspective was introduced into psychology and, later on, in psychiatry. Along the twentieth century, this approach was systematically developed by ethology. At first, this discipline has studied the behavior of animals in their natural environment, without any interference from the observer, but later on experimental interventions have been added, giving rise to the field of ethoexperimental analysis of behavior.

Defense Strategies and Emotions

The ethoexperimental analysis of antipredator defense conducted by Robert and Caroline Blanchard has established that the type of defense strategy a given animal displays is mainly determined by the presence or absence of the predator, by the distance between the latter and the prey, and by the availability of an escape route.

The first pattern of defense occurs when the predator is absent, but had been met in the past by the prey in the same environment; or, alternatively, the situation met is novel, bearing both potential rewards and hazards. In both cases, there is conflict between the opposed tendencies toward approach and withdrawal. The resulting defense strategy consists of cautious exploration aimed at risk assessment. The second defense pattern, named distal threat, occurs when the predator is present, but placed at a safe distance from the prey. If there is an exit, the animal quickly escapes, but if there is no such outlet, the

animal becomes motionless, to impair detection by the predator and tenses its muscles in preparation for further active responses, such as fight or flight. This posture is often named 'freezing.' Finally, when the predator is in close proximity or makes actual contact with the prey, either energetic flight or defensive fight takes place, if there is no exit.

Although the topography of the defensive responses varies widely (e.g., pigeons fly, while rabbits run away from danger), in virtually all species, antipredator defense is functionally organized from risk assessment to tense immobility, escape, defensive threat and, finally, defensive attack. The same defensive strategies are used when the threatening animal belongs to the same species, but in conspecific interactions a further strategy comes into play, namely, submission, which is important to establish social rank and, thus, minimize injury and death.

The levels of antipredator defense described above have been related to the three emotions in consideration: potential threat with anxiety, distal threat with fear, and proximal threat with panic. In addition, submission relates to shyness.

Neural Substrate of Anxiety, Fear, and Panic

The risk-assessment construct elaborated by Robert and Caroline Blanchard overlaps, to a large extent, with the concept of the 'behavioral inhibition system' (BIS) proposed by Jeffrey Gray. In its last account, published in Gray and McNaughton's (2000) *Neuropsychology of Anxiety*, the septo-hippocampal system, which also includes the dentate gyrus, the entorhinal cortex, the subiculum area, and the posterior cingulated cortex, is supposed to play the central role of detecting conflict between opposed behavioral tendencies (approach vs. withdrawal), what generates anxiety. The septo-hippocampal system is seen as responding to threat – conveyed by either learned or innate danger cues, or novelty – by interrupting ongoing behavior and increasing the level of arousal and attention for better information gathering. In human anxiety, the septo-hippocampal system would be responsible for the cognitive manifestations of anxiety, like worrying about future adversity, whereas the affective or arousal component would be mediated by the amygdala. The latter has been involved in fear learning and expression, as well as in the acquisition and recall of emotional memories, mainly by the work of Michael Fanselow, Michael Davis, James McGaugh, and Joseph LeDoux.

Following the same line of thought, when there is no tendency to approach the source of danger, the experienced emotion is not anxiety, but fear. The structures of the central nervous system (CNS) likely to be involved constitute the so-called 'brain defense system,' comprising

the amygdala, medial hypothalamus, and midbrain periaqueductal gray matter (PAG). Summing up their interplay, Michael Fanselow has suggested that the amygdala evaluates the type and magnitude of danger, the results of this evaluation being conveyed to the hypothalamus and PAG, which are in charge of organizing and executing adaptive defense reactions. More recently, this defense system, together with connected cortical areas, has been named by McNaughton and Corr as the 'avoidance defense system' considered as the neural substrate of fear and panic; in contrast, a parallel set of structures suggested to deal with anxiety, including the septum–hippocampus was named the 'approach defense system.'

The main substrate of the third level of defense, proximal defense, is likely to be the dorsolateral column of the PAG, as determined by Richard Bandler and co-workers by injecting excitatory amino acids into different regions of the PAG. Either electrical or chemical stimulation of the dorsolateral column of the PAG causes a flight reaction and activation of the autonomic sympathetic system in laboratory animals. In patients undergoing neurosurgery, electrical stimulation applied in or near the lateral PAG induces feelings of terror or impending death, accompanied by respiratory and cardiovascular changes similar to those of a panic attack. In the proposal by MacNaughton and Corr, the distinction between fear and panic is made within the avoidance defense system, as a function of the defensive distance. Distal threat (fear) would activate forebrain structures, such as the frontal cortex and amygdala, whereas proximal threat (panic) would activate the PAG. The forebrain structures are necessary for careful environmental analysis and threat evaluation, as well as planning of purposeful escape strategies based on previous learning; in contrast, the PAG would elicit vigorous, but poorly directed flight responses, required in situations of extreme danger. This proposal has received strong support from the results of a functional magnetic resonance imaging (MRI) study performed by Mobbs and coworkers in 2007, showing that brain activity shifted from the prefrontal cortex (PFC) to the midbrain PAG, as a virtual predator capable of inflicting pain to the participant grew nearer to a virtual prey, controlled by the participant. In addition, the PAG activity correlated with reported subjective degree of dread and decreased confidence of escape.

Within a broader perspective, the last results imply that human beings share, with nonhuman mammals, approximately the same defense strategies and underlying neural circuitry. Nevertheless, there is at least one peculiarity in the human brain that deserves special comment: the remarkable development of the PFC. Even in laboratory animals, several findings indicate that the medial PFC is involved in the coordination of defense reactions. Supporting these findings, it has been shown that the medial PFC has direct connections with subcortical,

diencephalic, and brainstem structures related to defense, such as the amygdala, hypothalamus, PAG, and dorsal raphe nucleus, the latter containing serotonergic neurons. In rodents, the medial PFC has been particularly related to extinction, characterized by a decrease in a conditioned response to a stimulus, when the previously associated unconditioned aversive stimulus is omitted. The medial PFC is proposed to have a dual role in fear extinction, retaining information that aversive cues are no longer fearful, and acting on visceral structures to reduce autonomic changes. The interference in extinction seems to involve projections from the ventromedial PFC to the amygdala; in addition, the ventromedial PFC can influence autonomic responses associated with defensive responses by means of its extensive connections with hypothalamic areas. The medial PFC can also modulate the activity of the hypothalamic–pituitary–adrenal (HPA) axis through its direct connections to the bed nucleus of the stria terminalis or to the periventricular nucleus of the hypothalamus and, indirectly, through its connections to the amygdala, which projects to the periventricular nucleus through the bed nucleus. Finally, the medial PFC projects directly to the PAG.

In the human species, the role of the PFC in anxiety disorders has recently been highlighted by Rachel Berkowitz and co-workers (2007), who remarked the structural and functional peculiarities of the human PFC. This brain region is responsible for the remarkable cognitive abilities that are characteristic of the human species. In particular, the capacity to foresee future events and the consequences of one's actions would cause concern, the major symptom of generalized anxiety disorder (GAD). On the other hand, this development of the PFC allows for enhanced cognitive control of emotions, as testified by several functional neuroimaging studies, showing that cortical forebrain regions are able to attenuate emotional responses at subcortical levels, suggesting a neural basis for modulating emotional experience through interpretation and labeling. These findings may help to understand how psychotherapy works.

Pharmacological Analysis

The distinction between GAD and panic disorder (PD), presently established in psychiatric classification manuals, was first made on a pharmacological basis. In the early 1960s, Donald Klein originally reported that panic patients were improved by long-term (3–4 weeks) administration of imipramine, an antidepressant agent. In contrast, benzodiazepine anxiolytics were known to be ineffective. Nevertheless, further clinical trials have shown that GAD can also be ameliorated by chronic administration of antidepressants, and PD responds to some potent benzodiazepines, like alprazolam, when

given chronically. Nevertheless, the fact that PD is resistant to low doses of benzodiazepines that are effective on GAD remains true. Moreover, biological markers of PD have been found, the most well known being that nearly two-thirds of panic patients experience a panic attack following a lactate injection, in contrast to very few healthy people or patients with other diagnoses.

Other anxiety disorders, such as social anxiety disorder (SAD) and posttraumatic stress disorder (PTSD) can also be improved by long-term antidepressant administration. Furthermore, selective inhibitors of serotonin (5-HT) reuptake inhibitors (SSRIs), administered for over 6 weeks, have been shown to improve obsessive-compulsive disorder (OCD), a clinical condition that is considered, although not unanimously, as a primary anxiety disorder. Controlled clinical trials by David Nutt and co-workers using tryptophan depletion to decrease brain 5-HT level, have shown that the therapeutic effect of SSRIs is reversed by 5-HT depletion in PD, SAD, and PTSD, suggesting that 5-HT mediates the clinical action of these drugs. Interestingly, OCD is not affected, indicating that SSRI treatment of this condition is less dependent on 5-HT availability.

Preclinical and clinical investigation of the role of 5-HT in anxiety has contributed to the view that GAD and PD are neurobiologically different. In animal models of anxiety that generate behavioral inhibition due to conflict between approach and avoidance tendencies, and are predictive of drug response for GAD, for example, punishment or conflict tests, it has been observed that drugs that decrease 5-HT neurotransmission have anxiolytic-like effects, whereas those that increase 5-HT are anxiogenic. In contrast, escape tasks that are predictive for PD, such as switching off electrical stimulation of the PAG, are impaired by the drug treatments that enhance 5-HT. This has led to the suggestion that 5-HT facilitates anxiety, but inhibits panic (**Figure 1**). The latter action is likely to be localized in the PAG, since recent results obtained by Hélio Zangrossi Jr. and co-workers in the rat have shown that chronic, but not acute, administration of drugs that improve PD, including several antidepressants and the antipanic benzodiazepine alprazolam, sensitizes 5-HT receptors in the PAG, the stimulation of which inhibits escape responses. This action may underlie the property shared by these drugs of reducing the number of panic attacks in PD patients.

Data obtained with two procedures of inducing experimental anxiety in human beings further support the view that anxiety and fear have a different neural basis. For instance, drugs that presumably enhance the functioning of 5-HT neurotransmission have been shown to facilitate conditioning of skin conductance responses to a loud noise, associated with anticipatory anxiety, but decrease the fear of speaking in front of a videocamera; the opposite effects on both tests have been determined by drugs

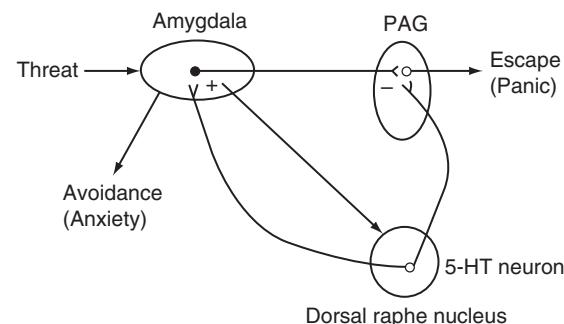


Figure 1 Dual action of serotonin (5-HT) in defense. Serotonergic neurons localized in the dorsal raphe nucleus project to both the amygdala and the periaqueductal gray matter (PAG). In the former structure, 5-HT is supposed to facilitate defense reactions to potential threat, related to anxiety, whereas in the PAG, 5-HT would inhibit proximal defense (freezing/fight/flight) related to panic attacks.

that impair 5-HT neurotransmission. The aversive conditioning test has been shown to have a pharmacological profile akin to GAD, whereas that of simulated public speaking resembles the drug response of PD, SAD, and PTSD.

Another important difference between anxiety and panic is in the activation of the HPA hormonal axis. Although situations that generate anticipatory anxiety release corticoids in the blood stream, panic attacks induced by selective panicogenic agents, such as lactate injection or CO₂ inhalation, or even natural panic, fail to activate the HPA axis. Interestingly, it has been reported that simulated public speaking does not seem to stimulate the HPA axis either.

Thus far, only one class of anxiety disorders has proved resistant to drug treatment, being otherwise responsive only to cognitive behavior therapy (CBT), namely specific phobias, such as claustrophobia (fear of closed environments), acrophobia (fear of heights), or arachnophobia (fear of spiders). These may be considered as pathologies of fear, partly due to overreaction to species-typical fear stimuli, as, for instance, closed spaces, spiders, and heights are for humans, and partly to associative learning or fear conditioning.

Overall, pharmacological analysis suggests the existence of three different neurobiological substrates for the defense-related emotions: one underlying anticipatory anxiety and GAD, which is sensitive to acute treatment with benzodiazepines as well as to chronic administration of antidepressants; another involved in PD, SAD, and PTSD, which is resistant to acute benzodiazepines, but responds to a chronic antidepressant regimen; and a third one that is drug resistant and underlies specific phobias. Therefore, such disorders may be viewed as dysfunctions of the anxiety, panic, and fear neural systems, respectively.

Neuroimaging Data

As already exemplified by the Mobbs *et al.* experiment described above, brain neuroimaging gives an important contribution to clarify the neural substrate of defense-related emotions by showing that anxiety involves the activation of forebrain structures, whereas panic engages the midbrain PAG. In the same vein, Rachel Berkowitz and co-workers have concluded from a thorough review of neuroimaging data that anxiety disorders that involve intense fear and panic, such as PD, SAD, and PTSD, seem to be characterized by PFC underactivity, thus disinhibiting the amygdala; in contrast, GAD and OCD, which involve worry and rumination, seem to be characterized by PFC overactivity. In agreement, a recent study aimed at assessing the effect of CBT in panic patients, using positron emission tomography (PET), carried out by Sakai and co-workers, has shown positive correlation between decreased activity in the left medial PFC and clinical improvement of anxiety and agoraphobia, as well as between decreased activity in the midbrain and reduction in the number of panic attacks during the 4 weeks separating the first scan, made before treatment and the second, made after CBT.

The latter study also testifies to another important contribution of neuroimaging, which is the demonstration that psychotherapy can change brain functioning in the same way as medication. This line of research started with the now-classical PET study by Lewis R. Baxter and co-workers in 1992 showing that treatment with either CBT or an SSRI could reduce activity in the caudate nucleus in correlation with improvement of compulsive symptoms.

Consonant with animal data indicating a pivotal role of the amygdala in defense-related emotions, several neuroimaging studies have shown amygdala activation by conditioned aversive stimuli, innate fear stimuli, and human faces expressing emotion, in particular fearful faces.

In many studies, anxiety-disorder patients have shown amygdala overactivation as compared to healthy individuals, and chronic administration of antidepressants has been shown to reduce amygdala activation by aversive stimuli. Nevertheless, increased activity of the amygdala may be present only at the onset of an experimentally induced state of conscious fear, with other regions such as the insula, thalamus, and brain stem-sustaining activity. As a result, Tracy Butler and co-workers (2007) have recently proposed that the amygdala works as a threat and novelty detector, whose initial, brief activity is accompanied by sustained tonic activity of other brain regions, which are responsible for maintaining the behavioral, autonomic, and metabolic responses to danger stimuli.

Further evidence has highlighted the role of the insula in anxiety disorders. In PD patients, functional studies have shown hyperactivity of the insula during experimentally induced panic attacks, and structural MRI investigations using an automated procedure of assessing volume (voxel-based morphometry) have shown an increase in gray matter volume of the insula in PD patients at rest. Since the insula has been involved in the process that ascribes negative emotional meaning to potentially distressing interoceptive stimuli, the reported alterations in the insula may be related to the abnormal evaluation of bodily signals shown by panic patients. One of the main goals of CBT is to reverse such overreactivity to interoceptive stimuli.

Conclusions

A synthesis of the above discussion is presented in **Table 1**.

It is suggested that anxiety disorders are dysfunctions of brain neural networks that control defensive strategies to different levels of threat, which evoke particular emotional states.

Table 1 Antipredator defense, related emotions, and anxiety disorders

Threat	Defense reaction	Critical brain structure	Emotion	Disorder	Drug treatment
Uncertain	Risk assessment	PFC Septum-hippocampus	Anxiety	GAD	Anxiolytics
Conflict	Behavioral inhibition	Amygdala			Antidepressants
Anticipated (CS)	Freezing (No exit)	Amygdala VPAG	Anxiety	Anticipatory anxiety	
	Avoidance	Amygdala	Conditioned fear	Specific phobias	None
Distal (US)	Escape	Medial hypothalamus	Unconditioned fear	Specific phobias	None
Proximal (US)	Flight or Immobility	DPAG	Panic	PD SAD PTSD	Antidepressants

CS = conditioned stimulus, US = unconditioned stimulus, PFC = prefrontal cortex, VAPG = ventral periaqueductal gray, DPAG = dorsal periaqueductal gray, GAD = generalized anxiety disorder, PD = panic disorder, SAD = social anxiety disorder, PTSD = posttraumatic stress disorder.

Returning to the opening question, the biological evolutionary approach illustrated in the preceding sections indicates that anxiety, fear, and panic are qualitatively different emotions.

See also: Active Avoidance and Escape Learning; Animal Tests for Anxiety; Behavior Adaptation and Selection; Brain Imaging; Communication of Emotions in Animals; Emotions; Evolution of Emotions; Fear, Anxiety, and Defensive Behaviors in Animals; Fear Conditioning; Fear: Potentiation and Startle; Neural Bases of Defensive Aggression; Neural Substrates of Conditioned Fear and Anxiety; Neural Substrates of Unconditioned Fear, Defense, and Anxiety; Neuropsychological Aspects of Anxiety Disorders; Stress and Emotionality; Subjective Experience and the Expression of Emotion in Man; Value of Animal Models for Predicting CNS Therapeutic Action.

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Neuropsychological Aspects of Anxiety Disorders

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Glossary

Generalized anxiety disorder – The disorder is characterized by various repetitive, uncontrollable, and distressing worries, accompanied by a range of physical symptoms. The definition of the syndrome has been recurrently modified through time, the disorder being still challenged as specific.

Obsessive-compulsive disorder – Recurrent intrusive and distressing thoughts (obsessions) and repetitive ritualistic behaviors (compulsions) characterize this disorder. Main theoretical cognitive models involve inflated responsibility and thought-action fusion as major features of the syndrome.

Panic disorder – The disorder is characterized by repetitive and sudden attacks of terror, accompanied by impairing physical symptoms, such as palpitations, sweatiness, faintness, or dizziness. Between episodes subjects feared the next attack; the state coined by the expression fear of fear.

Posttraumatic stress disorder – The hallmark characteristics of this disorder are distressing intrusive thoughts, nightmares, and vivid memories of the trauma associated with psychophysiological arousal. Other central features are avoidance of the stimuli related to the trauma and/or emotional numbing.

Social anxiety disorder – Individuals suffering from social anxiety disorder experience an intense, persistent, and chronic fear of being exposed in social situations. Distress is associated with anticipatory anxiety and *post hoc* rumination concerning social performance.

Specific phobia – Patients with specific phobia experience intense fear when they are confronted with the object of their phobia of which they develop an active avoidance. Numerous subtypes of phobia exist (e.g., spider and blood) which are associated with specific activations of the autonomic nervous system.

context. Different terms characterize the anxious spectrum as fear, concern, worries, apprehension, angst, terror, and anxiety. Taking into account the fact that anxiety is an emotion experienced by all human beings, the point is to distinguish normal anxiety from pathological anxiety. There is general agreement that the main attributes of pathological anxiety are to appear out of a relevant context, to be inappropriately intense or particularly chronic, to be associated with paroxysmal or chronic somatic symptoms and leading to incongruent behavior, and psychological, physical, or social impairment. Pathological anxiety, although a symptom belonging to a wide range of psychiatric disorders, is considered to be the central feature of only a few disorders. Therefore, the formal nomenclature of the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV) includes 13 categories of anxiety disorders of which the main ones are obsessive-compulsive disorder (OCD), posttraumatic stress disorder (PTSD), panic disorder (PD) with and without agoraphobia, social anxiety disorder (SAD), specific phobia (SP), and generalized anxiety disorder (GAD). A common feature of most anxiety disorders is the existence of acute episodes of heightened anxiety triggered by specific (SP, SAD, PTSD, OCD) or nonspecific (PD, GAD) threatening stimuli. The intensity of the acute episode may vary along with the nature of the disorder, the stimulus, and the individual. Specific and nonspecific cognitive strategies are employed to avoid or reduce both the occurrence and the level of the paroxysmal anxiety. For example, phobic patients actively avoid known threatening stimuli, although patients experiencing PD cannot employ such a strategy. The diagnostic reliability (inter-reliability and test-retest) of some anxiety disorders has been found to be low (e.g., for GAD) or moderate (SP and PTSD) even with the use of standardized interviews. Another problem is the high rate of comorbidity, principally with other anxiety disorders and with depression. Some epidemiologic studies have shown that more than half of the individuals with anxiety disorders present another psychiatric disorder at the time of assessment; more than 75% in the case of GAD which has been challenged as a distinct syndrome. Studies have reported several patterns of cognitive functioning among patients reporting high levels of state or trait anxiety. Their conclusions must be cautiously examined as they may not be extended to specific anxiety disorders without

Ordinary Fear and Pathological Anxiety

Fear is an archaic emotion common to numerous species. It plays a major role in adaptation to the environment. In humans, this includes physical, sociological, and cultural

evidence. The model proposed by Eysenck to explain some cognitive impairment among highly anxious subjects remains one of the most consensual. Eysenck put forward cognitive preoccupations with emotionally negative concerns in which preferential attention is given to the negative aspects of highly anxious subject's environment. This hypothesis is also known as the 'hypervigilance hypothesis' and is well supported by authors such as Beck and Bower. Moreover, some resources that are permanently allocated to tasks associated with these negative concerns may lead to impairment in executive function. In addition, some authors have suggested that individuals with anxiety disorders also tend, at various extents, to avoid or to inhibit deep processing of threatening information. This hypothesis, put forward in particular by Foa and Kozak, has been recently completed by the assumption that information processing among anxious individuals may be organized in two stages, with a tendency to increase attention to threatening information and then to avoid attention or to inhibit deep processing of the initially discriminated threatening information (hypervigilance-avoidance hypothesis).

Subjects with anxiety disorders display specific concerns and beliefs which in turn interfere with cognitive functioning. Morphologic and, moreover, functional neuroimaging can specify the cerebral structures involved in these processes. Executive functions, including inhibition, flexibility and planning, attention, judgment processes, and memory, have begun to be systematically studied for each anxiety disorder. In our presentation, we separate general cognitive impairments and specific cognitive biases for threatening stimuli. We also complete this presentation by giving an account on neuroimaging data.

From Psychiatric Categories to Cognitive Processing

All anxiety disorders present somewhat common clinical features: heightened sensibility to specific or general threatened stimuli, peaks of anxiety triggered by these stimuli, and cognitive or behavioral strategies of variable efficiency whose supposed aim is to cope with and/or to avoid the threatened stimuli. Beyond these general features, each anxiety disorder shows its own clinical specificities, OCD and PTSD displaying the more complex patterns.

Obsessive-Compulsive Disorders

The estimated lifetime prevalence of OCD in general population studies is 2.5–4%. Nevertheless, a large study using strict criteria and psychiatric interviews found lifetime prevalence lower than 1%. OCD is characterized by recurrent intrusive thoughts (obsessions)

and repetitive motor or cognitive ritualistic behaviors (compulsions). The main core of the ritualistic behaviors is to reduce the undue anxiety that obsessions generate, but they themselves may provoke anxiety and impairment. Fear of contamination/washing (washers) and pathological doubt/checking (checkers) are the most common combinations of symptoms. Complex cognitive models have been elaborated for OCD. The main theoretical models focus on the cognitive factors of inflated responsibility, thought-action fusion, and meta-cognitive beliefs.

Posttraumatic Stress Disorder

PTSD is triggered by a so-called traumatic event, which leaves the individual in an internal feeling of terror and of a total lack of control. Most individuals may experience a putative traumatic event in their lifetime, but according to the nature of the event and of the personal and social resources of the individuals, only a fraction of subjects will subsequently develop a PTSD. The hallmark characteristics of PTSD are the painful and distressing intrusive thoughts, nightmares, and vivid memories of the traumatic event associated with psychophysiological arousal. Intrusive memories, including flashbacks, contain contextual or core details of the traumatic event loaded with massive sensory inputs. A disorganized narrative memory of the trauma, but not of control events, characterizes a large part of PTSD patients. Brewin has hypothesized that memories associated with the trauma are encoded in a two-representation system – one verbal and the other contextual. According to the author, the former is voluntarily accessible and responsible for narrative memory; the latter responds automatically to sensory cues of the trauma and leads to sensory relivings. Other central features of the diagnosis are active avoidance of the stimuli related to the trauma and/or emotional numbing (e.g., feelings of detachment, disinterest, and restricted range of affect).

It has been suggested that PTSD symptoms result from both classical conditioning of emotional responses to extreme traumatic stimuli and operant conditioning of avoidance of traumatic stimuli. The traumatic experience overwhelms the emotional and cognitive system, promotes an excessive sensitivity to threatening stimuli, and then impairs fear-extinction processes. The threshold for stress is lowered and a large amount of stimuli may activate autonomous responses which in turn reactivate feelings and memories relative to the trauma.

General Anxiety Disorder

Lifetime prevalence of GAD has been estimated to 12%. The disorder is characterized by various repetitive, uncontrollable, and distressing worries, accompanied by

a range of physical symptoms. Individuals with GAD anticipate disaster, show intolerance for uncertainty, and they are especially disturbed by not being able to control the present or to predict the future. However GAD, as aforementioned, is discussed as a specific anxiety disorder and may be grossly described as a quite-durable (at least 6 months) high-anxiety state.

Panic Disorder

PD has a lifetime prevalence of 2–4%. It is characterized by repetitive and sudden attacks of terror, whose peak usually lasts around 10 min. It is accompanied by physical symptoms such as palpitations, sweatiness, shortness of breath, faintness, or dizziness. Between episodes, subjects worry intensely and dread the next attack; the state coined by the expression fear of fear. Repeated panic attacks may lead to social impairment, with a restriction of activities and agoraphobia. Central to the cognitive model of the disorder, it has been hypothesized that individuals developed an attentional bias toward cues associated with panic, with a special emphasis on bodily sensations. Agoraphobia in PD is generally understood as a consequence of panic attacks. Patients avoiding situations, in which help may not be readily available, present a quasi-similar pattern to phobics. The appraisal by the subject of its own coping abilities has also been put forward in the triggering of a panic attack. This hypothesis is known as the “theory of self efficacy.”

Social Anxiety Disorder

SAD is considered to be the most common anxiety disorder with a lifetime prevalence of 12–16%. Individuals with social phobia have an intense, persistent, and chronic fear of being exposed in social situations, in which they may be watched or judged by unfamiliar people. Physical symptoms of anxiety often accompany social phobia as other anxiety disorders. Distress in social phobia is associated with anticipatory anxiety and *post hoc* rumination concerning social performance. Self-focused attention leads to recollection of negative experiences, heightened attention to signs of threat, and misinterpretations of other's behaviors which in turn help to maintain social phobia.

Specific Phobia

SP has an estimated lifetime prevalence of about 12%. Individuals with SP experience intense fear when they are confronted with the object of their phobia and at the same time develop avoidance behaviors of contexts related to the phobic object. There are many subtypes of SP.

General Cognitive Impairments: Results from Experimental Studies

Cognitive impairments have been quite reliably found in individuals with OCD, particularly for executive functions and visual memory, and to a lesser extent with PTSD for attention and both verbal and visual memory. These impairments are generally understood as the result of a neural circuit dysfunction involving an orbitofrontal–subcortical network for the former and a brainstem and prefrontal network for the latter. They are detailed below. Fewer studies have addressed neuropsychological functioning in other anxiety disorders. Inconsistent results characterize PD and social phobia. Patients with SP and GAD generally show no deficit in general cognitive tasks.

Executive Functions

Executive functions refer to the ability to plan and carry out complex goal-directed behaviors. According to Miyake, shifting between tasks or mental sets, updating and monitoring of working-memory representations, and inhibition represent three latent variables which may potentially describe most of the executive functions. OCD patients show impairment in set shifting, set maintenance, and demonstrate lower abilities to learn from the changing responses brought by the environment. Such a deficit has been hypothesized to involve impairment in selective attention and obsessive checking, leading to an overloading of working memory. Deficits in neuropsychological tests measuring inhibition have also been quite regularly reported in OCD patients. Motor inhibitory processes have been explored with tasks such as ‘go/no go’ or “stop signal reaction time” and cognitive inhibition by tasks such as the “Stroop color-naming paradigm.” Inconsistent findings have been observed for planning and decision making in OCD patients although they tend to perform slower and to exhibit more perseverative errors than controls. Slowness and perseveration may be related to a lack of mental flexibility impairing organizational strategies. Deficit in shifting and inhibition have been considered as the hallmark of an orbitofrontal dysfunction.

No deficits in executive functioning have been consistently reported in other anxiety disorders except for PTSD, in which working memory has been quite reliably shown impaired, particularly when strategic cognition was also involved. Hence, in a plain task, such as “digit span forward” which focuses on passive storage of digits, patients generally exhibit no deficit contrary to the “digit span backward” that is more executive. In verbal or visuospatial tasks, which require maintenance and manipulation of short-term information, some deficits have been found although inconsistently. Individuals with PTSD generally failed to show impairments in set shifting.

Attention

No consistent deficits have been found in patients with OCD although they often exhibit a lack of confidence in keeping attentional focus. In contrast, an attention deficit has been quite reliably established for PTSD patients, particularly on measures of sustained attention and encoding, but not on measures of focused attention or attentional shifting. These deficits may reflect the frequent lack of concentration alleged by patients with PTSD.

Explicit and Implicit Memory

The terms, 'explicit memory' and 'implicit memory,' here refer to the recall or recognition of data that are expressed, respectively, with and without awareness of encoding. The exploration of explicit memory includes cued recall, free recall, and recognition of data. Many studies have established that OCD patients, particularly those with prominent checking symptoms (checkers), exhibit impairment for visuospatial memory. This memory impairment may result from an irrelevant organizational strategy involving lack of flexibility, perseveration, and checking. When reproducing a complex figure (Rey-Osterrieth complex figure test) to assess visual memory, OCD individuals tend to focus on irrelevant details during the initial copy of the figure which may explain their low scores. Concerning verbal memory in most studies, OCD patients perform as well as controls. Moreover, a study has shown that subclinical checkers exhibit better recognition for words than controls. Nevertheless, when memory tasks involve semantic strategies, especially if encoding is time limited, performances of OCD patients tend to decrease in immediate recall and recognition.

Regarding individuals with PTSD, many studies have identified decreased performance in verbal memory and learning. Although the deficits are commonly mild, delayed free recall has been found more frequently impaired than cued recall and recognition, which require less data manipulation. Initial acquisition in recall tasks has been shown impaired quite regularly when the memory task bears on semantically unrelated words (Rey auditory verbal learning test (RAVLT)) but not for words semantically linked (California verbal learning test (CVLT)). These conditions suggest that, similar to OCD, memory is more often impaired when searching and retrieving relevant information requiring more organization and manipulation of the data. Few studies have explored memory in other anxiety disorders and the results lack consistency. A small number of studies have found that PD patients exhibit performance impairments for recall of visual and verbal information and that SP patients exhibit recall deficits for verbal information.

Autobiographical Memory

Some studies have focused on 'autobiographical memory,' defined as recollections of one's own personal experiences that can be placed in a specific time and location. Autobiographical memory is narrowly attached to the construction (or the maintenance) of the self and is linked to the regulation of emotions and beliefs. Anxious participants asked to retrieve specific personal memories in response to neutral- or positive-trait cue words tend to respond slower and/or to be less specific than controls. Several studies have found evidence of overgeneral autobiographical memory for PTSD, OCD, and in a lesser extent for SAD. In case of overgeneral memories, answers usually did not refer to a specific episode, precisely localized in time and space. On the contrary, they either referred to an extended or a vaguely defined period or described habits or categories of events with quite wide-ranging descriptions. It has been hypothesized that overgeneral memories protect the patient from the unpleasant recall of painful or even traumatic events. However, when depressive symptoms were controlled for, fewer or no significant results emerged, emphasizing the importance of controlling for depression within the research field of anxiety disorders. A study showed that OCD checkers used less visual imagery to recall biographical information than controls which may contribute to explain the trend to overgeneral memory. However, for all individuals, difficulty in remembering the past may render it difficult to picture the future. There are no clear results regarding specificity of autobiographical memory in SAD. Most studies exploring autobiographical memory have failed to establish consistently a memory bias toward social threats in comparison to positive and neutral cues. Moreover, when cued with social threat stimuli, SAD patients take more time to recall a memory which tends to be more general than controls. In PD and SP, no impairment in autobiographical memory has been established. To the opposite, some studies show a better recall of vivid memories in response to threatening cues.

Reality Monitoring

OCD patients often express doubt concerning the reality of what they recall, uncertain if they have performed an action or merely imagine it. Nevertheless, studies of reality monitoring in OCD patients, checkers and noncheckers, have not reliably shown a real impairment but instead, a lack of self-confidence and an intolerance of uncertainty. The lack of confidence in reality monitoring has been significantly correlated with dissociative state in a recognition task study.

Cognitive Bias Relative to Threatening Stimuli: Results from Experimental Studies

Attention Biases

Most of the studies using experimental approaches to explore attentional processes in anxiety disorders have employed either facilitation or interference paradigms. Emotional analogs of the Stroop color-naming paradigm task and the dot-probe paradigm have by far been the two most employed. The classic Stroop task has been modified to test patients with an anxious disorder by examining the response time of the participant to name colors of neutral or negative emotional words. These negative words may refer either to general threatening contents (e.g. 'danger') or more specifically to the core of their anxious preoccupations (e.g. 'dust' in case of OCD Washers). Individuals with anxiety disorders often exhibit delayed color naming for emotionally threatening words compared to controls. The dot-probe paradigm measures the distribution of visual attention. Typically, subjects are instructed to press on a button as soon as they have detected in what part of a screen a dot appears. Dots are presented on a split screen just after the disappearance of a threatening or a nonthreatening (neutral) stimulus, usually a word. Generally, anxious patients answer faster when the dot is situated in the part of the screen where the threatening stimulus stood. In nonclinical population, the opposite result tends to be found. Emotional Stroop tasks demonstrate quite consistently attention biases for social phobia (with social threatening stimuli), OCD (with words specifically linked to their concern as dirt for washers, but not for general threatening or positive words), PTSD (specific and general threat words), PD (for general threat words, but not for panic-related words as those pointing the attack itself, the bodily sensations, or their feared consequences). Such facilitated attention to threat stimuli (e.g., faces) has also been reliably found with dot-probe paradigm and other tasks exploring attention biases in GAD, some subtypes of SP, SAD, and PTSD.

Stroop task and the dot-probe paradigm fail to differentiate between enhanced detection of threatening stimuli (attentional facilitation) and difficulties in disengagement from such stimuli (attentional interference). Various tasks such as the dot-probe detection task and the visual search task have been adapted in order to disentangle the two main mechanisms that may be involved in attentional bias for threat. They quite reliably found a reliable emotion-congruent attentional interference, but inconsistent results for emotion-congruent attentional facilitation to threat stimuli.

Judgment Bias

Anxious subjects tend to overestimate the threatening character of their environment. Some anxious individuals tend to increase the probabilities of the outcome of an adverse event even in neutral or positive circumstances, others disproportionately exaggerate its cost estimation, and others misinterpret stimuli or situations as a potential threat. A threatening appraisal of a situation may subsequently transform the attentional processes and lead the subject to focus on potential threats. This selective attention to putative threatening stimuli leads to an increased probability of misinterpretations of the environment. Biased judgment may play a role both in the etiology and the maintenance of anxiety disorders. Numerous experiments, most relying on the interpretation of ambiguous stimuli or situations, consistently evidence that anxious patients tend to report more aversive interpretations than controls. Priming has also been used to demonstrate the consequence of the tendency to misinterpret adverse stimuli or events. After an initial presentation of ambiguous sentences, words, or scenarios, patients tend to consistently choose a subsequent lexical task, target words related to the negative meanings of the previous ambiguous primes. Authors, such as Foa, consider that alternative meanings of an ambiguous event are activated automatically, regardless of the meaning coming to awareness depending on a system of mutual inhibition. They also postulate that, in that competition, an automatic preference to pejorative meanings may result from the repetition in the past of such a choice. Questionnaires exploring judgment processes have shown that social phobics and socially anxious individuals were more likely to interpret social scenarios as negative when the situations were rated as self-referent but not when the situation was quoted from the point of view of other's mind.

Memory Bias

An anxiety-linked advantage for memory of threatening information among anxious patients has frequently been speculated. This hypothesis is coherent with the frequent uncontrollable rumination or worrying that anxious patients present with regard to some specific situations or stimuli. Hence, individuals with social phobia could be overwhelmed with vivid recall of social humiliation, while patients with PTSD face intrusive and repetitive memory of a traumatic event through flashbacks and nightmares. Actually, results are more contrasted. With the exception of PTSD and to a lesser extent in PD, no memory bias has been evidenced for threatening stimuli that are not of central concern to their clinical condition. In PTSD, standardized episodic memory tasks have quite

reliably shown that patients retrieve less neutral and positive words than controls and, within PTSD group, more negative words even of nonspecific content were retrieved compared to positive and neutral words. Recognition tests appear less sensitive to these effects. In PD, a quite similar pattern has been established particularly when memory tasks involve deep encoding. In several studies using directed forgetting procedures, PTSD patients have not exhibited heightened forgetting of trauma words, but to the opposite, they have shown impairment in explicit memory for positive and neutral words. However, a bias for threatening stimuli may be evidenced in most anxiety disorders when the protocol engage the patient to self-reference, either by an experimental setting increasing his specific anxiety or with the use of a threatening material narrowly linked to his preoccupations. In OCD, a positive memory bias for threatening stimuli has been demonstrated with checkers if the patient perceived responsibility for the outcome of a particular check and with washers for objects which have been contaminated by the experimenter. In studies of PD patients in which encoding was self-referred, individuals exhibit a bias for threatening material. This effect has been interpreted in reference to the theory of self-efficacy. In social phobia, advantage for memory of threatening stimuli has been evidenced in experiments in which the level of social anxiety has been artificially heightened. Inducing the individuals to focus on themselves and their own competence in social performance involves self-reference and hence increases the probability of an interpretation bias which itself interferes in memory tasks. The importance of self-reference in all anxiety disorders is progressively taken into account. Moreover, some studies show that patients with GAD tend to demonstrate impairment in the retrieval of negative words of general content, but not if they are specific to their individual worries. This result may be understood with respect to the hypervigilance-avoidance hypothesis previously described.

An implicit memory bias for threatening stimuli has been displayed in some anxiety disorders (social phobia, PTSD, PD, and OCD), but some well-designed studies have also failed to replicate this result. Among the more frequent study protocols, we may cite the white noise paradigm relying principally on automatic processes and the word-stem completion, a less purely implicit task which may include some explicit resources.

Functional Neuroimaging

The study of human anxiety disorders has benefited from functional neuroimaging approaches with methods such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). They allow to measure across time the relative variation of metabolic activity, such

as indexed by glucose metabolic rate (PET) or regional cerebral blood flow (fMRI and PET). These indexes indirectly reflect regional neural activity. Provocation studies, resting state studies, and cognitive activation studies have been used to explore regional brain activity and, additionally, longitudinal studies comparing functional brain activity before and after treatment. These studies, in conjunction with other sources of knowledge, have brought some evidence that some network of structures play a critical role in anxiety disorders, namely an amygdala-centered network, a network involving the anterior cingulate cortex (ACC) and the medial prefrontal cortex (PFC), and a prefronto-striatum circuitry.

A general pattern of facilitated and heightened activation of the amygdala for threat-related stimuli has been established in PTSD, SAD, SP, and GAD, but not in PD and inconsistently in OCD. In PTSD, hyperresponsivity to threat-related stimuli, within the amygdala, may result from a defective top-down inhibition of the ventromedial PFC, but as suggested by studies of masked fear faces, the hyperresponsivity of the amygdala could be also automatically engaged. Most correlation studies using provocation paradigms have shown that activities of the amygdala and medial PFC are negatively correlated. Functional studies have also shown a diminished activation of the hippocampus during tasks involving both explicit encoding and retrieval tasks. In addition to these functional specificities, a reduction of hippocampal volume has been quite reliably found in adult PTSD patients. Animal studies suggest that the decreased volume results from cortisol-induced and glutamate-mediated neurotoxicity. However, a study involving twins suggests that hippocampal volume reduction is a predisposing factor. In resting state studies and in provocation studies, compared to controls, OCD patients reliably show increased activity in the orbitofrontal cortex (OFC), striatum, and thalamus, as well as in other regions although less consistently reported, such as the anterior cingulate and dorsolateral prefrontal cortices. Additionally, the hypothesis for primary striatal pathology in OCD finds support in some structural MRI studies which found a reduced striatal volume in patients. An increased activity in the amygdala and insula has been found in a minority of studies, particularly the one with washers. Several fMRI or PET studies have found, after a successful treatment of OCD symptoms, the normalization of the metabolism of the aforementioned regions.

Limitations and Directions for Future Research

Gathered data for anxiety disorders show general trends in cognition although contradictory findings appear. Often deficits could be evidenced only in part of the

studies and the frequent use of limited samples makes it difficult to exclude some potential biases such as comorbidity or current or past treatment. The heterogeneity of the experimental designs also weakens the ability to provide firm conclusions. The classification of anxiety disorders may also not reflect the cerebral underpinnings of such disorders. However, increasing correlation studies between neuropsychological assessment and various neurophysiological assessments will enlighten the cerebral basis of such disorders. They will provide indications on the course of cerebral activations at different stages. Another enthusiastic direction will consist to integrate self-reference in neuropsychological assessment which may help to gather hypotheses proceeding from both experimental cognition and clinical psychology studies.

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See also: Active Avoidance and Escape Learning; Animal Tests for Anxiety; Effects of Stress on Learning and Memory; Emotions; Episodic and Autobiographical Memory: Psychological and Neural Aspects; Fear Conditioning; Human Fear and Anxiety; Mouse Genetic Approaches to Psychiatric Disorders; Neural and Pharmacological Substrates of Aggression; Neural Substrates of Conditioned Fear and Anxiety; Neural Substrates of Unconditioned Fear, Defense, and Anxiety;

Psychiatric and Substance Use Disorder Comorbidity; Stress and Emotionality; Stress and Brain Morphology.

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Personality, Temperament, and Behavioral Syndromes

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Glossary

Behavioral syndromes – Groups of behaviors that covary in their expression across situations or contexts within a species or population.

Eysenckian personality dimensions – Three dimensions known as Neuroticism, Extraversion, and Psychoticism which were discovered by Hans Eysenck (1916–1997) and his collaborators through factor analysis. Neuroticism describes emotional instability, depression, and vulnerability; Extraversion describes sociability, excitement-seeking, and activity; and Psychoticism describes aggressiveness, risk-taking, and unusual beliefs.

Factor analysis – A data reduction technique to redescribe the correlations among observed variables with a smaller set of unobserved latent variables.

Five-Factor Model – The predominant model of personality, which holds that five independent dimensions are sufficient to describe individual differences in human behavior, affect, and cognitive styles. These dimensions include Neuroticism (the reverse sometimes labeled Emotional Stability) and Extraversion dimensions similar to those of the Eysenckian model. Other dimensions include Openness to Experience (sometimes labeled ‘Intellect’ or ‘Culture’), embodying intellectual curiosity, fantasy, interest in esthetics, and liberal values); Agreeableness which captures altruism, kindness, modesty, and cooperativeness; and Conscientiousness which involves diligence, orderliness, dutifulness, and achievement-striving.

Orthologous genes – Genes in separate species that are descended from a common ancestral gene.

Personality – Behavioral, emotional, and cognitive dispositions that are consistent across time and situations.

Proximate causation – Genetic, developmental, physiological, and environmental reasons for why a particular phenotype exists.

Quantitative trait locus – Regions of DNA containing or closely linked to genes or transcription regulators that influence a trait.

Temperament – Behavioral and emotional dispositions that emerge early in development and are consistent across time and situations. Because of its restriction to behavioral and emotional expressions and its early

emergence, this term is often used in studies of infants or nonhuman animals.

Traits – Any measured physical, behavioral, emotional, or cognitive characteristic of an individual within a species. In psychology traits specifically refer to nonability measures such as personality or temperament.

Ultimate causation – Evolutionary explanation of why a particular phenotype exists.

Introduction

That animals, like humans, exhibit consistent individual differences in character or personality has been a long-standing observation among pet-owners and researchers alike. Personality refers to mostly stable individual differences in behavioral, affective, and cognitive predispositions. More narrow terms used to describe stable individual differences include temperament and behavioral syndromes, which emphasize measured or observed behaviors to the exclusion of possible cognitive or affective traits. Temperament is an older term which derives from human psychology research and is used to refer to individual behavioral differences that are stable across time and situations, for example, activity. The term ‘behavioral syndrome’ is more recent and has its roots in the study of behavioral ecology. Behavioral syndromes are defined as sets of correlated behaviors that are consistent across multiple situations. This paradigm explicitly ties behavioral variation to differences in growth, reproduction, and survival. While the approaches encompassed by these three terms differ broadly in the kinds of data considered, the species they are used to study, the traits they are compared with, and the background of researchers who practice them, they share a common goal of understanding and explaining differences on the individual level.

One characteristic that distinguishes animal personality, temperament, or behavioral syndromes from other aspects of animal behavior include their bases within individuals. That is to say, these characteristics of individuals are not elicited or caused by environmental situations or schedules of reinforcement, but, instead, indicate how an individual animal typically behaves

across a variety of situations. A second characteristic is their relative stability over time; while there is a considerable amount of debate about how to interpret the magnitude of the effects, most researchers studying human personality would agree that, in adulthood, while there is some change, an individual's standing on a particular personality dimension at one point in time is a fairly good predictor of their standing on that personality dimension at a later point in time. A final, though not often mentioned, characteristic is the emphasis, at least in animal personality or behavioral syndromes research, of studying multiple correlated traits or facets that make up broader dimensions, sometimes referred to as factors, domains, or dimensions.

Measurement of Personality, Temperament, and Behavioral Syndromes

How to measure dimensions of personality, temperament, or behavioral syndromes as well as how to distinguish between relatively good and relatively poor dimensions is crucial to the study of personality in all species. Broadly, there are two approaches to assessing animal personality: ratings and behavioral coding.

Rating Approaches

Ratings-based approaches originate from methods often used in human personality research. They share in common a reliance on judges familiar with the individual animals, the use of questionnaires for rating personality, and asking judges to base their ratings on their overall impressions of the animals. Typically ratings on these questionnaires are made on five- or seven-point Likert scales, true-false questions, or similarly quantitative responses. Some questionnaires are based on psycholinguistic approaches to studying human personality and ask judges to rate the degree to which each animal can be characterized by a series of personality descriptor adjectives (e.g., anxious). In some questionnaires, these adjectives are supplemented by sentences which attempt to resolve ambiguity or define the descriptor within the context of the species' behavior. Other questionnaires are more behaviorally oriented, that is, they ask raters to describe the animal with respect to specific behaviors, such as rate of aggressive interactions, as opposed to descriptor adjectives.

Behavioral Coding Approaches

Behavioral coding is rooted in ethological approaches to the study of behavior and are more commonly used in animal personality research than ratings approaches. They involve actually objectively measuring behaviors,

for example, by recording their frequency or assessing the strength of the response to some stimuli. There are two major subtypes of behavioral coding approaches. One approach can be described as naturalistic. Here, observers watch an animal in its natural environment or a captive setting and record its spontaneously emitted behavior on an ethogram. The second approach can be described as experimental. This method involves changing the animal's environment in some way and recording the animal's response. For example, in novel-object tests a new or unusual object may be introduced into an animal's natural environment or enclosure and the reactions of the animal to the object as well as how closely it approaches the object are recorded. A similar method may categorize the animal's behavior when handled by experimenters.

Reliability

Prior to using a particular measure of animal personality, one must determine its reliability. Reliability refers to the proportion of the animal personality score that is accounted for by the characteristics of the subject, sometimes referred to as true score variance. The remaining variance is referred to as error and is accounted for by any other factors, including specific judges, time of measurement, situational effects, and any other effects.

A common misconception is that the behavioral coding approaches are more reliable than ratings approaches. No studies support this view, and, in fact, a recent study of captive chimpanzee personality directly compared the reliabilities of ratings and behavioral coding methods. This study found that the mean reliability of ratings was substantially higher than the mean reliability of codings derived from focal animal sampling.

Validity

In studying animal personality it is also important to show that a personality measure demonstrates convergent and divergent validity. That is to say, is a personality measure related in expected ways to other measures or outcomes?

Convergent validity

Convergent validity refers to the degree to which a personality measure is correlated with measures or tasks that should tap the same construct. For example, we would expect that a rating measure of extraversion and a behavioral coding measure of time spent in proximity to conspecifics should be positively correlated. In short, measures of the same construct should be related.

Divergent validity

Divergent or discriminant validity refers to the degree to which a personality measure is not correlated with measures or tasks that reflect different constructs. That is to

say, it is indicative of the extent to which supposedly different constructs are distinct. For example, we would expect that a rating measure of neuroticism should only show negligible correlations with measures such as time spent in proximity to conspecifics.

Comprehensiveness

Recently, some have emphasized the importance of comprehensiveness as another criterion by which to judge personality measures. This criterion asks whether a measure captures all the personality differences of the animal or is limited to a select number of domains. A bottom-up behavioral repertoire approach to studying animal personality is one way to address these issues. This method involves behavioral coding and may enable researchers to find domains or dimensions of personality differences that are not revealed by the use of top-down ratings-based approaches, which are seen to impose a personality structure from one species (typically humans) onto another.

Factor Analysis

Factor analysis refers to a family of data analytic methods that can reduce a large number of variables into a smaller set of variables. It is based on the premise that the relationships among a set of manifest variables or traits arise because of a common underlying set of causes or latent variables, which cannot be directly measured. In the study of personality, manifest variables refer to the individual items making up the rating scale or individual behaviors whereas latent variables are personality dimensions such as five-human-like domains found in chimpanzees and the chimpanzee-specific domain of Dominance. These analyses are used in the study of personality in humans and nonhuman animals, and are more commonly used in studies that measure personality via ratings as opposed to behavioral codings.

Exploratory Factor Analysis

Of the two types of factor analytic techniques, exploratory factor analysis is the most commonly used. These techniques consist of methods such as principal-components analysis and principal-axes analysis. These approaches have in common that the researcher does not pre-specify the nature of the latent variables, that is, which items they define. Instead, he or she determines the number of factors believed to be sufficient to explain the intercorrelations among variables, extracts these factors, and then interprets factors based on how strongly items reflect or load on these factors. This last procedure often first involves rotating the factors, which serves to rescale the loadings so that high

loadings are as close to 1 or -1 as possible and low loadings are as close to 0 as possible.

Confirmatory Factor Analysis

An equally important but more seldom used technique is confirmatory factor analysis. Here the researcher pre-specifies the nature of the latent variables, that is, indicates which manifest variables they believe will be explained by which latent factors. The researcher then tests whether the pre-specified factors adequately explain the intercorrelations among items. A researcher may also wish to compare multiple sets of pre-specified factors to determine which best explains the intercorrelations of the items with the fewest possible parameters.

This technique is most commonly used when researchers, based on prior studies using exploratory factor analysis, already have strong ideas concerning the nature of the factors or if they wish to compare the factors of two different samples or species. For example, confirmatory factor analysis was recently used to test whether the rhesus macaque personality factors identified in one sample replicated in a new sample.

Correlates of Animal Personality Measures

Behavior

A fundamental question regarding measures of animal personality, in particular those based on ratings, is whether they are related to behavior. There is considerable evidence in a range of primate species that measures of personality obtained via ratings are, in fact, correlated with behaviors in ways consistent with the definitions of the factors. For example, extraverted zoo chimpanzees are more oriented toward their conspecifics than they are to members of the viewing public whereas this pattern is reversed for introverted chimpanzees; langur monkeys rated as more agreeable engaged in less aggressive behavior and more grooming; and rhesus macaques rated high in sociability in one context engaged in more affiliative behaviors in another context up to 4.5 years later.

In nonprimate species, because measurements are usually made using behavioral coding, the interest instead is finding ecologically meaningful correlates between behavioral domains. In stickleback fish, aggression, activity level, and exploratory behavior were strongly correlated only in populations that were subject to predation. Recently, this research has been extended to include birdsong: male collared flycatchers who scored higher in tests of exploratory behavior or risk-taking behavior sang from lower and higher posts, respectively.

Well-Being

One well-known feature of human personality is its relationship to measures of subjective well-being. Although it is correlated with other personality dimensions, too, on average, more emotionally stable and more extraverted people are happier than less emotionally stable and less extraverted people. The nonhuman work on personality and well-being has been limited to studies of chimpanzees and orangutans. In the case of chimpanzees, greater well-being was positively related to the chimpanzee-specific personality domain, Dominance, a broad factor that incorporates aspects of low neuroticism, high extraversion, low agreeableness, and low conscientiousness. More extraverted and conscientious chimpanzees also were rated as being higher on well-being than their introverted and unconscientious counterparts. A later study of orangutan personality revealed that orangutans who were rated as extraverted, emotionally stable, and agreeable had higher well-being scores than their introverted, neurotic, and disagreeable counterparts.

Health

Human personality is related to health outcomes and in several studies with long follow-up periods, personality dimensions have been related to all-cause mortality. In particular, low conscientiousness is strongly related to earlier death, though high neuroticism, low agreeableness, and low extraversion have also been linked to greater mortality risk. Studies of the relationship between human personality and health outcomes are limited in that ethical considerations bar the experimental procedures that could elucidate the mechanisms underlying the relationships. However, work on this relationship in primate models is making progress in revealing the causal mechanisms underlying these associations.

Corticosteroids

Studies of personality dimensions in nonhuman species have revealed relationships between these dimensions and hormone levels. For example, one study examined adult male rhesus macaques that were assessed on four personality dimensions and plasma cortisol concentration. Consistent with some studies of human neuroticism, this study found that monkeys rated as being higher in excitability had lower levels of basal cortisol in the PM phase. This study also found that higher levels of the confidence dimension were related to significantly higher levels of cortisol throughout the AM phase and higher levels of cortisol throughout the beginning, but not end, of the PM phase.

Also, in a study of cortisol and sedation stress in young chimpanzees, individuals who scored higher on the

behavioral dimension mellow showed higher peak cortisol levels and a more substantial increase in cortisol over time. Another study of juvenile chimpanzees showed some evidence that the same dimension was related to testosterone levels, though the effects of the chimpanzees' actual rank may have been a confound.

Studies of great tit lines selected for being fast explorers or slow explorers that were exposed to stressors (a social defeat in this case) showed that the fast explorers displayed a greater reduction in activity levels than slow explorers. However, slow explorers also showed increases in fecal corticosteroid metabolites, whereas no such increase was revealed among the fast explorers.

Reproduction

Evidence from several studies suggests that personality may be related to variables associated with successful reproduction. Captive-born cheetahs that were rated as being higher in a dimension named tense-fearful were more likely not to breed than those who were rated as being lower on this dimension. Also, in an experimental study, successful mate pairings in dumpling squid were those in which females with intermediate or high scores on a shy-bold dimension were paired with males who also displayed intermediate or high scores on this dimension. On the other hand, shy females were able to successfully mate with shy, intermediate, and bold males. A similar boldness-shyness dimension also leads to differential reproductive success in bighorn sheep; bold ewes exhibit an earlier age at primiparity and greater weaning success. Among red squirrels, female activity level was related to their offspring's growth rate but did not influence dates of birth or litter size. In common lizards, clutch size was found to correlate positively with the ability of females to tolerate conspecifics (a measure of sociality). Social females that can deal with high population densities have more opportunities to mate and reproduce.

Causes

Proximate Causes

Genetic influences

Heritability

Human personality dimensions are approximately 50% heritable, but what does this mean? Roughly, heritability is the proportion of variation in a trait between individuals in the same population that is due to genes that are passed down from parents. Heritability is not a measure of how biological a trait is, only how much of the differences between individuals can be accounted for by differences in genes.

The importance of heritability for the study of personality is in informing both the proximate and

ultimate causes of individual differences. Low heritability may suggest strong selection for a particular level of a personality trait. Estimates of heritability vary wildly between species, traits, and even between studies of the same trait in the same species. On the low end, boldness and aggression in three-spined sticklebacks has been found to have no heritability while dominance in chimpanzees was estimated to be 66%. Like any trait, the heritability of personality is a function of both selection and the amount of genetic and environmental variability underlying it.

Genomics

Two genome-wide scans have been carried out on humans to detect quantitative trait loci (QTL) connected with personality differences. One study used linkage with microsatellite markers to detect larger regions of each chromosome that explained variance in Psychoticism, Extraversion, Neuroticism, and social desirability as measured by the Junior Eysenck Personality Questionnaire. A second study conducted a genome-wide association scan and measured the personality traits Neuroticism, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness using the Revised NEO Personality Inventory and identified several biologically plausible genes associated with each dimension.

Several other studies have considered personality or personality-like traits individually, particularly in conjunction with psychiatric disorders. The inability to replicate results from genome-wide association studies is not a fatal flaw for this type of research but simply an indication that other physiological and developmental evidence will be needed to determine how specific genetic variants result in personality and behavioral differences. A larger challenge is that the genes identified thus far explain such a small amount of phenotypic variance (on the order of 1%). Personality is indeed a complex trait in this sense, a product of many genes interacting with the environment.

Because of the complex genetic architecture of personality in humans, researchers often turn to model organisms, particularly mice. The strategy is to identify genes in the model species that are descended from a common ancestor with humans (i.e., the genes are orthologs). The assumption is that the orthologous genes carry out the same (or similar) functions in both species. Because the ultimate aim of studying individual differences with model organisms is usually in understanding psychiatric disorders, the focus is usually on traits such as neuroticism (sometimes called emotionality) that have strong correlates with anxiety- and depression-related disorders. For example, a region of chromosome 1 associated with emotionality in mice (as measured in novel-environment tests) was compared with the orthologous region that has been implicated in human neuroticism. A

key result from this comparison was that the exact disease-causing gene variants are unlikely to be shared across species.

A further outcome of investigations with mice is to separate genetically clusters of related behavioral phenotypes such as activity in open-field tests and mazes that are assumed to measure a single trait: anxiety. QTL analysis revealed a dimensionality of anxiety-related behaviors that was lost in a factor analysis of the phenotypic data.

Neurotransmitters and enzymes

There is mixed evidence for the role that variation in specific neurotransmitter transporters and receptors and related enzymes play in personality and behavior. Most work has centered around a small set of genes and their promotor: serotonin transporter (5-HTT or SLC6A4), dopamine receptor D4 (DRD4), and monoamine oxidase A (MAOA). Polymorphism in the serotonin transporter gene and its promoter region (5-HTTLPR) have been linked to neuroticism in humans and emotion in rhesus macaques. Variation in DRD4 has been associated with novelty-seeking in great tits, humans, and vervet monkeys, among other species. MAOA is a catalyst that breaks down the neurotransmitters serotonin, epinephrine, and norepinephrine. It has been linked to aggressive behavior in humans and several species of macaques.

However, various follow-up studies with humans have failed to replicate a link between 5-HTT and neuroticism and DRD4 and novelty seeking or extraversion. Rather than completely overturning the behavioral associations of these genes, these mixed results suggest that the pathway between genes and behavior is more complicated and not the action of single genes. Similarly, populations may differ in which gene variants are responsible for differences in personality and behavior.

Gene × environment interactions

The possibility that the environment may moderate the influence of genes on personality development has often been discussed and recently been explored. One area of emphasis has been on the MAOA gene. Low-activity MAOA genotypes have been related to aggression, anti-social behavior, and conduct disorder in humans. An orthologous gene (rhMAOA-LPR) was subsequently identified in rhesus macaques. A recent study of male rhesus macaques suggested that the effect of the low-activity genotype may be sensitive to the early rearing environment: mother-reared macaques with the low-activity genotype had higher aggression scores than mother-reared macaques with the high-activity genotype; peer-reared macaques with the high-activity genotype had higher aggression scores than peer-reared macaques with the low-activity genotype.

Ultimate Causes

Understanding variation in personality involves not just dissecting the genetic and environmental influences, but in discovering why such differences exist in the first place. This is a puzzle, namely because under direct selection one would expect the additive genetic variance of a trait to decrease. As such, multiple hypotheses have been offered to explain the maintenance of heritable variation in personality.

Neutral theory

The first possibility is that variation in behavioral, affective, and cognitive predispositions is the result of neutral evolution. Under this mode, all differences in a trait are due to mutations that are fixed or lost in the population through random genetic drift. The phenotypes resulting from each allele all have equal fitness, so there is no selection on the trait. However, the evidence connecting personality with differences in reproduction and health outcomes makes it unlikely that personality differences have zero fitness costs or benefits. Selection would have to be extremely weak to be overcome by genetic drift. More directly, there are many cases where, when measured, selection on personality traits have been found to be moderate. However, the neutral theory is not completely irrelevant for the evolution of personality. First, it reminds us that the origin of all genetic variation lies ultimately with mutation. Second, the neutral theory remains the null hypothesis against which all evolutionary propositions are tested.

Mutation-selection balance and balancing selection

After random drift, the next mechanism to consider is a balance between selection and mutation. If there is an optimal value of a personality trait (e.g., being moderate in neuroticism) that maximizes fitness, selection will move the population mean toward that optimal value. This elimination of genetic variation in the trait is balanced by the introduction of new variation via mutation. However, many characteristics of personality variation do not fit with the predictions of this explanation.

Another possibility that is better supported by the evidence is that some form of balancing selection accounts for the variation in personality traits. Thus, differing levels of the personality trait exist because each is related, in some way, to fitness benefits and costs. For example, in a species where females favor aggressive males, the benefit of being higher in some aggression-related trait may be offset by the higher likelihood of injury or death resulting from fights. Another example involves differences in life history within species, that is, that different levels of personality dimensions are

related to trade-offs between number of offspring reproduced and lifespan of the parent or viability of the offspring.

Environmental niches

Another possible way in which genetic variation in personality might be maintained is because the environment offers a multitude of niches in which different levels of personality may be successful. Thus, in a population there may be ways for individuals along all levels of extraversion or any other dimension to successfully survive and reproduce. Environmental heterogeneity can also lead to differing selection pressures among populations, such as whether stickleback behaviors were correlated resulted from differing predation rates. In addition to spatial variation, individual differences can also be maintained by temporal variation in selection pressure. For example, bold ewes are less susceptible to predation by cougars, but this only results in appreciable fitness differences when predation is high.

Conclusion

The abundance of individual differences is the only clear result that is consistent across all species where personality has been examined. The genetic architecture underlying personality and how its variation is being maintained are likely to differ between species, and a major goal will be to identify the broad patterns that explain the ultimate and proximate causes of individual behavioral proclivities. Measuring and validating personality and dissecting its complex genetic basis are key challenges in this area. What personality is and how best to study it will continue to provide much fuel for debate.

See also: Psychosocial Influences on Immunity; Molecular Psychology of Personality.

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Relevant Websites

- <http://ipip.ori.org> – International Personality Item Pool webpage.
- <http://www.primate-personality.net> – Jana Uher's Primate Personality and Social Relationships webpage.
- <http://www.animalpersonality.org> – Webpage of the Animal Personality Institute.

Behavioral Development and Socialization

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Glossary

Epigenesis – While different disciplines have different slants on the meaning of this term, what they all share in common is an attempt to capture the idea that developmental processes are context-dependent. In this view, gene products arise not only through the action of those genes, but also as a result of interactions with other genes as well as with nongenetic factors.

Evolutionary developmental biology – The core idea of this field of inquiry is that evolution acts through inherited changes in the development of the organism. From this perspective, the mechanisms that give rise to organisms over the course of their development are crucial for a full understanding of how evolutionary changes across generations are possible.

Homeostasis – This refers to the bodily mechanisms that regulate a number of physiological variables, such as blood pressure and blood glucose levels, within the narrow limits that the body requires. Such homeostatic regulation depends on negative feedback, where the output of the system affects the input. For example, an increased CO₂ level in the blood can lead to an increase in ventilation rate which, in turn, reduces the CO₂ level to the physiologically more appropriate level.

and nurture often subtly move to the conclusion that if we dig deeply enough we will find that the fundamental difference is made by a gene or a particular environmental input. Both conclusions are correct: hold the environment constant and we will find a critical contribution by a gene, and hold the genetics constant and we will find a critical contribution by the environment. Genes have evolved to function in a particular context, and that context includes other genes, subcellular and cellular processes, wider intra-organismic as well as inter-organismic interactions, and then, all manner of environmental influences from the physical world; that is, the epigenetic context within which genes functions are not only interesting, but also essential to our understanding of development. That epigenetic processes can have a profound influence on development is shown by the work arising from several laboratories that demonstrate that early experiences can be transmitted across generations. Molecular mechanisms, triggered by specific experiences, lead to particular genes being activated or inactivated and, in turn, these are transmitted to the next generation in their altered form. Therefore, it seems somewhat irrelevant to label any critical component of the network as more critical than any other, as all of the pieces working together are needed. Given this complex array of interactions, an important issue for the study of development, especially from an ethological perspective, is how these myriad influences manage to coalesce so as to produce consistent species-specific behavior, generation after generation.

A classic study, done by Gilbert Gottlieb, shows the subtle ways in which developmental context can lead to stable and consistent behavior across members of the same species. Upon hatching, ducklings are attracted to the species-typical maternal call. By rearing eggs so that the developing embryos do not experience hearing adult calls, such hatchlings are, nonetheless, able to show a clear preference for their species-typical maternal call. A lesser researcher would have jumped to the conclusion that the development of responsiveness to species-typical calls is impervious to environmental information. However, through an ingenious series of experiments, Gottlieb showed that the development of this responsiveness is dependent on hearing vocalizations, be they those of ducklings from neighboring eggs or those generated by the duckling itself. There are two major conclusions arising from these studies.

Introduction

For much of the last century, the study of behavioral development, similar to development more generally, was plagued by the nature/nurture problem. The problem of development was perceived as being one of apportioning the contributions of the genes (nature) and the contribution of the environment (nurture). By the end of the twentieth century and the beginning of the twenty-first, it became commonplace to accept that the nature-nurture debate had ended. Everyone seemed to be in agreement that development involves a continuous interaction of genetic and environmental information and that we should be focusing our attention on the interactive processes involved.

All modern textbooks on behavioral neuroscience and animal behavior subscribe to this interactive perspective. Interestingly though, even books written to expound the importance of the interactive relationship between nature

When Gottlieb examined the vocalizations of the developing ducklings, he found that they do not have any of the properties of the species-typical maternal calls. That is, experiences seemingly unrelated to what is being developed are necessary for that development to proceed. Many studies have confirmed this counterintuitive conclusion. For example, hatchling chicks denied of the opportunity to observe their own toes, fail to develop the ability to discriminate between food and nonfood items, and infant squirrel monkeys denied of the opportunity to feed on live insects, fail to develop the capacity to fear snakes. Most researchers are now sensitive to the fact that seemingly unrelated experiences may impinge on the development of the particular system they are studying, and that context matters when it comes to developing species-typical behavior. However, there is an underappreciated lesson to be learned from Gottlieb's work.

Consider the testing paradigm used by Gottlieb: a duckling is placed in a chamber with a speaker at each end, which gives the duckling a choice as to which sound to approach. The behavioral measure used is how much of the test period is spent near one speaker versus the other. In this context, what Gottlieb demonstrated is that ducklings exposed to their own vocalizations show a stronger preference for the species-typical maternal call than ones that hear no vocalizations whatsoever, and ones that hear their own vocalizations as well as those that emanate from neighboring eggs, show an even stronger preference. Finally, those exposed to their own vocalizations, those of their neighbors as well as those of adults, show the strongest preference of all. What Gottlieb did not report is that under altered environmental conditions, including that of not hearing anything, ducklings developed species-atypical behavior, such as showing avoidance of calls, or, for that matter, doing somersaults when hearing a call! While the environmental information affected the strength of the responsiveness to the species-typical maternal call, it did not create behavior unrelated to the theme of responsiveness to adult calls. In other words, development is constrained to generate particular themes, with contextual information – be it genetic or environmental – being used to achieve that end.

Passive and Active Forms of Constrained Developmental Change

An early proponent of the view that there are homeostatic processes that constrain developing systems to establish and maintain particular themes was Conrad Waddington, with his concept of canalization. He conceived of development to be similar to a ball rolling down a hill with grooves and protuberances; as the ball travels down hill, it is channeled to move in the paths of least resistance. The

height and angle of the slopes of the grooves ensure that once a ball begins a particular trajectory, it is difficult to shift to another. Studies have identified that evolved contexts can contain passive constraints that ensure that B can only occur because A has occurred or is present. However, in some situations, there may also be active processes that steer development along particular paths, compensating for the potentially disruptive influences of mild to moderately abnormal genetic or environmental information.

A beautiful example of the passive-contextual situation is illustrated by Ton Groothuis' studies on the development of social displays in black-headed gulls. Shortly after hatching, young gulls from all the nests in the colony cluster together and form a large, dense flock. A characteristic of these large gatherings of immature gulls is that they squabble: they peck and threaten one another. As the young ones grow, the rich repertoire of body signals and vocalizations that are used for communication as adults, gradually mature. If the young gulls are denied of the opportunity to squabble, the development of these signals is retarded or even incomplete. Although a logical inference would be that during the squabbling, the gulls are gaining valuable feedback from their opponents that helps shape the final form of the signals, it appears that the squabbling between the young gulls does not affect development in this direct manner. Rather, the squabbling elevates the circulating levels of testosterone in young gulls, and it is this extra testosterone that facilitates the development of their signals, probably by promoting the development of the relevant neural circuits. When young gulls, not housed socially, are treated with injections of testosterone, they not only develop the signals in the absence of squabbling, but they also do so prematurely – that is, at a younger age. Therefore, the reliable and normally present occurrence of squabbling ensures that the relevant hormones are kept at an appropriate level to effect the development of signaling behavior.

For some developmental processes, there may be active processes that compensate for disturbances in information. Consider the following possibility: embryos of all the vertebrate classes perform spontaneous movements. In experiments with chickens where these movements are inhibited, such as by injections of curare, a muscle paralyzing agent, the feet become gnarled and curled up, into postures similar to the 'clubfoot' that can occur in human infants. Indeed, clubfoot in humans is associated with a reduction in the experience of embryonic movements. Genetic mutations known to curtail such movements produce these abnormalities in the limb extremities, as does their arrest via environmentally induced means. Thus, whether due to genetic or environmental disturbance, disruption of embryonic movements leads to abnormal development in the joints of the feet. In

mammals, these movements begin by being produced spontaneously, but later, are also inducible by stimuli emanating from the embryo's own movements and those of its mother. In fish, they appear to remain primarily self-generated. Such class-level differences may arise from differences in opportunities for external stimulation to provide a reliable source of movement induction. These class-level differences illustrate how a reliably available environmental context can substitute for intrinsically driven behavioral change. Importantly, multiple sources of information can converge on the same processes to generate developmental changes – in this case, the formation of appropriately sculptured limb joints. But what are these common processes?

A clue to this question comes from understanding how collagen sheaths develop in the body muscles of fish. Collagen, a protein, is the major constituent of the fibrous network that encases the body in skin, and that forms the connective tissues, such as tendons, that link muscles to bones. Such fibers are under the dynamic control of a regulatory process. Fibroblasts are cells that move along collagen fibers and monitor the tension of those fibers. There is a range of stretching in such fibers that does not induce fibroblast action, but if the tension on the fibers is above or below that range, adaptive changes arise. Strands of collagen fibers that are stretched above a certain threshold are reinforced by adding more fibers and ones that are slack, below that threshold, are eroded. Thus, fibroblasts actively sculpt collagen fibers via a simple homeostatic device. Similarly, the collagen sheaths that separate the bundles of muscles (somites) along the body of a fish are shaped by the tensions produced by those muscles. During development, the spontaneous movements generate the forces that stretch these collagen sheaths, and so produce the context upon which fibroblasts monitor the differences in tension and so modify the development of the sheaths. Species-typical spontaneous movements acting on species-typical body morphology generate forces that induce the fibroblasts to sculpt the collagen sheaths into species-typical patterns. In turn, these developmental changes in sheath size, shape, and orientation lead to the development of muscle orientations that produce the species-typical patterns of force generation during swimming in posthatching fish. Again, either genetic mutations or altered environmental context can alter the production of the forces needed for the fibroblasts to do their job. Thus, if the disturbance is above the threshold that can be compensated for by the core homeostatic processes regulating development (the walls of Waddington's canals), then abnormal developmental outcomes emerge.

If the disturbances are within the tolerable range that the homeostatic mechanisms can provide compensatory change, then novel, but still functional, outcomes may arise. An intriguing example of this possibility has been

recently shown for the change in the beak size of Galapagos finches. A small, genetic change, leading to the prolonged production of a growth factor influencing embryonic beak development, leads to birds with larger beaks. However, while the growth factor influences the length of the beak, there are also compensatory changes that lead to beaks being deeper and alterations to the skull and muscles that accommodate these enlarged beaks. All these additional reconfigurations of beaks, skulls, muscles, and connecting tissues arise in compensation for the one change in the direction of the beak's growth; that is, the homeostatic mechanisms are sufficient to compensate for the one disturbance and produce a viable phenotype. This example illustrates that not all changes need to be specified by specific genes or environmental inputs, and that intrinsic homeostatic mechanisms are able to reorganize the developing system. In addition, it illustrates how developmental processes may play a key role in the evolution of new forms – small changes can modify how a common developmental tool-kit functions, and so how novel phenotypes can be generated. Applying the lessons that are being learned from the new field of evolutionary developmental biology (evo-devo) – which has been mostly focused on identifying the genetic side of that tool kit – to the evolution and development of behavior is still in its infancy, but this is certainly an exciting direction for the field to explore.

The homeostatic mechanisms, such as the fibroblasts monitoring tension in connective fibers, which enable species-typical organization to unfold during morphological development, have not yet been a major focus of study in behavioral development, and it is not until they are that we will be completely liberated from the temptation to subscribe a developmental event to the action of a gene or an environmental input. But recognizing that behavioral development, just like morphological development, involves physiological mechanisms that maintain stable homeostatic systems, is an important beginning, as it changes thinking of behavior as a privileged trait to one that is as biological as any other. Mechanisms that can modify the development of morphology are thus likely to apply to behavior also.

Types of Environmental Influences on the Development of Behavior

The example of embryonic movements alerts us to another valuable distinction when thinking about environmental influences on behavior. Fetal immobilization in young mammals, be they rats or humans, leads not only to abnormal joints, but also to a variety of other abnormalities, such as smallish lungs and stomachs. That is, embryonic movements are involved in the development of the structures engaged in the movement – the

move-your-feet-to-sculpt-the-joints-in-your-feet link – and ones not directly involved in the generation of the movements, lungs, and gastrointestinal tracts. The training of the system being performed makes sense, and such a direct link can focus our attention on the processes likely involved in the developmental transformation. This is not true for by-products that have no obvious connection – not only are we puzzled as to why the stomach is affected, but we are also ignorant as to why the absence of embryonic movements should lead to smaller, rather than larger, stomachs. Nonetheless, it is useful to separate those influences – be they genetic or environmental – that directly affect the developing system under study versus ones that do not. Perhaps, as our knowledge grows, we will one day be able to characterize meta-mechanisms that are triggered by these inputs and so understand the connections between them. We are not there yet, but at the conclusion of this article, an example of such a possible meta-mechanism is provided.

In our present state of ignorance, it is helpful to classify those behavioral systems directly sculpted by particular inputs and those indirectly influenced by such inputs. A useful term, proposed by Jerry Hogan, to capture this distinction is ‘prefunctional.’ A prefunctional behavior is one that needs not be rehearsed before it is expressed in its normal form – as was the case for the gull social signals. In other cases, future action is only possible because it was shaped by action-relevant experiences earlier in development – the proper formation of the limb joints arising from embryonic movements is an example. Both types of developmental outcomes, prefunctional and functional, likely depend on a host of indirect influences from environmental (and genetic) information. These indirect influences are well illustrated by the effects of embryonic movements on the development of internal organ size. Thus, by stating that a behavior is prefunctional is informative, without being burdened by any implication that it develops independently of environmental influences, as would be the case for most meanings of the terms ‘innate’ or ‘instinct.’ Prefunctional and functional components of behavior and their development are illustrated by work done on the development of dust bathing in chickens, a behavior that is used to remove excess oils and parasites from the feathers.

The dust-bathing sequence begins with the chicken scratching and pecking at the substrate. The chicken first drops one wing and then the other, while simultaneously shuffling its feathers. It then rolls on the ground, first onto one side, then the other, and finally squats, with its body feathers raised. More shuffling ensures that the dust released by the pecking, scratching, and wing movements is passed through the feathers to their base. Finally, the chicken stands up and gives its body a good shake to rid it of the excess dust. From hatching, it takes about 2 weeks for all of these behavioral elements to emerge, and

they appear in the chick in the order in which they are performed in the adult in the completed sequence. Thus, scratching the substrate emerges earlier in development than rolling onto the flanks. Furthermore, as these behavioral components emerge, the chicks are indifferent as to whether they are standing on sand or dust or on a wire-mesh floor devoid of any usable particles. That is, the behavioral components of dust bathing emerge independently of functionally useful feedback from the environment. In this sense, the actions of dust bathing emerge prefunctionally.

In contrast, after these behavior patterns emerge, the substrate on which the birds are reared can influence subsequent substrate preferences for dust bathing. Chicks raised with wood shavings prefer wood shavings to other possible substrates, such as sand or coal dust, and conversely, chicks raised on a substrate of coal dust prefer coal dust to other options. Clearly, the functional feedback experienced from the particular substrate material used influences the bird’s preferences for later substrate selection. Because both perceptual preferences for substrates and behavior-pattern formation may ultimately be traced back to requiring some important, indirectly acting, environmental input would seem to be confounding very different mechanisms. Rather, the identification of behavior that develops prefunctionally versus functionally provides us with valuable information as to the types of mechanisms that are likely to be involved and provides us with a starting point for designing research strategies.

Prefunctional versus Functional Developmental Change

The development of playful fighting in laboratory rats is sufficiently well understood to provide a useful illustration of how these distinctions can guide us in looking for specific mechanisms. Play fighting in rats involves attack and defense of the nape of the neck that, if contacted, is gently nuzzled with the snout ([Figure 1](#)). Such play fighting emerges in late infancy, just before weaning, peaks in the juvenile period and then continues well into adulthood, albeit at a low frequency. Similarly, there are age-related changes in the tactics of attack and defense that are used over this time period. These age-related patterns appear to unfold in the same manner whether animals have had prior experience with play fighting or not. That is, play fighting and the age-related changes that it undergoes are prefunctional. This does not mean that these changes are not dependent on environmental inputs.

In the case of age-related changes in the tactics of attack and defense, gonadal hormones and specific brain mechanisms are critical for the patterns to unfold as they do. In contrast, while gonadal hormones are important for determining the size of the juvenile peak in the frequency

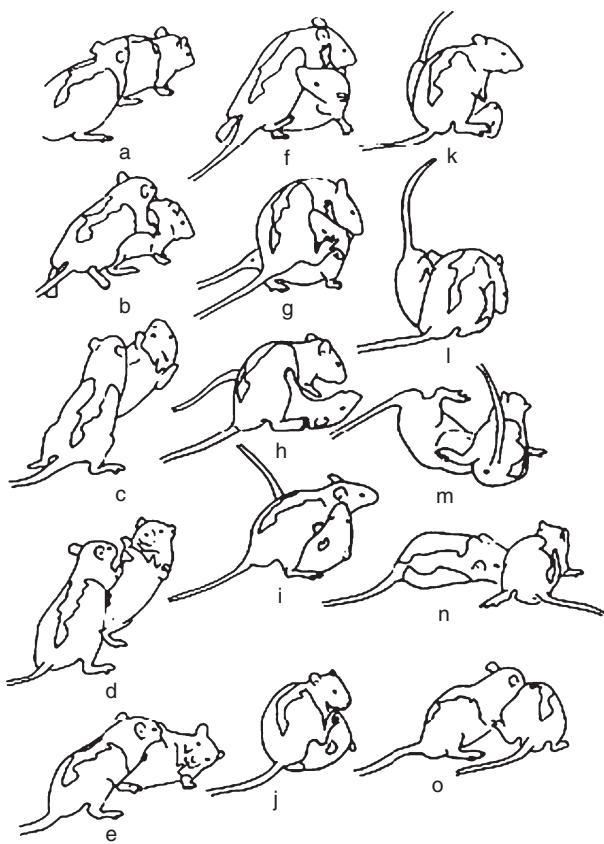


Figure 1 Two juvenile male rats, at about 35 days old, are shown engaging in a play fight. Note that the rat on the left lunges to reach the nape and the recipient acts to defend itself from such contact (a–h). Even when the defending rat is lying on its back, both it and its on-top partner continue to lunge at each other's napes (h–k). Finally, once the original defender pushes the attacker over and regains its standing posture, it, in turn, lunges at its partner's nape (l–o), continuing the play fight. From Pellis SM and Pellis VC (1987) Play-fighting differs from serious fighting in both target of attack and tactics of fighting in the laboratory rat *Rattus norvegicus*. *Aggressive Behavior*, 13(4): 227–242. © 1987, Wiley; reprinted with permission of Wiley-Liss, Inc. a subsidiary of John Wiley and Sons, Inc.

of play, they do not determine the shape of the age-related pattern. However, the experience of play fighting in the juvenile period has been shown to be critical for the development of postpubertal social skills. As young adults, male rats modify their play fighting and other social responses depending on the status of their partner – dominant males are responded to differently than to other subordinate males and females. Play-experience-dependent changes in social skills have been demonstrated to involve correlated changes in the anatomy of the neurons of the prefrontal cortex. Therefore, the development of social skills is functional, depending on experiences derived from engaging in social behavior. Indeed, the play fighting occurring in the juvenile period is organized differently to that which appears earlier or

later in development, and based on the form of those organizational differences, it is reasonable to infer that the pattern of play present in the juvenile period is organized so as to enhance the experiences that are relevant to the development of social skills. But these age-related changes in the organization of play fighting are not themselves dependent on functional experience to emerge as they do in the juvenile period. Thus, prefunctional changes in the organization of behavior are critical for the functional development of social skills.

It is worth reiterating that the prefunctional changes in play that emerge during the juvenile period are linked to particular environmental inputs; in this case, those inputs reside in the animals' own bodies in the form of particular levels of circulating gonadal hormones. But similar to other cases of prefunctional behavioral development, be it Gottlieb's ducklings, Groothuis' gulls, or changes in gut size in the absence of embryonic movement, knowing that there are indirect environmental influences on development is incomplete as an explanation for development. We still need to understand why those indirect environmental inputs produce the outcomes that they do, rather than a near infinite number of other possible outcomes. For behavioral changes that are functionally linked to behavioral development, the relevant environmental inputs usually have a more direct influence, shaping motoric, motivational, or perceptual capabilities that bias them to work one way rather than another. Practicing tennis, for example, can lead to a better performance on the court during an actual game, and some of this improvement can likely be traced to improvements in hand–eye coordination, but if practicing tennis improves chess playing, then where do we begin looking for the causal links? Thus, the distinctions between prefunctional and functional are sometimes a helpful guide to the researcher.

Conclusion

Instead of worrying about the sources of information relevant to behavioral development, we should focus on understanding the mechanisms by which information is incorporated into developing organisms and integrated to produce the outcomes that they do. Consider the classic work on visual development of mammals. The alternating neural columns on the visual cortex of a mammal, such as a cat, develop in response to competing inputs from the retinas of the two eyes. Thus, visual input is necessary for pattern formation in the cortex. However, before the eyes open, binocular anatomical patterning is developed in a part of the thalamus that receives input from the eyes (the lateral geniculate nucleus (LGN)), the relay point for visual information reaching the visual cortex. While visual information is not necessary in this case, information from the retina is needed. In this case, instead of light stimulating

the retinas, at the right age, the retina begins to be spontaneously active. Therefore, the patterning of both the LGN and the visual cortex depends on activity in the retina, but whereas for the LGN retinal activity is independent of incoming light, for the visual cortex, that retinal activity is triggered by light entering the eyes. It is possible that for the LGN spontaneous retinal activity is triggered by turning on some particular gene or cascade of genes, but what is important here? To understand the developmental changes in the brain, whether the source of the information or the common mechanism by which activity in retinal cells shapes the organization of neurons deeper in the brain?

The source of the critical information may be important in understanding the full context of the interacting developmental networks, and may also help guide researchers as to where to look next. However, the example of the patterning of the LGN and the visual cortex clearly shows that this is only a small part of the story. The processes by which organisms integrate information, and transform that information into particular outcomes is by far the bigger, and, as yet, less explored part of the story. The development of brains, not unlike the development of connective tissues, probably involves passive, contextually dependent processes, as well as active homeostatic mechanisms. The challenge for the future study of behavioral development is to identify and characterize those organismal processes that construct behavior in particular ways.

See also: Infant Bonding and Attachment; Social Bonding and Attachment.

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Birdsong and Vocal Learning during Development

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Glossary

- Imitation** – A song that is a good copy of a tutor song.
- Improvisation** – A song that resembles a tutor song, but which is substantially different in certain respects.
- Invention** – A song which cannot be traced to a tutor song.
- Sensitive period** – A relatively short period early in life when the bird is receptive to song memorization.
- Sensorimotor phase** – The phase during which the bird sings and tries to match its output to earlier-memorized songs; follows or overlaps the sensory phase.
- Sensory phase** – The phase of song learning during which the bird memorizes tutor song.
- Song** – A relatively complex vocalization used in interactions with males and/or female conspecifics. A single song (or strophe) is usually relatively short (usually 2–4 s) and is separated by a longer period of silence before the next song. In some cases, birds sing more continuously and individual songs can be quite long (e.g., 20 s or more in sedge warblers).
- Song crystallization** – A developmental phase in songbirds in which there is a transition in song from poorly structured vocal material (subsong or plastic song) to stereotyped, well-structured song patterns that are typical of adult birds.
- Song learning program** – The genetic-developmental program thought to underlie song learning in a species (or a race or population of a species).
- Song repertoire** – Defined in terms of song types or elements. Most birds form song elements into stable song types, but others (e.g., sedge warblers) improvise songs from their repertoire of song elements.

Bird Song as a Model System

Songbirds (oscine passerines) are one of the few animal groups in which individuals learn their vocal signals. Vocal learning has been found so far only in the humans, two other mammalian groups (cetaceans and bats), and two other avian groups (parrots and hummingbirds). Although other animal groups capable of vocal learning will probably be discovered in the coming years, vocal learning is clearly the exception in the animal kingdom: in most animals, individuals develop perfectly normal

species-typical vocal signals without having to hear models of these signals. Among vocal learners, songbirds stand out because of their complex vocal repertoires. Some songbirds show extraordinary powers of vocal mimicry, far exceeding human capabilities.

In the 1970s, it was discovered that songbirds possess a distinctive set of neural circuits dedicated to their vocal communication system. Research has since attempted to parse out the roles of different parts of the system in the perception, production, memorization, and learning of vocal signals. The bird song system has become a major model system in neurobiology in part because of its potential to provide insights into the neural mechanisms of learning and memory. The discovery of neurogenesis within the adult songbird song system, the first unambiguous demonstration of neurogenesis within the adult vertebrate central nervous system, was a key element in the establishment of the bird song system as a model system in neurobiology.

Parallels between Bird Song Learning and Human Language Learning

Yet another reason for the interest in the bird song system as a general model system is the number of parallels between vocal learning by songbirds and language learning by humans. These parallels were first pointed out by Peter Marler in a seminal 1970 paper. Six of these parallels are included here, with an additional parallel – social context – which will be discussed further in the section titled “Comparative differences in song bird learning.”

1. In both songbirds and humans, the vocal communication signals are learned.
2. This learning takes place during a sensitive period that begins soon after birth or hatching and concludes sometime later. There is some uncertainty as to when and how completely the sensitive period closes in humans, but it is generally accepted that second-language learning, unless started early in life, is considerably more difficult than first-language learning. The sensitive period for songbirds depends on the species, and is discussed in the section titled “Comparative differences in song bird learning.”
3. Dedicated neural systems underlie the perception, memorization, learning, analysis, and production of the vocal signals.

4. The sensory-memorization phase precedes the production phase. For example, infants understand more than they can say. Some songbirds do not even begin to practice vocalizing until they have memorized all the songs they will sing.
5. Related to the previous point, there is a babbling or subsong phase in which the individual practices vocalizing. The vocalizer presumably matches these productions to the models that were memorized earlier. The signals are refined until they ultimately reach the adult level.
6. Auditory feedback is essential for vocal learning, at least until the normal vocal repertoire has crystallized. Although deafening after this period can cause degradation of vocal signaling, auditory feedback after crystallization is generally less critical for maintenance of the vocal repertoire.
7. Vocal learning is inherently a social process.

Vocal Learning is a Social Process

The fact that people around the globe learn different languages is of course sufficient evidence that language is learned. The additional hypothesis that language must be learned in a social context seems self-evident. On the rare occasions that an individual has been isolated from human contact during development, language is inevitably deficient, but this of course is just one of the myriad clinical problems shown by such individuals. Extrapolation of this isolation test to songbirds is sometimes referred to as a Kaspar Hauser experiment, named after one such unfortunate human (although the story is partly apocryphal). Most songbirds when isolated from the sights and sounds of other birds from hatching until maturity develop abnormal songs (some exceptions are discussed below). Presentation of tape-recorded song to the isolated bird is often sufficient, however, for the bird to develop normal species song. It is this last fact that led many investigators to overlook this importance of social context for bird song learning.

While the importance of social contact is obvious for language learning in humans, the early studies of song learning in songbirds explicitly excluded social factors. They did so for both theoretical and experimental reasons. The original theoretical conception of song learning was derived from the classical ethological concept of imprinting, translated into the song-learning context by Thorpe, and then fully developed in the experiments of Marler and his colleagues. By analogy to the classical imprinting studies, it was supposed that the key stimuli for song learning would be very basic, processed by species-specific filtering mechanisms and that learning would occur during an early sensitive period. This view provided the rationale for the ‘tape tutor’ experiment, in

which all aspects of the species- and population-typical song-learning context were removed, the bird being isolated shortly after hatching and hearing song only through a loudspeaker in an isolation chamber. Besides fitting the theoretical view, the tape-tutor experiment also unquestionably provided more experimental control than would be possible were actual birds the song tutors. In a classic series of tape-tutor experiments, Marler showed that to develop normal song, a white-crowned sparrow male must hear conspecific song during an early sensitive period (roughly the second month of life); the bird will reject heterospecific song heard during this period, as well as conspecific song heard after the sensitive period.

Workers in the field became aware of the importance of social factors in song learning, however, with the discovery that birds learned more readily from live tutors than from tape-recorded song. Moreover, some of the rules of song learning derived from tape-tutor studies appeared to bend, if not break, when the song tutors were actual birds. For example, whereas tape-tutor studies had indicated that the sensitive period for white-crowned sparrow song learning closes at approximately 50 days, and that heterospecific songs are uniformly rejected, Baptista and Petrinovich showed that if a young white-crowned sparrow was exposed to a tape tutor through 50 days and thereafter exposed to a live tutor, the young bird would learn the song of the live tutor, and in some cases would do so even if he were a heterospecific tutor.

Field studies also provided a major impetus to the study of social factors. Although field studies cannot provide the experimental control of a laboratory study, they naturally bring into focus the social variables that are controlled out of laboratory experiments. To the question of when song learning occurs, field studies added the questions of where and from whom, and have given a new context for the questions of how many, which ones, and how accurately? Researchers doing the first field studies on song learning noted that learning appeared to occur later than indicated by the classical tape-tutor studies, post- rather than pre-dispersal, so that birds wound up learning songs not from their father and birds in the natal area, but from birds in the area where they would breed, often their neighbors of their first breeding season.

Despite the problems raised by field studies and by experiments with social tutors, the basic findings of the classical tape-tutor experiments have not yet been firmly contradicted in any species. In particular, although the sensitive period for song learning may extend much further into the first year for some species than was originally thought, for no species does it appear to be true that song learning is equally possible or equally likely at all points during the bird’s life. Moreover, even if a powerful heterospecific social tutor can overcome it, the preference for conspecific song found in tape-tutor

experiments, at least for some species, does suggest some form of tuning for conspecific song. Nevertheless, comparative studies of songbird species have revealed an amazing diversity in song-learning patterns, both between species and between different populations of the same species, and this diversity should warn us not to take any particular pattern of song learning, for example, that shown by white-crowned sparrows, as typical or fundamental. We return to this point in the section titled ‘Comparative differences in song bird learning’

What are the Social Variables in Song Learning?

Despite the recognition that social factors are critical in song learning, there is little understanding in the field of exactly how social variables shape song learning. The numerous comparisons of live versus tape tutors that have been made are usually indirect and often made across different studies. As researchers have pointed out, it is not at all clear what precise aspects of social stimulation influence song development, and indeed even whether the effects are ‘truly social’.

The difference between results derived from tape-tutor and live-tutor experiments can be viewed from another purely theoretical angle. The tape-tutor and live-tutor paradigms implicitly suggest different models of the song-learning process. The tape-tutor paradigm implies that song learning is essentially a process of overhearing or simple eavesdropping on a singing adult. In contrast, the typical live tutor setup – with the young bird stationed close to a singing adult bird – implies that the fundamental process involves direct interaction of the older bird (song tutor) with the young bird. However, both experimental setups are potentially unnatural: we do not know whether in nature the young bird learns from a song tutor singing solo and out of sight (as implied by the tape-tutor design), from a song tutor who is up close and interactive (as implied by the typical live-tutor design), or, perhaps, in some other way altogether.

A theory developed by Nelson and Marler combines the ‘simple eavesdropping’ and ‘direct interaction’ models of song learning by proposing that the first process describes the early phase of song learning, while the second process describes the later phase of song learning. According to this theory, in the first phase of song learning, the young bird memorizes many songs during the natal summer, many more songs than he will ultimately keep for his final repertoire. In the second, action-based phase of song learning, typically occurring early in the following spring, the bird counter-sings with his new neighbors as he tries to establish a territory, and selects from his earlier-memorized songs those that best match the songs of the birds he is now interacting with. Thus, the

Nelson–Marler theory incorporates the implicit models of both the tape-tutor and live-tutor paradigms: the early, memorization phase of song learning follows the simple eavesdropping model, while the later action-based phase conforms to the direct interaction model.

A third model, the social eavesdropping model, suggests an alternative way in which social interaction might affect song learning. The central idea is that the young bird learns by eavesdropping, not on solo singing, but on singing interactions between two or more birds. Recent field experiments on songbirds have shown that males base their decisions on whom to challenge and females their decisions on whom to mate with on information about the dominance relationship of the singing males, information which they extract when eavesdropping on singing interactions. The social eavesdropping hypothesis proposes that young birds too may use information they extract from singing interactions they overhear to decide which songs to learn or retain. The relative dominance status of the two birds might be one important dimension. This idea is similar to the social modeling theory, as developed by Pepperberg, which suggests that observation by the young bird of communication interactions between individuals who have mastered the communication system may be critical for vocal learning.

There is a second unique type of information a young bird could extract from the interactive singing (counter-singing) of two adults that he could not extract from solo singing of these same birds: contextual information relating to singing rules concerning the appropriate replies to particular songs in particular contexts. We take for granted that humans have to learn the rules of language, but animal communication systems – although simple compared to human language – do have their complexities, and do follow fairly intricate rules. In the study of bird song learning, the focus has always been on the learning of particular songs rather than the learning of how to use them, but the two processes may be intertwined. This is the case for human language learning of course. Although the attention in studies of human language learning has been focused on direct interaction, especially between parent and infant, it is necessarily the case that the infant can potentially learn much more about grammar and the rules of language by eavesdropping on conversations among older individuals.

Some evidence suggests that songbirds may preferentially learn by eavesdropping on singing interactions of other birds. In an experiment on young song sparrows (*Melospiza melodia*) using live tutors, Beecher and colleagues compared two types of song tutoring: that resulting from direct interaction with the song tutor, and that resulting from social eavesdropping, that is, overhearing the singing interactions of other birds. Subjects were exposed to the songs of four tutors during the early memorization phase (phase 1) of song learning and to

just two of them again in the later action-based learning phase (phase 2). Of the two tutors returning in phase 2 one became a subject's interactive tutor, while the other became the subject's overheard tutor, that is, was overheard interacting with another, yoked subject. Subjects learned (retained) more songs from their overheard tutor than their interactive tutor (about twice as many on average). This result is consistent with the social eavesdropping hypothesis, and not the direct interaction hypothesis.

In a totally different approach to the problem in the same species, Templeton and colleagues examined the response of juvenile male song sparrows in the field to simulated adult song contests and solo singing. Songs were presented from two speakers separated by 10 m and 50 m from the bird. Juveniles were more likely to move toward the speaker, approaching closer and more quickly, during the simulated singing interactions between the two birds than during solo song or control playback trials (solo song sparrow and heterospecific song, in fact, were equally unattractive to the birds). These results suggest that juvenile song sparrows are especially interested in eavesdropping song contests and that these types of social interactions may be particularly powerful tutoring events for song learning.

Comparative Differences in Song Bird Learning

The prevalent model of song learning is based on the classic studies of Marler on the white-crowned sparrow (*Zonotrichia leucophrys*). During the sensory or memorization phase, the young bird must hear tutor song, and during the sensorimotor phase, it attempts to match its vocal output to the songs memorized earlier. Learning can be demonstrated by isolating the bird during the sensory phase, or by deafening it just before the sensorimotor phase; both typically produce a bird that sings abnormal song. Since isolation and deafening are extreme manipulations, and because their outcomes are generally considered to be obvious, song learning is usually demonstrated instead by showing that the bird develops songs closely resembling the songs it was tutored on; this method is unambiguous so long as song learning takes the form of simple imitation (copying) of tutor songs (but as we will see, it does not always do so).

Despite the uniformity suggested by the outline of song learning just given, it has been long known that songbird species show many variations on this theme. However, in fact, the diversity of oscine song-learning programs is more extreme than is generally appreciated, and varies along at least the following five dimensions.

1. *When song is learned or how long the song repertoire is modified.* The period during which birds can learn songs ranges widely, from a brief sensitive period in the first few months of life (white-crowned sparrow), to the entire first year (chaffinch, *Fringilla coelebs*, indigo bunting) to throughout the lifetime (village indigobird, *Vidua chalybeata*, great tit, *Parus major*, pied flycatcher, *Ficedula hypoleuca*, willow warbler, *Phylloscopus trochilus*). Species in which birds add songs to their song repertoires after the first calendar year are referred to as open-ended learners, species in which they do not as closed-ended (or age-limited) learners. Although closed-ended learning has generally been the default assumption, in most cases there is no evidence to support this assumption: song-learning experiments typically are not extended beyond the first year, and longitudinal field data are rarely gathered. Thus, species assumed to be closed-ended learners might on closer inspection prove to be open-ended learners, as, for example, McGregor and Krebs discovered for great tits.

2. *How many songs a bird learns.* In about 70% of songbird species studied, males sing multiple song types. These song repertoires range in size from small (e.g., chaffinch, great tit, swamp sparrow, *Melospiza georgiana*, all <5) to moderate (e.g., song sparrow, western meadowlark, *Sturnella neglecta*, 10 or so) to large (e.g., western marsh wren, *Cistothorus palustris*, common nightingale, *Luscinia megarhynchos*, >100) to huge (e.g., brown thrasher, *Toxostoma rufum*, >1000). Small- to moderate-sized repertoires are most common. Several experiments in which species (song sparrows vs. swamp sparrows) or subspecies (eastern vs. western marsh wrens) with different repertoire sizes were raised in a common environment established that differences in repertoire size in these cases were due to genetically based differences in the underlying song-learning programs.

3. *Copying fidelity.* Although imitation is the hallmark of bird song learning – typically it is the criterion by which song learning is assessed – birds do not always copy tutor songs precisely. In different species (and sometimes in different populations of a species), song-learners appear to vary along a continuum ranging from imitation (faithful copying of tutor song), to improvisation (variations on the tutor material) to invention (bird develops species-typical songs that bear no obvious relation to the tutor material, and which might or might not even require song tutoring).

4. *Role of early song experience.* Because bird song learning was first demonstrated by showing that songbirds raised in isolation develop abnormal song, there has been a tendency to assume that isolation-rearing will always have this effect, and hence isolation conditions are usually omitted from song learning experiments. However, isolation does not always produce abnormal song. Three recently discovered examples are the grey catbird, the

sedge warbler, and the canary: these birds generate large, normal song repertoires when raised in song-isolation conditions. These birds probably need to hear themselves sing in order to develop their normal repertoires (though this has not been tested), but it is still surprising that they can develop their large repertoires of good species songs without ever hearing external conspecific song models.

5. Degree of canalization. In white-crowned sparrows and several other species, birds copy tutor material only if it fits tightly constrained species-specific parameters, and in these cases song learning would be classified as environmentally canalized (*sensu* Waddington). Other species are less selective as to what material they will copy for their songs. For example, in a common-environment experiment on two closely related species, Marler and Peters found that when presented with the same tape-recorded regime of song sparrow and swamp sparrow songs, song sparrows will copy heterospecific as well as conspecific elements, but swamp sparrows will not. Other species are capable of copying virtually anything they hear, the best-known examples probably being brown thrashers, northern mockingbirds (*Mimus polyglottos*), marsh warblers (*Acrocephalus palustris*), Indian hill mynahs (*Gracula religiosa*), and superb lyrebirds (*Menura novaehollandiae*).

Conclusion

The study of bird song learning is only about 40 years old, and is still in its relative infancy. Nevertheless, it is a system that has a tremendous potential as a model system, given (1) our considerable knowledge of the neurobiology of the songbird vocal control system, (2) its many parallels with human language learning, and (3) our ability to investigate how the system functions in both the lab and the field. A fourth potential advantage is simultaneously a significant problem: What do we do with the staggering diversity of song-learning programs observed in the songbird group (4000-plus species)? This diversity must give pause to any investigator who would consider

one particular songbird species – say the zebra finch (presently the most popular species in neurobiological studies) – as representing the songbird learning program. The field will need to address this problem and use this comparative variation as a research tool if we are to move into the next phase of this very fruitful research area.

See also: Animal Models of Learning and Memory; Behavioral Development and Socialization; Developmental Neurogenesis; Evolutionary and Developmental Issues in Cognitive Neuroscience; Neurogenesis and Memory; Social Communication; Social Learning and Behavior Transmission.

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Development and Language

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Glossary

Fusiform gyrus – The part of the inferior surface of the temporal lobe that is involved in processing visual word forms (i.e., orthography).

Inferior frontal gyrus – The part of the lateral frontal lobe and consists of three subsections. Pars opercularis is superior and posterior and is involved in phonological processing. Pars orbitalis is anterior and inferior and is involved in semantic processing. Pars triangularis is in between these two subsections and is involved in syntax processing.

Inferior parietal cortex – The part of the lateral parietal lobe that is involved in integration between representations and consists of three subsections. Supramarginal gyrus arches over the upturned end of the lateral fissure, angular gyrus arches over the superior temporal sulcus, and inferior parietal lobule is superior to these two gyri and lies below the intraparietal sulcus.

Middle temporal gyrus – The part of the lateral temporal lobe in between the superior and inferior temporal gyri that is involved in processing verbal meanings (i.e., semantics).

Orthography – The writing system of a language that describes the nature of individual symbols used (e.g., graphemes) and the rules for writing these symbols (e.g., spelling).

Phonology – The sound system of a language that describes the nature of individual sounds used (i.e., phonemes) and the rules for combining these sounds (e.g., speech).

Semantics – The meaning system of a language that not only includes parts of words (i.e., morphemes) and individual words, but can also span phrases, sentences, and discourse.

Superior temporal gyrus – The part of the superior temporal lobe that is involved in processing auditory word forms (i.e., phonology).

Syntax – The grammar system of a language that includes the rules for constructing sentences.

have put forth the interactive specialization approach to functional brain development in humans. According to this approach, over development, there is increasing specialization of brain regions and pathways into systems that have different computational principles. Once these specialized systems have started to develop, there is increasing integration between these systems. This is likely to be a cascaded process because studies have shown that abnormal interactions between systems affect their development. The interactive specialization approach builds upon research on the networks of dynamical systems. Specifically, small-world networks have clusters of tightly interconnected nodes (e.g., specialized areas) with few long-distance connections between nodes (e.g., integration). These networks have several beneficial principles, including enhanced signal-propagation speed, computational power, and synchronizability.

The interactive specialization approach is supported by a body of behavioral and neuroimaging literature. Increasing specialization with development is consistent with the finding that there is synaptogenesis and pruning of synapses over childhood into adolescence that occur at different time points for different brain regions. Increasing specialization is also consistent with meta-analyses showing that brain activation patterns become more focal over development with greater activation of task-relevant regions and less activation of regions irrelevant to task performance. These age-related changes in the brain are consistent with behavioral evidence showing lack of specialization in infants and young children, for example, in the processing of color and motion. Increasing integration with development is consistent with the finding of gradual increases in white matter tracts over childhood into adolescence and with electroencephalogram evidence showing increasing coherence (i.e., correlation) between brain regions. There is also behavioral evidence for increasing integration between specialized systems, for example, in numerical development. In support of developmental changes in both specialization and integration, resting-state functional magnetic resonance imaging (fMRI) has shown age-related decreases in short-range connections suggestive of specialization and age-related increases in long-range connections suggestive of integration. This article presents data from neuroimaging in language and reading which suggest that these processes of specialization and integration characterize functional brain development in humans.

A Model of Development

Some have argued that a single cognitive process may involve the functional integration of many specialized areas. Consistent with this, Johnson and his colleagues

Over the past few years, a working model has been formulated of how auditory and visual word representations may be processed in the brain. This has been done using spelling judgments to index orthographic processing, rhyming judgments to index phonological processing, and meaning-relatedness judgments to index semantic processing, in both the visual and auditory modalities. This model suggests a left lateralized network with orthographic processing involving visual association areas, including fusiform gyrus; phonological processing involving auditory association areas, including superior temporal gyrus; and semantic processing involving amodal association areas, including middle temporal gyrus. Mappings between these representational systems seem to be mediated in part by heteromodal areas in inferior parietal cortex. These posterior systems are strongly interconnected with anterior systems in the frontal lobe that seem to play a critical role in modulating posterior representations. Studies have suggested that the anterior inferior portion of inferior frontal gyrus (i.e., pars orbitalis) is involved in semantic processing, whereas the posterior superior region of inferior frontal gyrus (i.e., pars opercularis) is involved in phonological processing. A region in between (i.e., pars triangularis) seems to be involved in syntax processing.

Representational Systems

Orthography

Behavioral research shows that reading acquisition is marked by greater elaboration of orthographic representations involving increases in the number of lexical representations, in the precision of these representations, and in the interconnectivity among these representations. fMRI studies show age-related increases in activation in fusiform gyrus when presented with words or nonwords in the visual modality (see **Figure 1**), suggesting a greater functional elaboration of this system. In addition, event-related potential (ERP) studies show an amplitude increase from kindergarten to second graders in early occipital responses when reading words and magnetoencephalography (MEG) studies show that early occipitotemporal activation when processing visual words is delayed in children compared to adults. Adults also show greater selective activation than children in left fusiform gyrus when processing visual word forms as compared to auditory word forms, suggesting that adults have a more specialized system for orthographic processing in response to task demands. In contrast to adults, children show greater overlap in brain activation when processing visual or auditory word forms. Although visual word forms seem to become more specialized, studies show increasing connectivity between orthographic and phonological representations over childhood. Altogether,

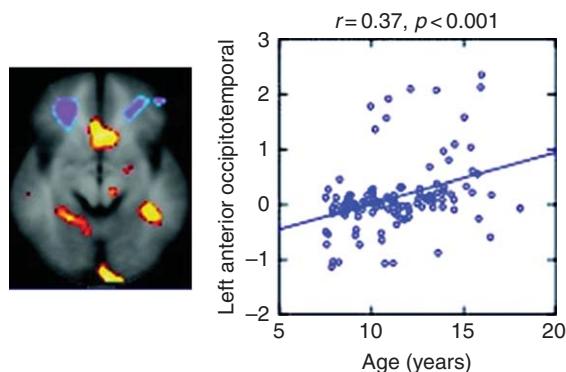


Figure 1 Developmental increases in fusiform gyrus during orthographic processing. Left brain image: correlation map between age and activation during a nonword rhyming task (partialed for accuracy). Areas in red and yellow indicate a positive correlation (note left anterior occipitotemporal cortex), and blue and purple indicate a negative correlation. Right scatter plot: correlation between age and left anterior occipitotemporal region of interest. From Shaywitz BA, Skudlarski P, Holahan JM, et al. (2007) Age-related changes in reading systems of dyslexic children. *Annals of Neurology* 61: 363–370.

these results suggest that orthographic representations become more elaborated and specialized with development, but that they also become more interactive with phonological representations.

Greater elaboration of fusiform gyrus has also been shown in learning studies in adults and greater specialization of fusiform gyrus over development has been demonstrated in other cognitive domains. Training in face recognition results in increased activation in fusiform gyrus, suggesting that elaboration of representations with learning occurs in other cognitive domains. Over development, increasing specialization in fusiform gyrus has also been shown during face processing. Although children and adults show a similar amount of activation in fusiform gyrus, it is distributed differently. Adults show greater activation in right medial as compared to lateral fusiform gyrus, whereas children show about an equal amount of activation in lateral and medial regions. In addition, studies have shown developmental increases in selectivity of responses to faces, natural objects, and manufactured objects. These studies suggest that elaboration and specialization of representations may be general characteristics of learning and development in the brain.

A greater role of left fusiform gyrus in orthographic processing seems to be related to developmental and learning-related reductions in activation in right fusiform gyrus. Developmental decreases in right fusiform gyrus activation have been shown for an implicit reading task that involved determining whether there was an ascending letter in visually presented words. Studies have also shown that learning to read mirror-reversed text in adults is associated with decreases in activation in inferior temporal regions in the right hemisphere and increases of

activation in left fusiform gyrus. Some have suggested that reading acquisition is initially a right-hemisphere process relying more on global visual forms, with greater engagement of the left hemisphere with increasing skill as analytic mappings are made between letters/symbols and phonemes/syllables.

Phonology

As with orthographic processing, phonological processing seems to be characterized by increasing specialization of representations. Six-month-olds show large ERPs over both left occipital and left temporal regions when listening to speech. During the first 3 years of life, there is a gradual reduction of the left occipital response so that at 36 months children show a reliable response to speech stimuli only over the left temporal region. In an ERP study that presented known and unknown auditory words to infants, 13- to 17-month-olds showed a differential response to these word types over frontal, parietal, and temporal sites in both hemispheres, whereas 20-month-olds showed a differential response only over temporal and parietal sites in the left hemisphere. Similar ERP effects have been reported when contrasting known words with nonsense auditory words. fMRI studies of auditory speech processing have similarly shown developmental increases from 21- to 39-month-olds in superior temporal gyrus activation and simultaneous decreases in occipital, frontal, and cerebellar regions (see **Figure 2**). The consolidation of the activation over left auditory cortex in all of these studies suggests that this region is becoming specialized for processing speech early in development. However, this increasing specialization seems to continue through childhood.

Older children show substantial overlap in brain activation associated with processing auditory or visual word forms. In contrast, adults show greater selective activation of left superior temporal gyrus when processing auditory as compared to visual word forms. There is also a developmental increase from childhood to adolescence in the connectivity between primary auditory cortex and superior temporal gyrus during spelling tasks in the auditory modality and during auditory narrative comprehension. In addition, there are developmental increases in left superior temporal gyrus activation during processing linguistic prosody. All of these findings suggest that the superior temporal region becomes more specialized for phonological processing over development.

Several studies with adults have shown increased activation in the superior temporal region associated with learning new phonological or auditory information. Adults learning Chinese tones, for example, show increased activation in left superior temporal gyrus. Increased activation in left superior temporal sulcus has been observed in French speakers learning to associate English auditory words with pictures. Widespread increases in activation in bilateral superior temporal gyrus have been shown when native Japanese speakers learn the non-native /r-l/ contrast of English. Finally, ERP studies show enhanced auditory cortical representations with musical training in children, in skilled adult musicians, and in adults learning new phonemic distinctions. All of these studies indicate that learning new auditory information is associated with increased activation in superior temporal regions, which may be associated with increasing elaboration of these sound-based representations.

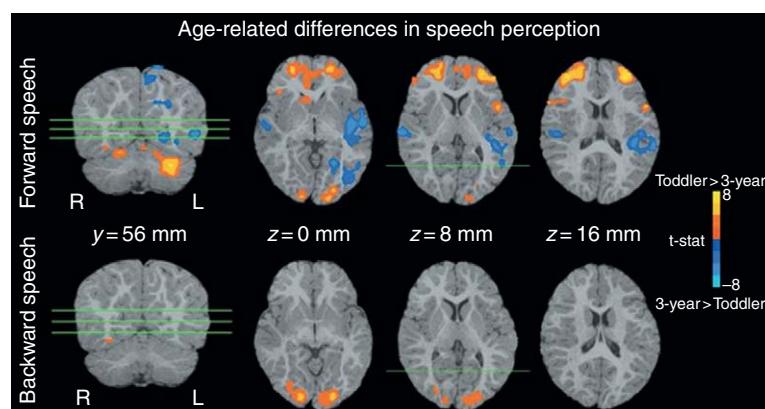


Figure 2 Developmental increases in superior temporal gyrus during phonological processing. Top row: 3-year-olds showed greater activation for listening to forward speech compared to rest as compared to toddlers in bilateral superior temporal gyrus and other regions (blues), and toddlers showed greater activation in other regions (reds). Bottom row: 3-year-olds and toddlers did not show a difference in superior temporal gyrus for backward speech compared to rest. From Redcay E, Haist F, and Courchesne E. (2008) Functional neuroimaging of speech perception during a pivotal period in language acquisition. *Developmental Science* 11(2): 237–252.

Semantics

As with orthographic and phonological processing, semantic processing seems to be characterized by increasing elaboration with development and learning. Behavioral studies have shown both a larger number of lexical entries and greater connections among these entries in older compared to younger children. Developmental increases in activation in middle temporal gyrus have been observed during a narrative comprehension task in the auditory modality. Developmental increases in activation in middle temporal gyrus have also been reported for semantic association judgment tasks in both the visual and auditory modalities (see **Figure 3**). Studies have also reported greater activation in middle temporal gyrus for weakly associated word pairs as compared to strongly associated word pairs, suggesting that developmental increases in this region are associated with semantic processing *per se* and not some other nonlexical process. Greater activation for weakly associated pairs may result from more extensive activation of the semantic system to identify distant relationships. The elaboration of semantic representations in middle temporal gyrus over development seems to be similar cross-linguistically, as age-related increases in this region have also been demonstrated in Chinese.

Learning studies suggest that the elaboration of semantic representations are not associated with development alone, as studies on adults have shown that semantic training results also in greater activation in middle temporal gyrus. Studies have also reported that higher

accuracy among children is correlated with greater activation in middle temporal gyrus on tasks that require a judgment of semantic association of word pairs and on semantic plausibility judgments to sentences, suggesting that children with lower accuracy have less elaborated semantic representations. Since children with lower accuracy seem to have underdeveloped semantic representations, they may rely to a greater degree on retrieval and selection mechanisms. A large body of research in adults suggests that greater activation in inferior frontal cortex is associated with more effortful retrieval or greater selection demands. The finding that lower accuracy is correlated with greater activation in inferior frontal gyrus during semantic tasks suggests that these children engage controlled processing to a greater degree. This interpretation is consistent with studies showing connectivity from inferior frontal gyrus to middle temporal gyrus is stronger in children with lower accuracy during semantic tasks in both the auditory and visual modalities.

Behavioral research suggests that as children become more skilled readers, they rely less on semantic representations for rapid word recognition. As the mapping between orthography and phonology becomes more automatic, semantic representations may have less opportunity to influence this rapid process. This behavioral effect is supported by brain imaging studies that show only children, and not adults, activate middle temporal gyrus during rhyming and spelling tasks in the visual and auditory modalities. Children also show greater middle temporal involvement while processing

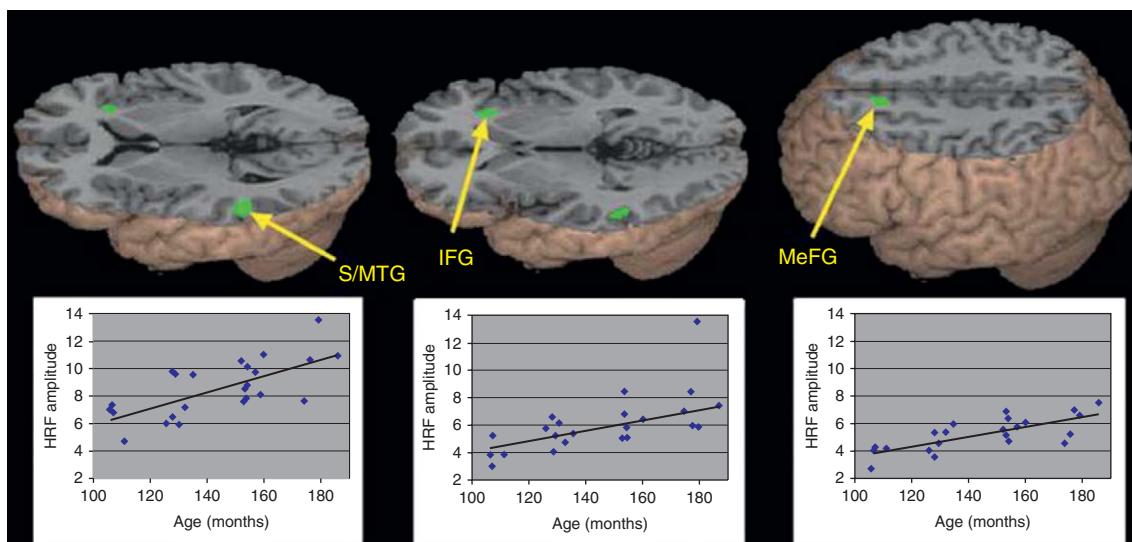


Figure 3 Developmental increases in middle temporal gyrus during semantic processing. Top brain images: correlation between age and activation during a meaning relatedness judgment to auditorily presented words in left middle temporal gyrus among other regions (green). Bottom scatter plots: correlation between age and activation in left middle temporal gyrus and other regions. From Chou TL, Booth JR, Burman DD, et al. (2006) Developmental changes in the neural correlates of semantic processing. *Neuroimage* 29: 1141–1149.

synonyms during a phonological task involving homophone judgments (e.g., plain–plane) to visual words. The middle temporal gyrus activation suggests that children are activating semantics during tasks that do not require access to these representations for correct performance. The reduction in activation in middle temporal gyrus with development suggests that semantics may play a reduced compensatory role as reading acquisition proceeds. This is not to imply that high-skill readers have a less elaborated semantic system, but rather that their more developed orthographic and phonological processing allows semantics to have less influence on rapid word recognition.

Syntax

Studies examining grammatical processing also suggest increasing specialization with development. An ERP study of toddlers examined activation associated with processing closed class words (e.g., prepositions and conjunctions) that are purported to index-syntactic processing. Twenty-month-olds showed similar negative potentials over the left and right hemispheres, 28- to 30-month-olds showed a more prominent negative potential over the right hemisphere, whereas the 36- to 42-month-olds showed only a negative potential over the left frontal region. Other studies suggest that these left anterior ERP negativities associated with syntactic processing show a prolonged developmental time course, because they appear to be qualitatively different between children and adults until about 6 years of age. Both the more diffuse bilateral activation and the consolidation of responses in left frontal regions in younger children suggest increasing specialization with development. Studies that have directly compared syntax to semantic processing using fMRI are consistent with these ERP studies. They report that children show greater overlap in activation in inferior frontal gyrus as compared to adults (see **Figure 4**), again suggesting developmental increases in specialization.

Greater involvement of left inferior frontal gyrus in syntax processing is also associated with language learning in adults. There is greater activation in inferior frontal gyrus for grammatical as compared to ungrammatical strings after learning an artificial grammar; and there is greater activation in inferior frontal gyrus over training sessions when learning an artificial grammar. Studies suggest that the increase of inferior frontal gyrus activation with learning is specific to grammatical and not ungrammatical forms in these artificial languages. Further, learning syntactic rules that follow universal grammar results in increased activation in inferior frontal gyrus, but learning rules that do not follow universal grammar does not result in increases in activation in inferior frontal gyrus. Altogether, these results suggest that inferior

frontal gyrus plays an increasing role in syntactic processing with learning and development.

Parietal Integration

One of the central skills associated with reading acquisition is the development of automatic and accurate mappings of orthographic to phonological forms. This increase in automaticity is associated with increasing elaboration of the connections between these systems at different levels, including grapheme–phoneme, onset–rime, and whole-word connections. Several studies in adults suggest that inferior parietal cortex is involved in extracting statistical regularities between orthography and phonology. Studies also show developmental differences in inferior parietal cortex during tasks that require mapping between orthography and phonology. Adults show greater activation than children in this region during rhyming tasks in the visual modality and spelling tasks in the auditory modality. Developmental differences on similar tasks have been replicated when comparing younger to older children (see **Figure 5**). Developmental increases in inferior parietal cortex have also been shown in Chinese for a rhyming task in the visual modality. Altogether, these studies suggest a developmental increase in the elaboration of the system for mapping between orthography and phonology.

The change of activation in inferior parietal cortex in adults when learning new relationships between orthography and phonology seems to be similar to the developmental differences reported for this region. Studies have shown that learning to associate new orthographic information with phonological information modulates activation in this region. For example, Japanese speakers learning to make associations between Korean letters and speech sounds produced more activation in angular gyrus early in training and functional connectivity of this region with posterior inferior temporal gyrus increased with learning. Another study compared the neural correlates of three training approaches to learning nonwords. Both phonological (rhyming judgments) and orthographic (letter judgments) training produced greater activation in angular gyrus as compared to semantic training, consistent with the involvement of this region in mapping between letters and sounds.

Inferior parietal cortex is a heteromodal region that appears to be involved in the integration of many different representational systems, not just orthography and phonology. There are developmental increases in activation in inferior parietal lobule during semantic association judgments to both visually and auditorily presented word pairs. This age-related increase has also been demonstrated for Chinese in meaning tasks in the visual

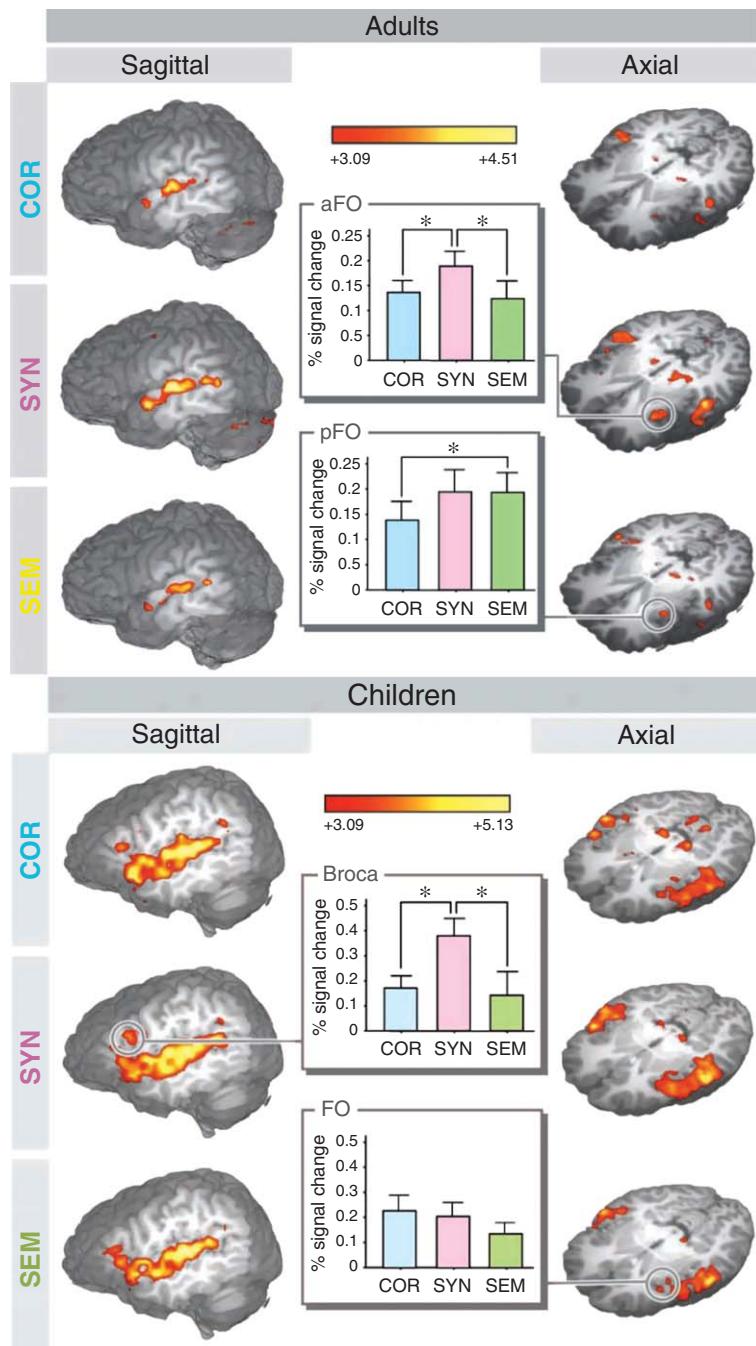


Figure 4 Developmental increases in specialization of inferior frontal gyrus during syntax processing. (Top) Adults showed greater activation in anterior frontal operculum (aFO) for syntax violations (SYN) compared to semantic violations (SEM) or correct sentences (COR). They showed greater activation for semantic violations compared to correct sentences in posterior frontal operculum (pFO). (Bottom) Children did not show differences in frontal operculum for the different sentence types. From Brauer J and Friederici AD (2007) Functional neural networks of semantic and syntactic processes in the developing brain. *Journal of Cognitive Neuroscience* 19(10): 1609–1623.

modality. Activation in inferior parietal lobule is also modulated by association strength, with strongly associated pairs producing greater activation in this region as compared to weakly associated pairs. The modulation by association strength suggests that the developmental

increase in activation of inferior parietal lobule is directly related to semantic processing and not to some other nonlexical process. The locus of activation in inferior parietal cortex for semantic tasks is different from the locus for rhyming tasks, suggesting that this region has

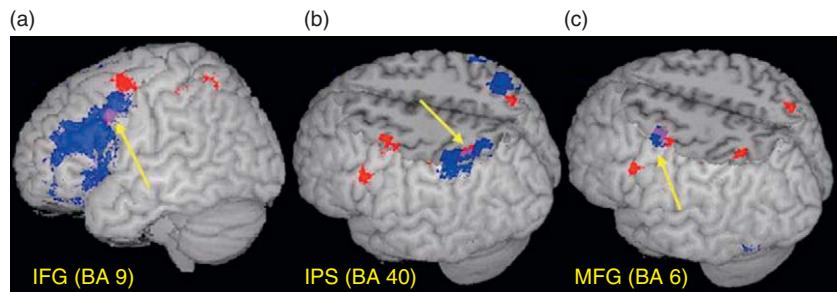


Figure 5 Developmental increases in inferior parietal cortex during a task requiring integration between representations. Developmental increase in activation in a rhyming task in the visual modality (red) overlaid on other maps. (a) Blue represents conflicting (e.g., pint–mint, grade–laid) versus nonconflicting (e.g., lake–cake, press–list) words in inferior frontal gyrus. (b) Blue represents correlation between the differential activation for conflicting compared to nonconflicting conditions and accuracy in conflicting conditions in intra-parietal sulcus. (c) Blue represents developmental increase in activation in the perceptual condition in middle frontal gyrus. From Bitan T, Cheon J, Lu D, Burman DD, Gitelman DR, Mesulam MM, and Booth JR. (2007) Developmental changes in activation and connectivity of phonological processing. *Neuroimage* 38: 564–575.

distinct areas involved in different kinds of integration, both of which show developmental increases in activation. This specialization within inferior parietal cortex may be similar to that shown for the anterior inferior portion of the inferior frontal gyrus in semantic processing and for the posterior superior portion of the inferior frontal gyrus in phonological processing.

Frontal Modulation

One of the most robust neuroimaging findings is that increased activation of the inferior frontal gyrus is associated with both development and learning. Developmental increases in activation have been shown in cross-sectional fMRI studies for silent verb generation to auditorily presented concrete nouns, verbal semantic fluency to auditorily presented categories, category judgments to visually presented words, ascending letter judgments to visually presented words, and for rhyming judgments to visually and auditorily presented words. Developmental increases have also been shown in longitudinal fMRI studies during verb generation to auditorily presented words. Developmental increases have also been established in Chinese for meaning judgments to visually presented words. Most of artificial grammar learning studies in adults also show increases in inferior frontal gyrus activation. Although there are many studies showing acquisition and maturational effects in the inferior frontal gyrus, the specific role of this region in learning and development has not yet been clearly defined.

Dynamic causal modeling (DCM) has been used to clarify the nature of the developmental increase in activation in inferior frontal gyrus. This technique allows the examination of patterns of effective connectivity between brain regions by looking at how the directionally specific influence of one brain region on another is affected by an

experimental manipulation. In a study with adults, a spelling task in the visual modality was marked by converging influences from other brain regions on intraparietal sulcus, whereas a rhyming task in the visual modality was marked by converging influences on superior temporal sulcus, suggesting that these regions are sites of integration for processing task-selective information – orthographic processing for spelling and phonological processing for rhyming. In both tasks, modulating influences also converged on inferior frontal gyrus. As each task also modulated the influence of inferior frontal gyrus on the task-selective region, it was proposed that inferior frontal gyrus is involved in top–down modulation of task-selective regions in a way that differentially enhances their sensitivity to task-relevant information. A developmental study showed that the preferential influence of inferior frontal gyrus on the task-specific integration site was stronger in adults than in children. In other words, there were developmental increases in the modulation of inferior frontal gyrus on intraparietal sulcus for spelling and on superior temporal sulcus for rhyming. This result suggests that stronger activation in inferior frontal gyrus in adults reflects their relatively greater top–down modulation of posterior task-selective regions. A later study replicated this developmental increase, within children and adolescents, in the effective connectivity of inferior frontal gyrus on superior temporal sulcus during a rhyming task in the visual modality. This study additionally showed that this developmental increase was specific to words with conflicting orthographic and phonological representations (e.g., pint–mint, grade–laid) as compared to nonconflicting representations (e.g., lake–cake, press–list) – suggesting the enhancement of relevant representations when irrelevant representations are conflicting (see **Figure 6**). The weaker top–down modulation in younger children also suggests that they may be more strongly driven by perceptual input. Consistent with this,

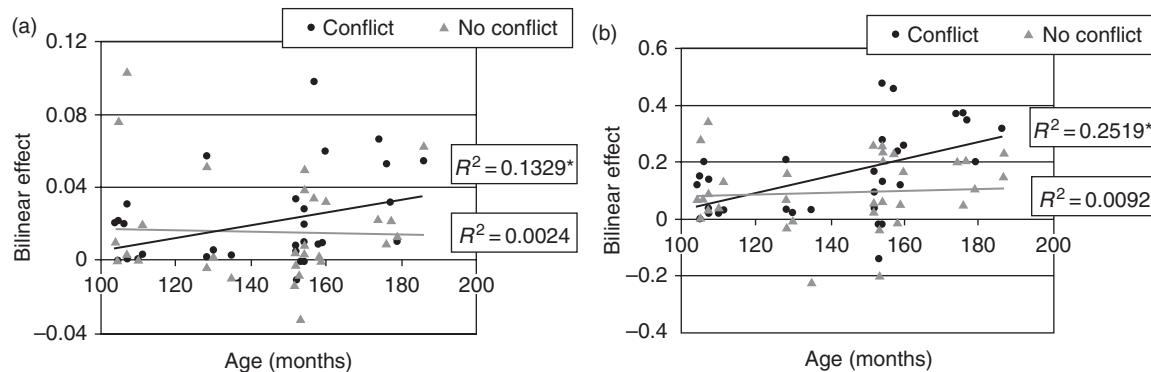


Figure 6 Developmental increases in effective connectivity of inferior frontal gyrus on posterior regions. Correlation of age with the modulatory effects of conflicting and nonconflicting conditions on the converging connection from inferior frontal gyrus (a) and fusiform gyrus (b) into lateral temporal cortex during a rhyming task in the visual modality. The proportion of variance in the modulatory effect explained by the correlation with age is presented. From Bitan T, Cheon J, Lu D, Burman DD, Booth JR. (2009) Developmental increase in top-down and bottom-up processing in a phonological task: An effective connectivity, fMRI study. *Journal of Cognitive Neuroscience* 21(6): 1135–1145.

connectivity from fusiform gyrus to superior temporal sulcus also shows developmental increases for conflicting representations.

The weak modulation of prefrontal cortex on posterior regions in children may explain the ineffective cognitive control and increased susceptibility to interference from irrelevant stimuli that has been observed in children. Weaker top-down modulation may also be the underlying mechanism for the diffuse and less fine-tuned brain activation patterns observed in children compared to adults. In similarity to the developmental effects, learning in adults seems to be associated with increasing connectivity. Learning an artificial grammar is associated with increasing connectivity of left inferior frontal gyrus with left parietal lobe and right inferior frontal gyrus. Increasing connectivity with learning has also been shown in other cognitive domains, such as spatial and object processing.

The developmental increase in connectivity as revealed by DCM is consistent with several other lines of research. Neuroimaging studies have shown developmental increases in white matter through adolescence in left, but not right, arcuate fasciculus that connects anterior and posterior language regions in the left hemisphere. Neuroimaging studies have also shown increases through adolescence in white matter of the corpus callosum that connects the right and left hemispheres. Together, these studies suggest that development is characterized by prolonged increases in white matter and this may allow for the more efficient and rapid communication between brain regions. These developmental differences in white matter appear in contrast to studies that show synaptic pruning through adolescence and neuroimaging studies that show complex patterns of increases and decreases of

gray matter over development. These changes in synaptic pruning and gray matter may be associated with specialization, rather than integration, processes.

Conclusions

This article reviewed what is currently known about the neural substrate of reading and language development. Development seems to be characterized by increasing elaboration and specialization of orthographic, phonological, semantic, and syntax representations to specific regions in the left hemisphere. These principles of elaboration and specialization seem to be characteristic of other cognitive processing domains and many of these age-related changes also seem to characterize learning of written and oral language in adults. However, studies are needed that directly compare learning in adults to developmental changes in order to more thoroughly evaluate the skill-learning hypothesis that assumes the neural basis of behavioral and cognitive development in children is similar to skill acquisition in adults.

Effective connectivity is an advance over conventional analyses because it can determine the directional influence of one brain region on another and allows an examination of the dynamics of neurocognitive networks. Work on effective connectivity suggests that prefrontal cortex plays an increasing role over development in top-down modulation of posterior representational systems in reading and language. The neuroimaging literature is broadly consistent with the interactive specialization approach that argues human functional brain development is characterized by increasing specialization of brain regions as well as increasing integration between

brain regions. However, further research should elucidate how the processes of specialization and integration influence one another at different age points, in different brain regions and languages.

See also: Brain Imaging; Developmental Neurogenesis; Dyslexia (Developmental); Evolutionary and Developmental Issues in Cognitive Neuroscience; From Sensation to Perception; Hemispheric Specialization: Language, Space, and Sexual Differentiation; Implicit Learning and Memory: Psychological and Neural Aspects; Language and Communication – Brain Substrate; Plasticity in the Primary Auditory Cortex: Substrate of Specific Long-Term Memory Traces; Vision.

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Developmental Neurogenesis

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Glossary

Apoptosis – A pattern of programmed cell death, involving a series of specific biochemical events leading to characteristic cell morphology and death.

Neurogenesis – The process by which neurons are generated during development (and adulthood), through mitosis of precursors.

Neurulation – An early step of organogenesis in vertebrate embryos, beginning with the formation of the neural plate and ending with the closure of neural tube (the future nervous system).

Patterning genes – Families of proteins controlling transcription of other genes and playing a key role in the control of neuronal fate during development.

The diverse cognitive, social, emotional, sensorimotor, and vegetative functions of the brain depend on multiple networks of interacting neurons, which are surrounded by (and actively communicate with) supportive glial cells. These functional networks emerge from neuron populations that arise during the earliest periods of embryonic development. In this article, we describe the morphogenetic, molecular, and genetic mechanisms that regulate the generation and movements of cells required to elaborate region-specific populations whose interconnections form functional networks. We highlight general developmental principles including roles of patterning genes and signaling centers in brain formation.

Morphological Development of the Nervous System

Nervous system development is traditionally viewed as a sequence of events including precursor cell proliferation, mitotic cell cycle withdrawal, cell migration, axon and dendrite formation, neurotransmitter system expression, axon outgrowth to targets, synapse elaboration, and selective neuron survival based on appropriate target innervation. Separation of these events into discrete processes is more a pedagogical convenience than a fact, since many of these processes occur simultaneously, not only within brain regions, but also in the same cell. This is important to recognize, since it alters the way in which we

build conceptual models about possible effects of changing one process, and how it may in fact alter several concurrent processes.

Several principles guide our understanding of brain development. First, different brain regions and neuron populations are generated at distinct times and exhibit specific temporal schedules, a fact that influences the consequences of developmental insults. Second, the sequence of cellular processes comprising ontogeny predicts that abnormalities in early events necessarily lead to differences in subsequent stages, at times very distant from the initial insult. Third, specific molecular signals, such as extracellular growth factors, play roles at multiple developmental stages of the cell. Thus, changes in expression or regulation of a ligand or its receptor, by environmental insults or genetic mechanisms, will have effects on multiple developmental and maturational processes. In this review, we illustrate developmental processes relevant to both humans and rodents, since interspecies translation is a valuable goal.

The Neural Plate and Neurulation

The nervous system of the human embryo first appears between 2.5 and 4 weeks of gestation. During development, emergence of new cell types, including neurons, results from interactions between neighboring layers of cells. On gestational day 13, the embryo consists of a sheet of cells. Following gastrulation (day 14–15), which forms a two-cell-layered embryo consisting of ectoderm and endoderm, the neural plate region of the ectoderm is delineated by the underlying mesoderm, which appears on day 16. The mesoderm forms by cells entering a midline cleft in the ectoderm called the primitive streak. After migration, the mesodermal layer lies between ectoderm and endoderm and induces overlying ectoderm to become the neural plate. Induction usually involves release of soluble growth factors from one group of cells, which in turn bind receptors on neighboring cells, eliciting changes in nuclear transcription factors, which control downstream gene expression. In some cases, cell–cell contact-mediated mechanisms are involved.

The neural plate, whose induction is complete by 18 days, is a sheet of columnar epithelium and is surrounded by ectodermal epithelium, which will form the skin. After formation, the edges of the neural plate elevate, forming the neural ridges. Subsequently, changes in intracellular

cytoskeleton and cell–extracellular matrix attachment cause the ridges to merge in the midline and fuse, a process termed neurulation, forming the neural tube, with a central cavity presaging the ventricular system (**Figure 1**). Fusion begins in the cervical region at the hindbrain level (medulla and pons) and continues rostrally (anteriorly) and caudally (posteriorly). Neurulation occurs at 3–4 weeks of gestation in humans, and its failure results in anencephaly rostrally and spina bifida caudally.

Regional Differentiation

After closure, the neural tube expands differentially to form major morphological subdivisions that precede the major functional divisions of the brain. These subdivisions are important developmentally since different regions are generated according to specific schedules of proliferation and subsequent migration and differentiation. The neural tube can be described in three dimensions, including longitudinal, circumferential, and radial. The longitudinal dimension reflects the rostrocaudal organization, which most simply consists of brain and spinal cord. Organization in the circumferential dimension, tangential to the surface, represents two major axes: in the dorsoventral axis, cell groups are uniquely positioned from top to bottom. On the other hand, in the medial-to-lateral axis, there is mirror-image symmetry, consistent with right–left symmetry of the body. Finally, the radial dimension represents organization from the innermost cell layer adjacent to the ventricles to the outermost surface, and exhibits region-specific cell layering. At 4 weeks, the human brain is divided

longitudinally into the prosencephalon (forebrain), mesencephalon (midbrain), and rhombencephalon (hindbrain). These three subdivisions or vesicles divide further into five divisions by 5 weeks, consisting of the prosencephalon which forms the telencephalon (including cerebral cortex, hippocampus, and basal ganglia) and diencephalon (thalamus and hypothalamus), the mesencephalon, (midbrain), and the rhombencephalon yielding metencephalon (pons and cerebellum) and myelencephalon (medulla). Morphological transformation into five vesicles depends on region-specific proliferation of precursor cells adjacent to the ventricles, the so-called ventricular zones (VZs), as well as programmed cell death (see below).

In the circumferential dimension, organization begins very early and extends over many rostrocaudal subdivisions. In spinal cord, the majority of tissue comprises the lateral plates, which later divide into dorsal or alar plates, composed of sensory interneurons, and motor or basal plates, consisting of ventral motor neurons. Two other diminutive plates, termed the roof plate and floor plate, are virtually absent in maturity, however, play critical regulatory roles as growth factor signaling centers in the embryo. Indeed, the floor plate induces midbrain precursors to differentiate into dopamine-secreting neurons of the substantia nigra. Similarly, the roof plate induces dorsal neuron cell fate in the spinal cord. In the absence of roof plate, dorsal structures fail to form, such as cerebellum, and midline hippocampal structures are missing as well. Finally, in the radial dimension, the organization of layers is subdivision specific, produced by differential proliferation of VZ precursors and cell migration, described below.

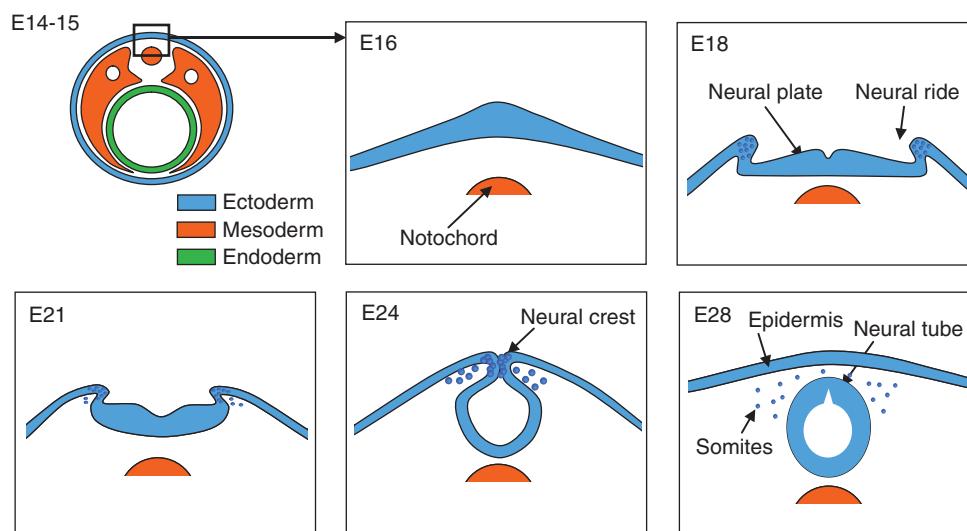


Figure 1 Mechanisms of neurulation. Neurulation begins with the formation of a neural plate in response to soluble growth factors released by the underlying notochord. The neural plate originates as a thickening of the ectoderm that results from cuboidal epithelial cells becoming columnar in shape. With further changes in cell shape and adhesion, the edges of the plate fold and rise, meeting in the midline to form a tube. Cells at the tips of the neural folds come to lie between the neural tube and overlying epidermis, forming the neural crest that gives rise to the peripheral nervous system and other structures.

The Ventricular and Subventricular Proliferative Zones

The distinct patterns of precursor proliferation and migration in different regions generate the radial organization of the nervous system. In each subdivision, final population size depends on the interplay of regulated neurogenesis with programmed cell death (see below). Precursor proliferation occurs primarily in two densely packed regions during development. The primary site is the ventricular zone (VZ) lining the walls of the entire ventricular system, and contributes to all brain regions. For select regions however, including cerebral cortex, hippocampus, and cerebellar cortex, precursors from the VZ migrate to secondary zones where they generate a more restricted range of cell types.

In the early embryo, neural tube VZ progenitors are arranged as a one-cell layer thick, pseudostratified neuroepithelium. The bipolar VZ precursors have cytoplasmic processes that span from the ventricular to the outer pial surface of the brain. During the cell cycle, the VZ appears multilayered, or stratified, because cell nuclei undergo movements, called interkinetic nuclear migration. New cells are produced through the cell cycle, which comprises four stages: mitosis (M), when nuclei and cells divide; G1, when cells double in size before dividing again; S-phase, when cells synthesize DNA and replicate chromosomes; and a brief G2 period, followed by M phase. Precursor cell division (M phase) occurs at the ventricular margin, producing two new cells (Figure 2). The progeny then reenter G1 as they move outward toward the pia. Under the influence of extracellular signals, these cells become committed to another round of division, marked by entry into S-phase, which occurs near the upper VZ margin. After replicating DNA, during G2, the nuclei move back down to the ventricular surface where they undergo mitosis and divide. The role of nuclear migration is not known, though it may allow nuclei access to environmental cues produced

by postmitotic cells that effect subsequent proliferation and gene expression.

At the earliest stages, VZ cells divide to increase the pool of progenitors before producing postmitotic neurons. Subsequently, during the prolonged period of neurogenesis, with each cell cycle on average, a cell divides giving rise to both a postmitotic neuron and another precursor that continues to divide, serving as a stem cell. At the end of neurogenesis, precursor division gives rise to two postmitotic neurons only, greatly increasing neuron production and depleting the precursor pool. The newly born neurons do not remain in the VZ, but instead migrate out to their final destinations, such as the cerebral cortical plate, traveling along the processes of radial glial cells (Figure 3). Like the bipolar VZ precursors described above, radial glia have one process associated with the ventricular surface and the other reaching the pial surface, a morphology consistent with the recent discovery that radial glia are in fact the dividing VZ precursors that produce cerebral cortical neurons. These neurons generated within localized VZ domains, known to express distinct patterning genes migrate to specific functional areas, though active debate remains concerning the relative roles of early expressed VZ genes versus in-growing thalamic afferents in determining cortical neuronal fate and function.

In addition to this general plan of neurogenesis in the VZ, secondary proliferative zones produce specific neuron populations in particular regions. For example, in cerebral cortex and thalamus, the subventricular zone (SVZ) produces astroglial cells, which can generate both oligodendrocytes and astrocytes. Moreover, recent studies in rodents, and likely relevant to humans, demonstrate convincingly that excitatory neurons of the upper cortical layers (2–4) are generated in the SVZ from precursors that do not bear radial processes, indicating distinct sets of regulatory mechanisms. In the hippocampus, secondary zones include the hilus, and, after puberty,

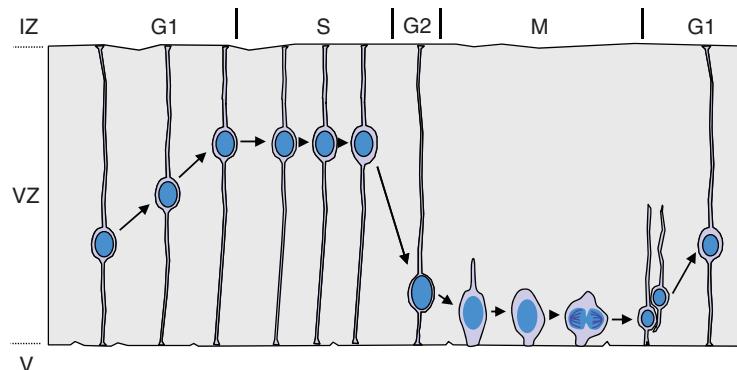


Figure 2 Interkinetic nuclear migration in the ventricular zone. During each cell cycle, nuclei move from the ventricular surface at G1 to the border of the ventricular zone where it enters S phase. Nuclei move down during G2, and reach the ventricular surface where they undergo mitosis. Asymmetric division leads to the generation of a postmitotic cell that leaves the ventricular zone to produce a neocortical neuron, while the remaining stem cell continues to divide. IZ, Intermediate zone; VZ, ventricular zone; V, ventricle. Modified from Jacobson 1991.

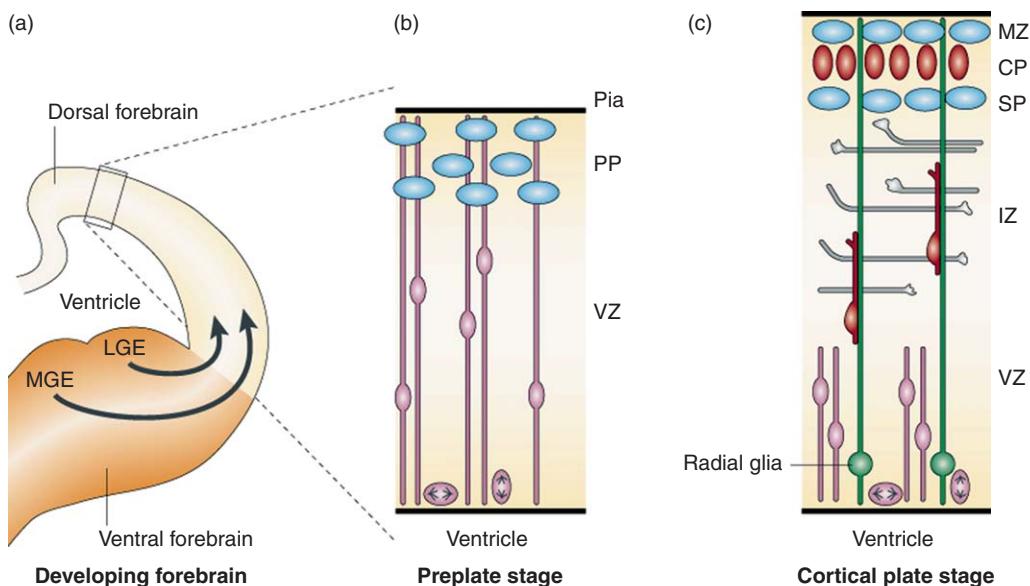


Figure 3 Schematic drawing of radial and tangential migration during cerebral cortex development. (a) A coronal section of one-half of the developing rat forebrain. The dorsal forebrain gives rise to the cerebral cortex. Medial ganglionic eminences (MGEs) and lateral ganglionic eminences (LGEs) of the ventral forebrain generate neurons of the basla ganglia and the cortical interneurons. The arrows indicate the tangential migration route for γ -aminobutyric acid interneurons to the cortex. The boxed area (enlarged in (b) and (c)) shows the developing cortex at early and late stages. (b) In the dorsal forebrain, the first cohort of postmitotic neurons migrate out from the ventricular zone (VZ) and create a preplate (PP) below the pial surface. (c) Subsequent postmitotic neurons will migrate along radial glia through the intermediate zone (IZ) and take position in the middle of the preplate, creating a cortical plate (CP) between the outer marginal zone (MZ) and inner subplate (SP). Ultimately, the CP will be composed of six layers that are born sequentially, migrating in an inside-to-outside pattern. Horizontal processes in the IZ represent axon terminals of thalamic afferents. From Nadarajah B and Parnavelas JG (2002) Modes of neuronal migration in the developing cerebral cortex. *Nature Neuroscience* 3: 423.

the subgranular zone that produces dentate gyrus granule neurons life long, the basis of adult neurogenesis. Finally, in newborn cerebellum, the overlying external germinal layer (EGL) generates granule neurons for several weeks in rodents and for 7–20 months in humans. After neurogenesis is complete, the VZ differentiates into ciliated epithelial cells forming the ependymal lining. Underlying this layer, undifferentiated cells of the SVZ, referred to as subependyma, have been identified as a neural stem cell population, capable of proliferating and generating neurons and glia throughout life.

Radial and Tangential Patterns of Neurogenesis and Migration

Throughout the nervous system, newly generated neurons normally migrate away from proliferative zones to achieve final destinations. There are three patterns of migration, including two radial patterns that are observed on cells originating from the VZ, referred to as inside-to-outside and outside-to-inside, and a third involving non-radial or tangential migration of cells, some of which originate in secondary proliferative zones.

In developing cerebral cortex, the most well-characterized mechanism is radial migration from underlying VZ to appropriate cortical layers in inside-

to-outside fashion. In addition, however, inhibitory γ -aminobutyric acid (GABA) interneurons that are generated in ventrally located medial ganglionic eminences (**Figure 3**) reach the cortex through tangential migration in the intermediate zone along axonal processes or other neurons. The phylogenetically older hypothalamus and spinal cord exhibit the reverse order of cell generation. The first formed postmitotic neurons lie superficially and late-generated cells localize toward the center. Furthermore, cells do not always lie in direct extension from their locus of VZ generation. Rather, some groups of cells migrate to specific locations, as observed for neurons of the inferior olfactory nuclei. Another distinct pattern is observed in the hippocampus, which involves both radial and nonradial patterns of neurogenesis and migration. The pyramidal cell layer, Ammon's Horn CA 1–3 neurons, is generated in a typical outside-to-inside fashion in the dorsomedial forebrain for a discrete period, from 7 to 15 weeks of gestation in human, and exhibits complex migration patterns. By contrast, the other major population, dentate gyrus granule neurons, starts appearing at 18 weeks, and exhibits prolonged postnatal neurogenesis, originating from several migrating secondary proliferative zones. The neurons in developing cerebellum also exhibit both radial and tangential migration. Purkinje cells leave the fourth ventricle VZ and exhibit radial migration,

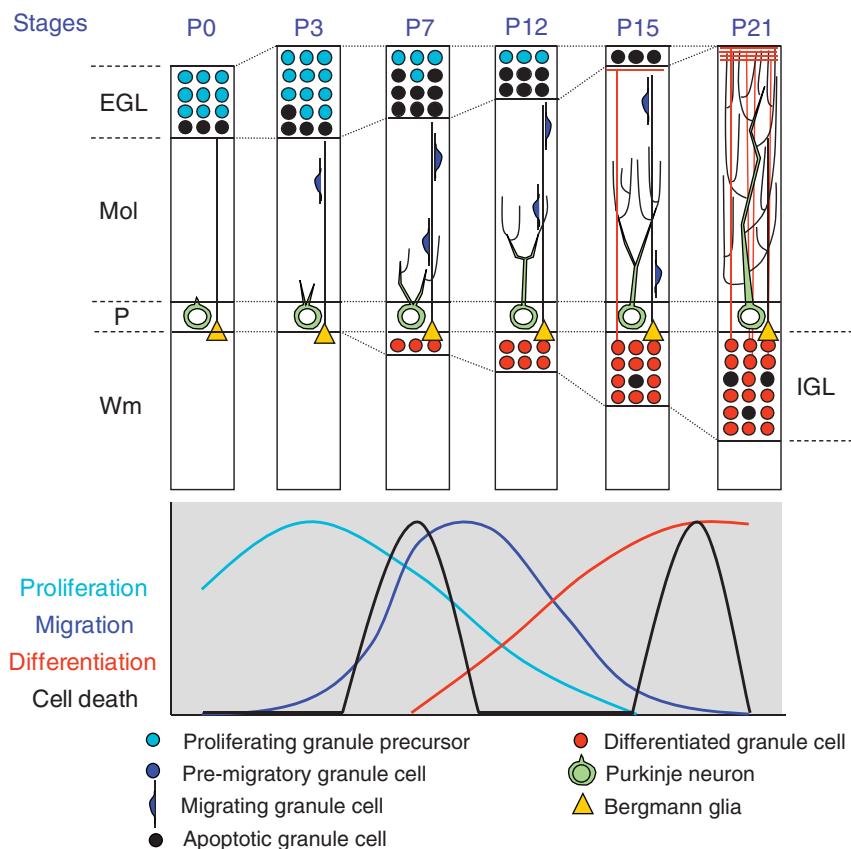


Figure 4 Neurogenesis, migration, and differentiation of granule cells during cerebellar development. Granule cell precursors proliferate in the external germinal layer. After exiting the cell cycle, they migrate through the molecular layer and past the Purkinje neurons to reach the internal granule layer where they differentiate and make synapses. Neurons that do not migrate properly or that do not establish proper synaptic connections undergo apoptosis. EGL, external germinal cell layer; Mol, molecular layer; P, Purkinje cell layer; IGL, internal granule cell layer; Wm, white matter.

whereas other precursors from the rhombic lip migrate tangentially to cover the cerebellar surface, establishing the EGL, a secondary proliferative zone. From EGL, newly generated granule cells migrate radially inward to create the internal granule cell layer (**Figure 4**). Finally, granule interneurons of olfactory bulb exhibit a different kind of migration, originating in the SVZ of the lateral ventricles overlying the striatum. These neuroblasts divide and migrate simultaneously in the rostral migratory stream in transit to the bulb, on a path comprised of chains of cells that support forward movements.

Neuronal Differentiation

After newly produced neurons and glial cells reach their final destinations, they differentiate into mature cells. For neurons, this involves outgrowth of dendrites and extension of axonal processes, formation of synapses, and production of neurotransmitter systems, including receptors and selective reuptake sites. Most axons will become insulated by myelin sheaths produced by oligodendroglial cells. Many of these events occur with a peak period from

5 months of gestation onward. During the first several years of life, many neuronal systems exhibit exuberant process growth and branching, which is later decreased by selective pruning of axons and synapses dependent on experience, while myelination continues for several years after birth and into human adulthood. To innervate diverse distant targets, neurons extend axons, which travel a complex route, passing through various structures. The growth cone is the structure located at the tip of axon, which ensures proper elongation in response to numerous guidance molecules. The growth cone has rod-like extensions called filopodia that bear on their surfaces receptors for specific guidance cues present in extracellular matrix (**Figure 5**). Interactions between filopodial receptors and environmental cues cause growth cones to move forward, turn, or retract.

Developmental Cell Death

Developmental cell death is a reproducible and spatially and temporally restricted death of cells that occurs during the organism's development. Programmed cell death

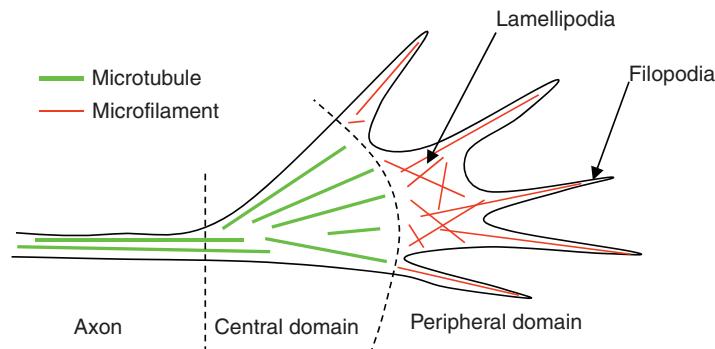


Figure 5 Structure of the growth cone. The cone is subdivided into two domains: the central domain, which contains mitochondria and microtubules, and the peripheral domain, which contains veil-like lamellipodia and spike-like filopodia. In the lamellipodia, microfilaments consisting of actin form a meshwork, while in filopodia they have the same orientation. Cell surface receptors on growth cone processes sense extracellular guidance cues to control navigation.

occurs at multiple stages, interacting with neurogenesis and differentiation with precise and complex mechanisms. Three types of developmental cell death have been described: (1) phylogenetic cell death that removes structures in one species that served evolutionarily earlier ones, such as the tail or the vomeronasal nerves; (2) morphogenetic cell death, which sculpts the fingers from the embryonic paddle, is required to form the optic vesicles and the caudal neural tube; and (3) Histogenetic cell death, a widespread process that allows removal of select cells during development of specific brain regions. Developmental cell death not only contributes to eliminate differentiated neurons that failed to establish proper connections, but also occurs in neural precursors and immature neurons.

Based on morphological criteria, three types of programmed cell death have been described. The first type, apoptotic cell death, is the most common, and is characterized by chromatin condensation and membrane blebbing, followed by nuclear fragmentation and cell shrinkage. Autophagic degeneration involves contiguous groups of degenerating cells and features autophagic vacuoles and pyknotic nuclei. Much less common are nonlysosomal disintegration and cytoplasmic-type cell death, forms that exhibit similarities to necrosis. As apoptosis, is the major type of developmental cell degeneration, underlying molecular mechanisms has been extensively examined. Mechanisms of apoptotic cell death are divided into three phases (Figure 6). First, a regulatory phase, termed ‘initiation,’ involves integration of multiple death and survival signals. These signals converge toward common components, such as initiator caspases, cysteine-containing aspartate-specific proteases that serve as a switch to initiate (or not) cell degeneration. Subsequently, in the case of cell death ignition, the second phase called ‘execution’ begins. During the execution phase, effector enzymes such as caspases-3 and -7 are activated and cleave specific substrates, leading to the last and irreversible step of

programmed cell death called ‘apoptosis.’ Apoptosis refers to the final events of programmed degeneration, when exposed chromosomal DNA between the nucleosomes is cleaved by a caspase-activated DNase (CAD), cytoskeletal components are disassembled, and plasma membranes swell into vesicles termed apoptotic bodies. The cell is then dismantled and phagocytosed without any release of its contents, which would otherwise induce a damaging inflammatory response.

Regulators of Developmental Processes

Morphological conversion of the nervous system through the embryonic stages, from neural plate through neural tube to brain vesicles, is controlled by interactions between extracellular factors and intrinsic genetic programs. In many cases, extracellular signals are soluble growth factors secreted from regional signaling centers, such as the notochord, floor or roof plates, or the surrounding mesenchymal tissues. The precursor’s ability to respond (competence) depends on cognate receptor expression, which is determined by patterning genes whose proteins regulate gene transcription.

Patterning Genes

The term patterning genes connotes families of proteins that serve primarily to control transcription of other genes, whose products include other transcription factors or proteins involved in cellular processes, such as proliferation, migration, or differentiation. Patterning genes exhibit combinatorial interactions as well as distinct temporal sequences of expression and function, acting in a hierarchical fashion. At the most general level, regionally restricted patterning genes participate in specifying the identity, and therefore function, of cells in which they are expressed. Initially, nervous system induction begins at

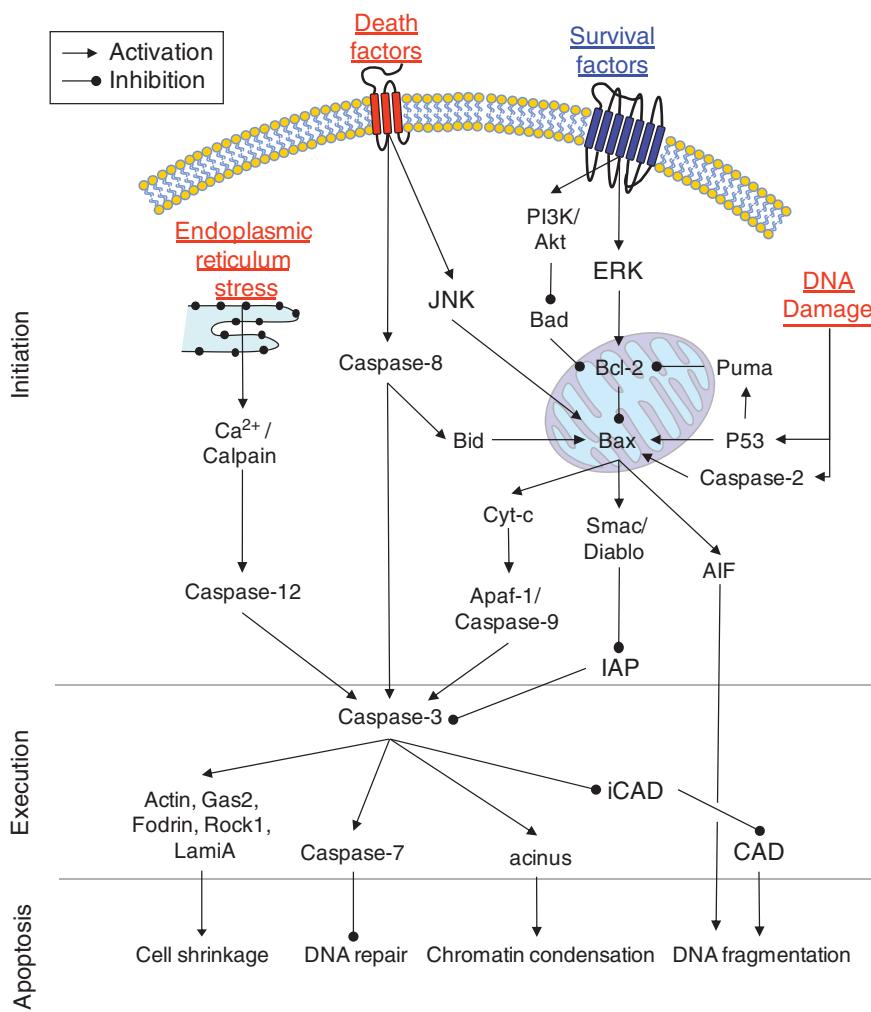


Figure 6 Regulation of apoptosis. Various positive and negative signals are integrated to trigger caspase activation. Caspases are present in cells as inactive zymogens and are converted into their active form through cleavage of the proenzyme. Each caspase cleaves its substrates at specific aspartate residues; thus, initiator caspases cleave effector caspases leading to their activation. AIF, apoptosis inducing factor; Apaf-1, apoptotic protease-activating factor-1; Bax, Bcl-2 associated protein; Bcl-2, B cell lymphoma-2; CAD, caspase-activated DNase; Cyt-c, cytochrome-c; ERK, extracellular-regulated protein kinase; IAP, inhibitor of apoptosis proteins; JNK, c-jun N-terminal kinase; PI3K, phosphatidyl inositol triphosphate kinase; Smac, second mitochondria-derived activator of caspases = DIABLO.

the neural plate stage when the underlying mesenchyme, surrounding epidermal ectoderm, and the notochord produce signaling molecules that affect the identity of the neighboring cells, leading to neural tube formation and closure. Subsequent subdivisions of the brain, and of cerebral cortex specifically, are identified by regionalized gene expression in the proliferative VZ of the neural tube, a ‘protomap’ that leads to subsequent differentiation of distinct types of neurons in each mature (postmitotic) region. Thus, the protomap of the embryonic VZ apparently predicts the cortical regions it will generate, and may instruct the hierarchical temporal sequence of patterning gene expression. It appears that the different genes underlie multiple stages of brain development, including (1) determining that ectoderm will give rise to

nervous system (as opposed to skin); (2) defining the dimensional character of a region, such as positional identity in dorsoventral or rostrocaudal axes; (3) specifying cell class, such as neuron or glia; (4) defining when proliferation ceases and differentiation begins; (5) determining specific cell subtype, such as GABA interneuron, as well as projection pattern; and (6) defining laminar position in the region, such as cerebral cortex.

Patterning genes have been intensively studied in the telencephalon, where signaling centers are localized to the edges of the cortex. In the dorsal midline, there are several centers, including the anterior neural ridge, an anterior cranial mesenchyme secreting FGF8 and Shh, among others, the roof plate, and at the junction of the roof plate with the telencephalic vesicle, the cortical hem

Table 1 Regulation of neurodevelopment by extracellular factors

Extracellular factors	Proliferation		Migration		Differentiation		Survival	
bFGF	↑	Cortex	Cerebellum	Hippocampus	—	—	↑	Nigrostriatum
IGF-1	↑	Cortex	Cerebellum		—	—	↑	Cortex
EGF	↑	Cortex	Adult	SVZ	—	—	↑	Cerebellum
TGF- β	↓	Cortex	Cerebellum		—	—	↓	Cortex
Shh	↑	Cortex	Cerebellum		↑	Cerebellum	—	Cerebellum
PACAP	↓	Cortex	Cerebellum		↓	Cerebellum	↑	Cerebellum
GABA	↓	Cortex			↑	Cortex	—	—
Glutamate	↓	Cortex			↑	Cortex	↓	Pyramidal neurons
						Cerebellum	↑	Mature neurons
						Granule neurons	↓	Immature neurons
TNF- α	↓	Neurons			—	—	↓	neurons
BDNF	—	—			↑	Cerebellum	↑	Neurons
Wnt	↑	Embryonic	stem	cells	—	—	↑	Cortex
NT3	↓	Cortical	stem	cells	↑	Cortex	↑	SVZ
LIF/CNTF/gp130	↑	Cortex	Embryonic	stem	cells	—	—	Spinal cord
						Axon guidance	—	—
						Cortex	↑	Cortex
						Astrocytes	—	—

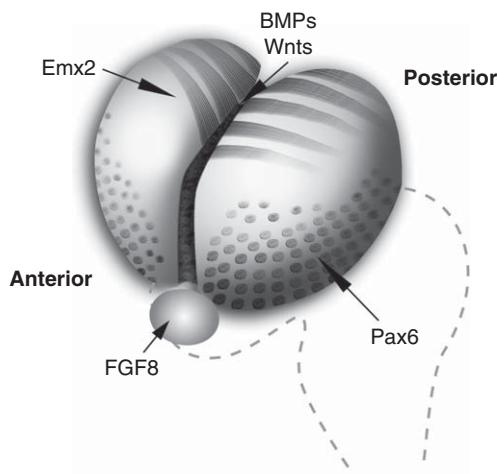


Figure 7 Patterning genes and signaling centers in the developing cerebral cortex. This schematic diagram shows a lateral-superior view of the two cerebral hemispheres of the embryonic mouse, sitting above the midbrain and hindbrain (broken lines). The anterior-lateral extent of Pax6 gene expression is indicated by circles. The posterior-medial domain of Emx2 expression is indicated by stripes. The genes exhibit continuous gradients of expression that decrease as they extend to opposite poles. The signaling factor fibroblast growth factor 8 (FGF8) is produced by and released from mesenchymal tissue in the anterior neural ridge, which regulates Pax6 and Emx2 expression. In the midline, bone morphogenetic proteins (BMPs) and Wingless-Int proteins (Wnts) are secreted from other signaling centers, including the roof plate and the cortical hemis. Courtesy of E. DiCicco-Bloom and K. Forgash.

(**Figure 7**). Other factors originate laterally at the junction of the dorsal and ventral forebrains, as well as from midline basal forebrain structures themselves. These inductive signals tend to work in opposing pairs of gradients, such as BMPs dorsally and Shh ventrally, or retinoic acid rostrally and FGFs caudally. Altogether, the synthesis, release, and diffusion of these inductive growth signals from signaling sources produce concentration gradients that impose distinct neural fates on responsive cells, carried out by inducing discrete regions of transcription factor gene expression.

Regulation of Neurodevelopment by Extracellular Factors

In addition to interacting with patterning genes to specify different brain regions, extracellular factors serve to regulate directly cell proliferation, migration, differentiation, and survival (**Table 1**). Extracellular factors are known to stimulate or inhibit proliferation of VZ precursors and originate from the cells themselves, termed autocrine, neighboring cells/tissues, or paracrine, or from the general circulation, as in endocrine, all sources known to affect proliferation in prenatal and postnatal developing brain. However, in addition to stimulating reentry of cells into

the cell cycle, termed a mitogenic effect, extracellular signals also enhance proliferation by promoting survival of the mitotic population, a trophic action. Stimulation of both pathways is necessary to produce maximal cell numbers. A number of mitogenic growth factors are now well characterized, including (1) those stimulating proliferation, such as basic fibroblast growth factor (bFGF), EGF, IGF-I, Shh; (2) those inhibiting cell division, such as pituitary adenylate cyclase-activating polypeptide (PACAP), GABA, and glutamate, and members of the TGF superfamily; and (3) those such as neurotrophins (BDNF and NT3) that promote survival of both precursors and newly generated progeny. The effects of mitogenic signals depended critically on the stage-specific program of regional development. In the cerebral cortex VZ of the embryonic rat, proliferation is controlled by pro-mitogenic bFGF and antimitogenic PACAP, which are expressed as autocrine/paracrine signals. At later stages, when gliogenesis predominates, bFGF affects glial numbers selectively. Similar to cerebral cortex, later generated populations of granule neurons, such as in cerebellum and hippocampal dentate gyrus, are also sensitive to growth factor regulation.

More generally, extracellular growth factors thus not only interact with local patterning genes, but also control precursor cell proliferation, differentiation, and survival during brain development. Since these processes respond to growth factors, there is also the possibility that traumatic events (e.g., hypoxia, maternal/fetal infection, and toxicant exposure), inflammatory conditions and pharmacotherapies, which alter growth factor expression, may have an impact on the developmental processes in unforeseen ways to contribute to brain dysfunction.

See also: Environmental Influences on Adult Neurogenesis; Evolutionary and Developmental Issues in Cognitive Neuroscience; Genes and Behavior: Animal Models; Perinatal Influences on Behavior and Neuroendocrine Functions.

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Perinatal Influences on Behavior and Neuroendocrine Functions

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Glossary

Circadian rhythms – Biochemical, physiological, and behavioral processes occurring in a cyclic fashion with a period of about 24 h (*circa diem* = about a day). A circadian rhythm is roughly a 24 h cycle of living entities, including plants, animals, fungi, and cyanobacteria. The term ‘circadian,’ coined by Franz Halberg, comes from the Latin *circa*, ‘around,’ and *diem* or *dies*, ‘day,’ meaning literally ‘approximately 1 day.’ The formal study of biological temporal rhythms, such as daily, tidal, weekly, seasonal, and annual rhythms, is called chronobiology. Circadian rhythms are endogenously generated, and can be entrained by external cues, called *Zeitgebers*, the primary one of which is daylight. These rhythms allow organisms to anticipate and prepare for precise and regular environmental changes.

Epigenetics – Heritable changes in phenotype (appearance) or gene expression caused by mechanisms other than changes in the underlying DNA sequence. These changes may remain through cell divisions for the remainder of the cell’s life and may also last for multiple generations. Nongenetic factors cause the organism’s genes to behave (or ‘express themselves’) differently. The best example of epigenetic changes in eukaryotic biology is the process of cellular differentiation. During morphogenesis, totipotent stem cells become the various pluripotent cell lines of the embryo which, in turn, become fully differentiated cells. In other words, a single fertilized egg cell – the zygote – changes into the many cell types including neurons, muscle cells, epithelium, blood vessels, etc., as it continues to divide. It does so by activating some genes while inhibiting others.

Neuroplasticity – Is the changing of neurons and the organization of their networks and, thus, their function by experience. This idea was first proposed in 1892 by Santiago Ramón y Cajal – the proposer of the neuron doctrine though the idea – was largely neglected for the next 50 years. The brain consists of nerve cells or neurons (and glial cells) which are interconnected, and learning may happen through changing of the strength of the connections between neurons, by adding or removing connections, or by adding new cells.

‘Plasticity’ relates to learning by adding or removing connections, or adding cells. During the twentieth century, the consensus was that lower brain and neocortical areas were immutable in structure after

childhood, implying that learning only happens by changing of connection strength, whereas areas related to memory formation, such as the hippocampus and dentate gyrus, where new neurons continue to be produced into adulthood, were highly plastic. This belief is being challenged by new findings, suggesting all areas of the brain are plastic even after childhood. Furthermore, studies determined that environmental changes could alter behavior and cognition by modifying connections between existing neurons and via neurogenesis in the hippocampus and other parts of the brain, including the cerebellum. Decades of research have now shown that substantial changes occur in the lowest neocortical processing areas, and that these changes can profoundly alter the pattern of neuronal activation in response to experience.

Prenatal stress – Prenatal stress refers to stress imposed on the fetuses via the pregnant mother. Prenatal maternal stress is exposure of an expectant mother to distress, which can be caused by stressful life events or by environmental hardships. The resulting changes to the mother’s hormonal and immune system may harm the fetus (and after birth, the infant’s) immune function and brain development. In the context of this article, prenatal stress was accomplished by submitting pregnant females to individual physical restraint thrice daily (at 09:00, 12:00, and 17:00 h) for 45 min in transparent plastic cylinders (7 cm diameter and 19 cm long) and exposure to bright light. This was performed daily during the last week of pregnancy until delivery. Control pregnant females were left undisturbed in their home cages.

Sleep-wake cycle – Daily successions of sleep and wake episodes as defined by electroencephalographic recording. A sleep disorder (somnipathy) is a medical disorder of the sleep patterns of a person or animal. Some sleep disorders are serious enough to interfere with normal physical, mental, and emotional functioning. A test commonly ordered for some sleep disorders is the polysomnogram. Due to rapidly increasing knowledge about sleep in the twentieth century, including the discovery of rapid eye movement (REM) sleep and sleep apnea, the medical importance of sleep was recognized. The medical community began paying more attention than previously to primary sleep disorders, such as sleep apnea, as well as the role and quality of sleep in other conditions. By the 1970s in the USA,

clinics and laboratories devoted to the study of sleep and sleep disorders had been founded, and a need for standards arose. Sleep medicine is now a recognized subspecialty within internal medicine, family medicine, pediatrics, otolaryngology, psychiatry, and neurology in the USA. Certification in sleep medicine shows that the specialist: "has demonstrated expertise in the diagnosis and management of clinical conditions that occur during sleep, that disturb sleep, or that are affected by disturbances in the wake-sleep cycle. This specialist is skilled in the analysis and interpretation of comprehensive polysomnography, and well-versed in emerging research and management of a sleep laboratory."

Introduction

Early adverse events induced long-term alterations in behavioral and neuroendocrine responses to stress. Early environmental triggers or stressors may thus have a permanent rather than a transient effect on the organism. In fact, the activity of nongenetic factors (epigenetics) early in life that result in the permanent organization or imprinting of physiological systems is known as perinatal programming. In the human, intrauterine growth retardation and low birth weight are considered indexes of prenatal stress, and these intriguing findings have spawned the fetal origin hypothesis of adult cardiovascular disease, and more recently, metabolic syndrome. When pregnant females are submitted to extreme conditions like stress and/or undernutrition, which induce reduced birth weight, their offsprings display persistent alterations in metabolic and endocrine function that are associated with emotional and cognitive performance through life. Interestingly, postnatal manipulations such as cross-fostering, handling, and environmental enrichment can counteract these effects, while other manipulations such as neonatal corticosterone administration and maternal separation have effects similar to those of prenatal stress.

Maternal glucocorticoids – one of the most important actors of the hypothalamic–pituitary–adrenal (HPA) axis – have been proposed to mediate the relationship between low birth-weight and various medical conditions such as hypertension, type 2 diabetes, ischemic heart disease, and different types of cognitive and behavioral disorders in adulthood. Glucocorticoids act both at the periphery and in the brain to reorient energy resources of the organism. Glucocorticoids bind to two types of intracellular receptors – mineralocorticoid and glucocorticoid

receptors (MRs and GRs, respectively). Activation of these receptors modify, by genomic mechanisms, the transcription of key regulatory proteins. Glucocorticoids play an essential role in development and many bodily functions, including inflammation, adaptive immunity, cognitive processes, and response to stress. While short-term activation of the HPA axis allows adapting to the challenge, in the long run this can be detrimental for the organism. Resistance to glucocorticoids can occur in many diseases, including autoimmune disorders, respiratory disease, lymphoma, and depression. Thus, chronic environmental exposure to abnormal concentration of glucocorticoids, as in repeated stress episodes, can have permanent effects in animals and humans. Epigenetic regulation (DNA methylation, histone acetylation, and histone methylation) is also a crucial process for the normal development and function of the nervous system, ranging from cell differentiation and neuronal plasticity to behavior such as learning and memory, sleep, and anxiety. Developmental dysregulation of the epigenome under the effects of glucocorticoids could be at the origin of various pathological conditions, including psychiatric disorders. Recent findings point, for instance, to an epigenetic regulation of GRs in the hippocampus of suicide victims with a history of childhood abuse.

Perinatal life is a period of increased plasticity, especially for the stress system, and is therefore particularly sensitive to epigenetic factors. Thus, events occurring early in life, acting through abnormal maternal hormones and/or behavior, can alter the trajectory of the programming of the HPA-axis response to stress. For example, increased mothering behavior was found to produce stable alterations in DNA methylation and chromatin structure. The resulting increase in transcription of the GR gene impacts on the feedback regulation of the HPA axis. Furthermore, early adverse life events that expose the organism to high levels of glucocorticoids have strong, long-term effects on the behavioral and neuroendocrine response to stressors, including growth, metabolism, reproduction, and the inflammatory/immune response. In order to study the response to stress while avoiding the obvious ethical dilemmas that are involved in human experimentation, different animal models have been developed since 1957 to assess the effects of perinatal stress on the activity of the HPA axis and circadian rhythms.

Another facet of the animal model of perinatal stress is the occurrence of several behavioral and neuroendocrine abnormalities in adult animals born from stressed mothers that parallel those found in human depression. These abnormalities can be reversed by chronic antidepressant treatment.

Early Life Events, HPA Axis, and Related Behaviors

Exposure to prenatal stress results in increased responsiveness of the HPA axis to stress. Levels of both types of corticosteroid receptors (MRs and GRs) are reduced in the hippocampus of adult offspring, revealing a possible mechanism for the deficit of the HPA-axis feedback-process by which glucocorticoids downregulate their own production. These abnormalities are sex dependent. Moreover, prenatal stress accelerates age-dependent alterations in the HPA axis. Indeed, the period of hyporesponsiveness of the HPA axis is abolished in newborn rats that have been prenatally stressed and circulating glucocorticoid levels are increased in middle-aged prenatally stressed animals when compared to those of controls. Female prenatally stressed rats durably exhibit attenuated corticosterone secretion in response to an intense inescapable footshock. In males, prenatal stress also results in the hyporesponsiveness of the HPA axis when animals are exposed to an alcohol challenge. These results indicate that the HPA-axis alterations induced by prenatal stress vary according to sex, as well as to the nature and intensity of the stressor. The hyperactivity of the HPA axis observed both in male and female prenatally stressed rats is accompanied in adult rats by increased anxiety-like behavior in males, and an increased behavioral response to novelty in both males and females. Data on the impact of prenatal stress in females are more controversial. In general, the effects of perinatal restraint stress (PRS) on anxiety are less marked in females and

possibly even contrary to those in males, as evidenced by the decreased anxiety-like behavior observed in prenatally stressed females (**Figure 1**). In contrast, depression-like behavior has been observed both in males and females. Prenatally stressed male rats also show a decrease in benzodiazepine receptors in the hippocampus, which could explain the high level of anxiety reported in this gender.

Prenatal stress alters not only reactive adaptation (the stress response), but also predictive adaptation by changing the circadian rhythms. In fact, prenatal stress increases corticosterone secretion at the end of the light period in both males and females, and throughout the diurnal cycle only in females. These effects could be mediated, at least in part, by a reduction in hippocampal MRs/GRs at specific times of the day. Alterations in circadian corticosterone activity are associated with disturbed circadian behavioral cycles, increased REM sleep, and sleep fragmentation. Enhanced levels of corticosterone are positively correlated to anxiety-like behaviors and sleep disorders. Altered sleep may be a mediator of the relationship between anxiety and eating disorders. Excessive fetal exposure to glucocorticoids could also be a common early-life factor which accounts for the association among hyperinsulinemia, hypertension, and hyperleptinemia often seen in individuals of low birth-weight. There is also some clinical evidence on the possible association of leptin levels with depression. In this regard, it is interesting to note that prenatal stress alters feeding behavior and metabolism. A possible explanation for this effect is that the postnatal leptin surge functions as

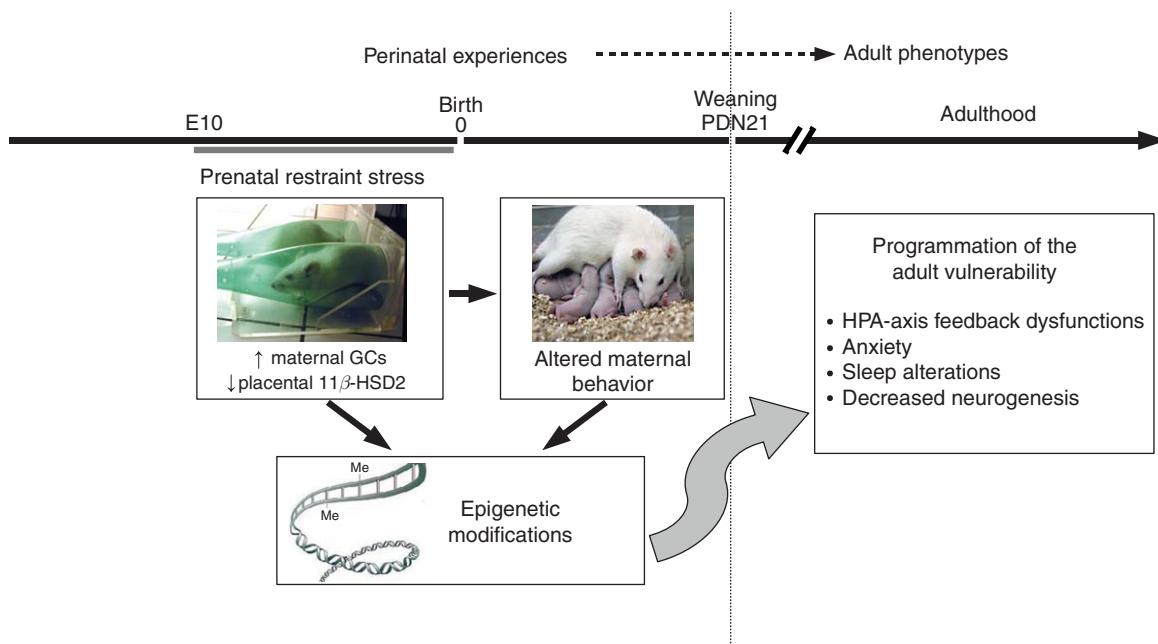


Figure 1 Perinatal experiences contributing to adult phenotypes. GC = glucocorticoid; HSD = hydroxy steroid dehydrogenase.

a developmental factor for hypothalamic projections involved in energy balance and prenatal stress reduces leptin levels in the offspring. Prenatally stressed male rats also display decreased plasma growth hormone (GH) levels at birth and hypoglycemia associated with a reduction in the expression of glucose transporter proteins (GLUT1) in the placenta at the same stage. Together, these findings indicate that maternal stress induces long-lasting disturbances in feeding behavior and increase the risk for type 2 diabetes mellitus. These developmental alterations could be due to the adverse glucocorticoid environment as well as to changes in maternal behavior and interaction with pups during the neonatal period.

Prenatal stress also increases the vulnerability to psychostimulants and other substances of abuse. It also has no effect on the average ethanol preference of female rats, as measured by the consumption of a 5% alcohol solution, either in the basal situation or after a procedure of conditioned fear. However, the strong inter-individual differences in baseline ethanol preference become exacerbated, in the sense that the high-ethanol-preferring rats that have been prenatally stressed still consume ethanol at a high level after conditioned fear whereas their nonstressed controls decrease their preference. Prenatal stress has no effect on ethanol preference in low-alcohol-preferring rats. These results suggest that early adverse events could induce epigenetic modifications that lead to an increased vulnerability to alcohol preference in adult female rats. PRS did not modify alcohol preference in males. Surprisingly, however, chronic alcohol consumption tended to impair spatial recognition in control male rats, but improved memory performance in male PRS rats. This effect of alcohol could be mediated, at least in part, by metabotropic glutamate (mGlu1) receptors, given that chronic ethanol exposure increases these receptors in PRS rats.

The impact of prenatal stress on glucocorticoid secretion and behavior is apparent earlier than its impact on cognition. Cognitive alterations are observed later in life and could be one consequence of early HPA-axis hyperactivity. Interestingly, a positive correlation between adrenal weight and latency to reach the hidden platform in the Morris water maze test was found in old prenatally stressed female and male rats. The effect of prenatal stress on spatial memory in females varies as a function of age, probably in relation to hormonal fluctuations. Prenatal stress alters memory in juvenile female rats but improves it in young adults.

Prenatal Stress and Hippocampal Plasticity

The influence of prenatal stress on neuronal plasticity has been studied in the hippocampus, where it reduces neurogenesis and increases the expression of the

polysialylated form of the neural cell adhesion molecule (PSA-NCAM) and brain-derived neurotropic factor (BDNF), probably as a compensatory effect of decreased neurogenesis. Prenatal stress reduces also the expression and activity of type-5 mGlu5 receptors in the hippocampus. This is relevant because mGlu5 receptors are implicated in the regulation of both synaptic plasticity and neurogenesis, in addition to anxiety. Prenatal stress also affects the expression of Homer proteins, critical to glutamatergic signaling, in the brains of offspring. Numerous proteins and RNAs have been implicated in epigenetic control. Among these, genes encoding tissue-specific transcription factors contribute to epigenetic status but have functions that are not necessarily required to establish or to maintain this status *per se*. Prenatal stress acts at both the transcriptomic and proteomic level with a reduction in the phosphorylation of cAMP response element binding (CREB) proteins, and downregulation of several proteins involved in signaling, protein processing, and cell-proliferation cascades. As discussed above, several lines of evidence point to epigenetic mechanisms as mediators of the long-term propagation and amplification of pre/perinatal stress, which could lead to behavioral changes via alterations on neuronal plasticity. This hypothesis is supported by the fact that stress-signaling nuclear hormone receptors, such as MR and GR, are key transcriptional factors with mainly negative activity. It is thus very conceivable that their inhibitory activity is memorized through epigenetic DNA methylation patterns in the regulatory regions of glucocorticoid target genes, leading at least in part to the effect called perinatal programming. MR and GR both bind to glucocorticoid-response elements (GREs), but only the GR can interfere with the transcriptional activity of other DNA-bound transcription factors, such as activator protein-1 (AP1) complex, nuclear factor-kappa B (NF- κ B), phosphorylation of CREB (pCREB), etc., inducing a general reorientation of cell activity. Over the last decade, a variety of studies have established a firm link between chromatin and transcription. One such example is the extensive work on various histone post-translational modifications which correlate strongly with dynamic transcriptional activity. Various protein modifications finely tune the cellular functions of each protein. Understanding the relationship between post-translational modifications and functional changes is essential for the comprehension of signaling at this level. Altogether, these observations make glucocorticoids a perfect candidate able – from early during development until all along through life – to program from the single cells to the entire organism, via DNA transcription and remodelling, protein expression, metabolism and circadian regulation, to behavior such as feeding and sleep. Until now, mechanistic studies on epigenetic influence of perinatal programming have

mainly focused on the role of serotonin as an epigenetic factor able to act on chromatin remodeling.

Prenatal Stress as an Animal Model of Depression: Predictive Value

Chronic treatment with antidepressants is effective in reverting alterations induced by prenatal stress at the behavioral and neurobiological level. Prenatally stressed rats, therefore, represent an interesting animal model for the evaluation of the efficacy of pharmacotherapeutic interventions in psychiatric disorders such as depression. Furthermore, results obtained in the last decade have proven the validity and the interest of this rat animal model as a model of epigenetic programming. In summary, prenatally stressed rats display long-term impairment of feedback inhibition in the HPA axis and prolonged stress-induced corticosterone secretion, which has been attributed to the observed decrease in central GRs. This is associated with a generalized disorganization of circadian rhythms and several behavioral abnormalities that are positively correlated to glucocorticoid levels and include anxiety, immobility in the forced swim test, learning impairments, and paradoxical sleep. Concomitantly with the dysfunction in the feedback inhibition of the HPA axis, prenatally stressed rats display higher hippocampal expression of the early activation gene *c-fos* under basal conditions and a blunted Fos-protein response after exposure to stress.

Conclusion

Prenatal stress leads to long-term consequences at the behavioral and neurobiological level in the offspring of stressed mothers, with increased vulnerability to anxiety- and depression-like behavior, drug administration, and memory impairment. Among the mechanisms involved in these behavioral alterations, the hyperactivity of the HPA axis plays a crucial role in both the reactive (response to stress) and predictive (biological rhythms) adaptive processes. This altered adaptation or allostatic load is characterized in prenatally stressed male rats by decreased neurogenesis, altered mGlu and ionotropic glutamatergic neurotransmission, changes in brain GRs, and increased Fos, PSA-NCAM, and BDNF expression. Prenatally stressed female rats seem to be less sensitive in terms of hippocampal plasticity. Despite the hyperactivity of the HPA axis, they display reduced anxiety-like behavior and improved learning. As far as mechanisms are concerned, the high maternal corticosterone levels and altered maternal behavior following prenatal stress probably contribute to the long-term effects that are apparent in the offspring through epigenetic modifications.

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See also: Effects of Stress on Learning and Memory; Environmental Influences on Adult Neurogenesis; Measuring Stress; Perinatal Influences on Behavior and Neuroendocrine Functions; Psychoneuroendocrinology of Stress; Regulation of the HPA Axis by Acute and Chronic Stress; Stress and Emotionality; Stress and Reward.

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Emotions

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Glossary

Affects – These are subjectively experienced feelings whose primary experience characteristics are various types of positively and negatively valenced arousal states in the brain that can be monitored by various approach and avoidance behaviors. Affects can occur in three major domains: (1) emotional arousals, (2) sensory stimulations, and (3) bodily homeostatic imbalances (yielding the three major types of affects – emotional, sensory, and body regulatory). Among the emotional affects, those emerging from SEEKING, LUST, CARE, and PLAY are primarily positive, and those emerging from RAGE, FEAR, and GRIEF are primarily negative.

Conditioned place preference (CPP) – A common behavioral test to evaluate whether animals have had rewarding or punishing experiences (positive or negative affect), as determined by whether they exhibit preferences or aversions to spatial locations (e.g., two- or three-chamber choice boxes) where they had specific types of experiences.

Dual-aspect monism – This is an ontological view, assuming that certain natural processes have both easily measurable and hidden aspects, and it provides an epistemological approach to study hidden aspects of nature. In other words, a measurable aspect of a phenomenon, such as the neural circuitry that generates emotional-instinctual behavior patterns, is used as a proxy to estimate the neural circuitry that is critical for generating the affective state that corresponds to the measurable behavior.

Endophenotype – A simpler component of a complex behavior pattern that can facilitate analysis of the underlying neurobiological mechanisms. The concept has been used extensively in psychiatry as a special kind of biomarker that can replace conceptual entities such as psychiatric syndromes and thereby help divide complex psycho-behavioral symptoms into clear and stable phenotypes that can be more definitively linked to underlying neural networks and genetic substrates.

Instincts – These are functional processes that are largely built into the organizational-functional structure of the brain. They are inherited tools for living, and hence are strongly controlled by the way genes construct the functional architecture of the brain. This does not mean that they are not refined and fine-tuned by their use during early development.

Limbic system – A system of functionally related neural midline structures in the brain that are primarily involved in distinct emotional and motivated behaviors. The concept started with the Papez circuit (the interconnected loop of hippocampus, fornix, mammillary bodies, mammilo-thalamic tract, dorsomedial thalamus, cingulate gyrus and frontal cortex, cingulum bundle and back to hippocampus), and elaborated by Paul MacLean. With additional anatomical work and functional analysis of related structures, especially the amygdala, hypothalamus, and periaqueductal gray, an extended limbic system concept emerged to highlight the territory in the brain that needs to be studied to understand basic emotions. The concept has been largely verified by human brain imaging studies as the primary regions of the brain that are critically important for the generation of primary-process emotionality.

Neuropeptides – Types of protein-like molecules made in the brain that serve as neurotransmitters or neuromodulators as well as hormones in the endocrine system. They are made up of short sequences of amino acids (usually 3–40) that are active in controlling information flow between neurons and also commonly function as hormone-type regulators of visceral processes within the gastrointestinal system.

Periaqueductal gray (PAG) – The neural tissue, also known as the Central Gray surrounding the central canal (aqueduct of Sylvius) in the midbrain. It is a convergence zone for all of the primary-process emotional systems of the brain, and appears to be the lowest region of the brain from which coherent emotional responses can be evoked by localized electrical and chemical brain stimulation. The ability of higher brain regions, such as amygdala and hypothalamus to maintain various emotional responses, such as rage, depends on the integrity of the PAG.

Posttraumatic stress disorder (PTSD) – This is a chronic anxiety disorder that emerges from repeated exposure to stressful and terrifying events. It is a common ailment from exposure to warfare, and used to be known as shell-shock and also arises from assaults, rape, and traumatic accidents. In humans, it is accompanied by chronic feelings of anxiety, fear, terror, and helplessness, and is often accompanied by sleep problems, irritability, and depression. It can be modeled in animals by various stressors that chronically change

the emotional phenotype of animals to persistent wariness.

Primary-process emotions – Primary-process emotions arise from evolutionarily provided subcortical operating systems (i.e., they are genetically provided networks that generate instinctual emotional behaviors and the associated affective states). In contrast, secondary-process emotions reflect basic emotional learning and memory processes as reflected, for example, in classical and operant conditioning; and tertiary-processes are the higher emotional functions, best studied in humans, that include thoughtful deliberations commonly based on episodic/autobiographical memories and capacities for symbolic thought and communication.

The Neuroscience of Emotions

'Emotion' is a way for labeling a very special set of brain-mind processes characterized by unique ways of behaving, feeling and thinking, accompanied by a host of autonomic changes in the body that sustain emotional behavior patterns. The behavior can be instinctual (i.e., largely programmed genetically via specific brain networks), although it connects up with a large variety of learning capacities. The study of emotions is one of the most important endeavors in behavioral neuroscience and among the most difficult and neglected. It is important because it links directly to major human concerns where emotional feelings are excessive, as in most psychiatric disorders. It is difficult because the concept of emotions is integrally linked to internal feeling states (affects). It is neglected because the attribution of internal psychological states to animals is still considered taboo because of the stigma of anthropomorphic thinking. This dilemma needs to be resolved by data rather than debate.

Even though emotional behaviors can be easily monitored in animals, it is debatable whether affective experiences are open to scientific inquiry in nonhuman species. This dilemma has been largely resolved if one is willing to accept various reward and punishment paradigms, especially conditioned place preference (CPP) and conditioned place aversion (CPA) paradigms as indicative of the existence of internal affective states. However, additional problems arise from the great variety of approaches to emotional theorizing that exist in the massive literature on human emotions, with most ideas being at high cognitive levels (attributions) which are unworkable in the behavioral neuroscience laboratories. However, if the defining characteristics of emotions are their distinct affective and behavioral states, our inability

to penetrate the cognitive-experiential states of other animal is no great problem. It is the definition of the affective state that is psychologically most crucial. Let us consider the diversity of theoretical perspectives in the field – before moving to the problem of dealing with internal affective-emotional states in other animals.

To simplify, we can categorize theories of emotions into three major types: (1) basic emotion theories, (2) componential theories, and (3) social constructivist theories. The basic emotion view holds that all mammalian brains contain a variety of emotional networks that generate a variety of distinct behavioral and physiological patterns accompanied by emotional feelings; hence, emotional systems are evolved tools that animals inherit in order to respond effectively to a variety of archetypal life challenges that face all higher organisms. The componential view suggests that a variety of physiological, behavioral, and psychological components are developmentally constructed into coherent emotional response systems, with higher regions of the brain providing the integrative ability to make wholes out of the many component parts. The constructivist view is that higher cognitive processes, arising from learning, thinking, and living in specific cultural contexts determines our emotional complexities from very simple beginnings, namely the neurobiological ability to have two primitive bivalent types of experiences – primal positive and negative affects.

Many of the controversies surrounding these views arise from different investigators being interested in at least three different levels of analysis of this complex problem. At the foundational primary-process level, the big question is whether there are genetically in-built emotional systems in the brain, and if so, what is their nature? The most effective way to resolve this question is using localized brain stimulation to evoke emotional behaviors, and to evaluate whether animals care about such states – namely, will they turn them on or off, and whether they can serve as rewards or punishments. Of course, soon after birth, practically all animal actions include acquired aspects, so at this secondary process level, investigators inquire how animals learn new emotional responses, both behavioral and physiological (classical conditioning of fear responses is an excellent modern example of this kind of work). Finally, at the highest level of analysis, it is clear that certain organisms think about their place in the world, so investigators of human emotions must consider tertiary process – how various higher cognitive, thoughtful mental processes change during emotional states.

Clearly, research on animals can effectively clarify the first two aspects of emotionality, while only human work can clarify higher mental changes that emerge during emotional arousals. At the basic level, one must characterize the natural unlearned behavior patterns that

characterize emotional episodes and their neural controls, and that requires a primary-process, neuroethological approach.

The Classic Biological Theories of Emotions

Modern ethological studies of emotional behavior patterns were inaugurated by Charles Darwin in his 1882 work on *The Expression of Emotions in Humans and Animals*. In line with this seminal work, basic emotion theory in humans has been based on a close study of the characteristic facial emotional displays that are evident in all human cultures that have been studied. This has led investigators such as Paul Ekman and Cal Izard to postulate anywhere from six to eight basic emotions (fear, anger, joy, sadness, interest, and sexuality at a minimum). Although social constructivists have correctly indicated that culture often modifies the display rules that people use to express emotions, the analysis of ethologically indexed activities of basic emotional circuits in animals generally supports the conclusions reached by facial analysis in humans. The fact that the animal neuroethological work and human facial analysis have converged, working completely independently of each other, affirms the correctness of the basic emotion view of primary-process emotionality across mammalian species. The constructivist views work best at tertiary-process levels.

Perhaps the most famous conjecture about emotions was the componential-constructivist James–Lange theory that postulated that emotions arose from higher brain mechanisms reading-out the peripheral bodily changes when animals were aroused. To this day, this theory remains without solid neuroscientific support, even though it is clear that peripheral influences, especially hormones such as adrenalin, cortisol, thyroxin, and many other circulating factors, can modify emotional intensities. In fairness to James, it should be mentioned that he had subsidiary theories such as the conclusion that every instinctual behavior was accompanied by changes in affective feelings. In any event, Walter Cannon’s rebuttal of the James–Lange theory highlighted brain networks, especially thalamic ones, as being the key to understanding emotionality.

This idea was made more elaborate by the postulation of widespread emotional networks by James Papez (neuronal loops from hippocampus via the fornix to mammillary bodies, mammillothalamic tract to dorsomedial thalamic regions which project to medial frontal cortical regions, including cingulate gyrus, which transmit information back to the hippocampus via the cingulum bundle). This loop was postulated merely on anatomical considerations and the fact that rage

accompanies hippocampal damage following viral infection with rabies. Only the medial thalamus, frontal cortex and anterior cingulate remain as major players in emotionality to this day, and their participation in social emotions such as separation distress is well established.

The heuristic value of the Papez circuit idea was affirmed by scientists who damaged the core of the temporal lobes (amygdala and ventral hippocampus). The resulting Kluver–Bucy syndrome, where animals were emotionally fearless, hypersexual, and undiscriminating in their feeding and social habits, suggested that such higher brain areas did disrupt emotional stability.

The Papez circuit idea also served as the foundation for the visceral-nervous system or limbic system concept of Paul MacLean. The limbic system is a midline subcortical–cortical locus for primary-process emotional processing that has been amply supported by modern brain imaging with everyone now including insula, orbitofrontal cortex, medial frontal, and anterior cingulate cortices in the control of emotionality and most probably participating in higher (cognitive) regulatory capacities as well. However, all these higher brain functions are supported by even more ancient subcortical emotional networks.

Emotions and Brain Stimulation

The best causal evidence for various primary-process emotional networks in the brain has arisen from localized electrical stimulation of the brain (ESB) studies. Various emotional-action states can be evoked by ESB (*vide infra*), and when animals are asked if they like or dislike such brain arousals, they routinely indicate that they do as indicated by their eagerness to turn on (self-stimulate) or turn off (escape) such evoked arousals. They also exhibit CPPs and CPAs to environments associated with such internal affective events. At times one will get fearful behaviors but animals do not learn to avoid the stimulation using operant tasks (lever presses), but they do avoid such states using CPA paradigms.

Thus, not only is it clear that various basic emotional behaviors emerge from arousal of diverse emotional networks concentrated in these ancient midline regions of the brain (well connected with higher medial cortical structures), but it is equally clear that such arousals are accompanied by affective (positively and/or negatively valenced) internal feeling states. Humans commonly give us verbal reports of corresponding feelings of emotional arousals when stimulated in these brain regions, especially the subcortical parts of these networks. It is noteworthy that the emotional states evoked from higher brain regions such as the amygdala and hypothalamus are dependent on the lower integrative regions of the brain such as the periaqueductal gray (PAG) of the midbrain.

Indeed, stimulation of these lower reaches provokes the most powerful behavioral and affective effects at the lowest current levels indicating the primacy of the lower levels in the hierarchical control of emotionality.

Still, the evidence-based conclusion that animals experience emotional states is not yet universally accepted in behavioral neuroscience. Most investigators prefer to use the traditional terminologies of rewards and punishments, but it is becoming increasingly clear that such global concepts need to be broken down into distinct varieties. Indeed, it is possible that the concept of reinforcement largely reflects the way affective processes operate within the brain. This proposition can finally be tested since we know quite a bit about the neurochemistries that control distinct emotional–instinctual behaviors.

Affects clearly come in many different varieties. In addition to emotional affects, there are a large variety of sensory affects such as the desirability of sweetness, and homeostatic affects such as hunger and thirst. Although such processes exist in animal brains, the fact that they cannot be directly observed has led to the general acceptance that anthropomorphic–subjective terminologies (first-person experiences) are not acceptable in traditional third-person observational sciences. However, the living mammalian brain is not a rock or cabbage; it does apparently have a point of view. It does have various feelings. Affective states are now widely accepted in human brain research, and the best evidence indicates that they are elaborated by subcortical brain regions shared by other mammals. That is a clarion call for scientists to begin considering blending third- and first-person observations. If such experiences also do, in fact, exist in animal brains, a compromise must eventually be found between the internal and external perspectives to certain cross-mammalian brain functions such as the basic affective states.

Emotional Feelings as Envisioned by Different Modern Schools of Neuroscience

Modern investigations of emotions in animal models reflect the diversity of approaches evident in human research. Some feel that bodily changes are the most important aspect of the problem. Others believe that emotional feelings are a very critical dimension. Yet others are most fascinated by change in learning and associated cognitive issues. Although all agree that these levels of analysis are important, they disagree dramatically over the most difficult problem of emotional research, how affective feelings emerge from brain emotional arousals.

The Behavioral Neuroscience Approach to Emotions

Prominent investigators, such as Joe LeDoux and Edmund Rolls, who take a strict behavioral neuroscience perspective, have claimed that emotional feelings are secondary to behavioral issues in emotion research, and suggest that potentially only humans have internal emotional experiences. LeDoux has suggested that emotional feelings can only arise when sophisticated higher working memory systems such as those found in the dorsolateral prefrontal cortex, process unfelt emotional information from below to generate a conscious experience of emotions. Rolls suggests that emotional feelings may only exist in organisms with enough higher brain power for the symbolic processing that permits humans to speak – for him language is an essential feature for humans to experience emotional feelings. Such perspectives lead many behavioral neuroscience investigators to restrict their research to the description of spontaneous and learned behavioral and physiological changes accompanying emotional arousals. Talk about emotional experiences in animals is discouraged as being scientifically irrelevant. They subsume all such nervous system functions under the concepts of positive and negative reinforcements.

The Cognitive Neuroscience Approach to Emotions

The analysis of human emotions using modern brain imaging (functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) studies) has motivated cognitive scientists to vigorously study brain changes during various emotional tasks. Abundant work has now implicated a large number of brain regions that dramatically overlap the classic limbic system concept in processing emotional states (including most commonly arousals in anterior cingulate, insular, and various frontal cortical regions, especially medial frontal and the more ancient orbitofrontal regions). There is a general consensus that emotional feelings arise from high, cognitive regions of the brain. The most seminal statement of this view is Antonio Damasio's somatic marker hypothesis, a variant of the James–Lange perspective, which proposes that emotional feelings arise when bodily commotions during emotional states map onto the somato-sensory regions of cortex. Damasio's work with PET imaging has also highlighted arousals of many of the subcortical regions relevant for basic emotions in animal brain-stimulation studies, but his theoretical view has been that arousals within these ancient circuits are not sufficient to experience emotional arousals.

The Affective Neuroscience Approach to Emotions

A final approach to emotional feelings is based on the idea that ingrained emotional action tendencies and basic emotional feelings arise from essentially the same networks concentrated in subcortical regions of the brain. Paul MacLean and Jaak Panksepp represent this tradition, and both have proposed that emotional feelings arise from subcortical regions that integrate unconditional emotional behaviors and associate physiological changes. This is premised on the simple fact that brain sites from which various emotional behavior sequences are evoked are, by all measures used, capable of sustaining diverse reward and punishment processes.

The Affective Neuroscientific, Dual-Aspect Monism View of Emotional Feelings

The confluence of the neural substrates for basic emotional behaviors and basic emotional feelings within the brain provides a straightforward strategy for unraveling how primary-process affective states are engendered within the brain. This simple strategy is called dual-aspect monism, and envisions that some aspects of coherent brain function are easily observed (unconditioned behaviors) while other corresponding aspects (feelings) are not. Thereby, brain mechanisms for unconditioned emotional behaviors can be used as a proxy for monitoring primary-process emotional feelings: for instance, the neural substrates of instinctual angry behaviors reflect the substrates of angry feelings; similarly, natural playful behaviors reflect socially joyful playful feelings, and so forth. This strategy, easily falsified, allows the translation of facts about basic emotional circuits that can only be well studied in animal models to generate predictions about how basic human emotional feelings are constructed.

In this affective neuroscience view, various basic emotions are concentrated in sub-neocortical regions of the brain. The number of basic emotions is determined by how many distinct emotional patterns can be evoked by localized electrical stimulation of subcortical brain regions. At present there is substantial evidence for brain networks that mediate anxiety (FEAR), anger (RAGE), sexual arousal (LUST), maternal nurturance (CARE), separation distress (PANIC) and the social joy arising from energetic physical engagement during youth (PLAY), as well as subtle ones that have not been fully conceptualized in most human theories – for instance a generalized appetitive motivational SEEKING system that mediates exploration, foraging, and the search for and interest in life-supporting resources. This system is

rather reflexively still called ‘the brain reward system’ by many.

Indeed, from this neuroethological perspective, the ascending so-called mesolimbic/mesocortical dopamine ‘reward’ system should not be called ‘the reward system of the brain’ but the appetitive motivational SEEKING or ‘wanting’ system that allows animals to pursue each and every kind of positive reward and also to seek safety from punishments. The ‘reward-prediction error’ hypothesis of this system may be misguided since it conflates correlates and causes: recordings from the source dopamine cell bodies in the ventral tegmental area (VTA) only tell us what the system is listening to, they do not necessarily tell us anything about the ‘information’ or ‘state control process’ that the system is passing onward in the brain. As Walter Freeman has emphasized, each level of neural activation reinterprets its inputs. Information does not simply get passed on within the brain but different levels of organization in the brain may speak their own functional languages. A main language of ascending VTA dopamine systems is the mediation of appetitive eagerness as opposed to the rewards from consuming resources such as food and water.

The emotional primes above are capitalized to label specific neural networks that mediate both the spontaneous behavioral expressions of those emotions as well as the associated feelings. Since science only studies the parts of nature, and never the wholes, the capitalizations also highlight that the emotional circuits are important primary-process parts of emotional wholes that are typically discussed in human research. In other words, these brain parts may be critical neural components for the construction of emotional wholes, suggesting how various levels of theorizing in human research (*vide supra*) could be integrated.

Emotion-Cognition Interactions

There is general consensus that the emotional feeling and cognitive information-processing aspects of the brain are highly intertwined. Most emotions in humans are aroused by cognitive attributions – evaluation of the meaning of events. However, from a neuroscience perspective, cognitions and basic emotions also need to be separately conceptualized and studied. This has become an enormous dilemma in modern human brain imaging of emotional-affective processes. If people are exposed to various emotional stimuli and one tries to concurrently evaluate affective change, then one may be conflating the affective changes that are occurring with the cognitive judgments about those affective changes. Indeed, Georg Northoff and his group advise that to minimize such confounds, cognitive judgments should always be harvested after affective brain scanning sessions are

finished. In doing this, the prominent finding is that arousals in many of the higher brain regions that light-up during emotional tasks are negatively correlated with affective change, while changes in lower subcortical regions, highlighted by animal brain stimulation studies, are positively correlated to affective change. This supports the idea that basic affects are, in fact, mediated by the subcortical networks that control instinctual emotional behavioral arousals.

Such findings are consistent with abundant animal data, where self-stimulation and aversion are evoked more easily by electrical and chemical stimulation of subcortical rather than cortical regions of the brain. Indeed, there is abundant data from human brain imaging that higher cortical processes typically tend to inhibit affective arousal, and that intense emotional feelings are often accompanied by reduced neocortical arousals. This suggests that higher and lower brain regions implicated in emotional processing are in a seesaw-type balance, and that intense emotional feelings arise from ancient subcortical regions that all mammals share in a remarkably homologous form. Indeed, abundant data from PET imaging of emotional feelings in humans are consistent with this conclusion. In short, it appears that while subcortical regions are more influential in generating emotional feelings, higher neocortical regions elaborate cognitive awareness and thoughtful contemplations of those feelings, processes that often go astray in psychiatric disorders.

The Neurochemistries of Emotions

Perhaps the most powerful understanding of how the brain controls specific emotions will arise from the analysis of the underlying neurochemical substrates. Although early in the neurochemistry revolution, it was thought that chemical coding of behavior did not exist in the brain, the discovery of hosts of neuropeptides indicates that there is, in fact, such neuromodulatory coding of large-scale emotional and motivational ‘state reactions’ of the brain. Still, it is true that the classic neurotransmitters such as glutamate, γ -amino butyric acid (GABA), acetylcholine, dopamine, norepinephrine and serotonin, which participate in all animal behaviors, do not specifically control any single emotion. They modulate all emotions.

Much greater emotional specificity is found among the neuropeptide networks. Of the basic emotional systems noted above, it is clear that substance P figures heavily in RAGE circuitry. FEAR circuitry includes corticotrophin releasing factors, alpha-melanocyte stimulating factor, cholecystokinin, and a host of less well studied neuropeptides such as diazepam binding inhibitor. LUST circuitry is heavily influenced by luteinizing hormone-

releasing hormone, oxytocin and vasopressin. CARE circuitry includes oxytocin, as well as prolactin and endogenous opioids. PANIC/separation-distress circuitry is regulated by the above social neuropeptides as well as very robustly inhibited by mu-opioids. PLAY, the least studied system, is regulated by opioids and also apparently by thyrotrophin releasing hormone and insulin-like growth factor-1. The SEEKING circuitry receives input from many of the above neuropeptides but also has more intrinsic controls through neuropeptides such as neurotensin and orexin excitatory influences, and dynorphin inhibitory ones. The specific motivational systems such as thirst are heavily regulated by angiotensin, and energy balance by a host of neuropeptides including leptin, ghrelin, melanocortin, orexin, neuropeptide Y, and agouti-related peptide. Genetic analyses of emotions, especially with the assistance of knockout mice, are now much more feasible than ever before.

All of these chemistries are new targets for human psychiatric drug discovery, and all need to be evaluated more extensively for affective-emotional changes in humans. So far there is only abundant data for endogenous opioids and oxytocin. However, it is clear that the former can reduce sadness and depression, while the latter produces warm-loving social feelings and intensifies feelings of confidence and trust. It is to be anticipated that some of these neurochemical networks will allow more precise regulation of emotional distress in psychiatric patients than is presently possible.

Basic Emotions and Psychiatric Disorders

It is generally recognized that most psychiatric disorders reflect the imbalances in the emotional dynamics of individuals. If so, the various psychiatric syndromes, which are medical concepts, need to be related to imbalances of basic emotional circuits. In other words, the emotional networks provide a uniquely important set of endophenotypes for future discussions of a variety of emotional disorders. Since each of the basic emotions is regulated quite specifically by neuropeptide circuits, one can imagine a new era of psychiatric medicinal development that is based on our understanding of and ability to manipulate the underlying neuropeptide circuits, such as agonists for those that contain endogenous opioids (for reducing grief/sadness), oxytocin (for promoting pro-social motivations), neuropeptides such as neurotensin (for promoting appetitive arousal and interest), and antagonists for corticotrophin releasing factor (CRF) (for reducing stress, anxiety, and depression), antagonists for cholecystokinin (CCK) (for reducing posttraumatic stress disorders (PTSDs)), substance P (for reducing anger and irritability), dynorphin (for reducing depression and lassitude), just to name a few.

Basic emotions are fundamental powers of the human mind that are of utmost importance for both mental health

and mental disorders. All mammalian brains inherit a variety of emotional dispositions as ancestral tools for living. Among the underlying neural networks, those for SEEKING, FEAR, RAGE, LUST, CARE, PANIC/GRIEF, and PLAY, are of critical importance for generating primary-process affective states – basic psychological states such as urgent interest/desire, anxiety, anger, eroticism, nurturance, sadness, and joy. These fundamental emotional powers of the mind, which are closely affiliated with a variety of bodily states and non-specific brain arousal systems (e.g., norepinephrine and serotonin), concurrently generate distinct emotional action tendencies as well as raw feeling states that rapidly get linked to a variety of events in the world through classical conditioning and other basic learning mechanisms.

When these ancient emotional forces of the human brain become tempestuous – dysregulated beyond our understanding – overwhelming psychological problems can emerge. In humans, emotional imbalances are invariably accompanied by cognitive changes – entangled in attributions, ruminations, and all sorts of hopes, plans, and worries. The FEAR system is especially important for promoting generalized anxiety disorders, neurotic disorders, and specific phobias. One can produce symptoms of PTSD simply by repeatedly stimulating this system, leading to increased limbic permeability whereby minimal stimuli develop the capacity to resurrect full-blown PTSD symptoms. New affect balance therapies may help strengthen one's emotional muscles in ways that can counteract the effects of various past traumas and perhaps inoculate emotional circuits against future adversities.

In short, the continued behavioral neuroscientific study of the evolutionary complexities of basic emotional processes may yield enormous dividends for understanding the foundation of human nature and the neural vicissitudes that lead to psychiatric disorders. What a deep neurobiological/psychoneurological understanding of brain emotional systems, and their associate affects, provides is a vision of brain functions that has enough cross-species ecological validity to allow translating between foundational mental states in animals and primitive psychological forces that contribute substantially to human psychiatric disorders.

Animal research has now clearly indicated that the basic emotions along with their raw feelings (affects) are concentrated subcortically, arising from evolutionarily dedicated brain circuits. Indeed, from a global perspective, there appears to be a seesaw balance between these ancient tools for living and the higher cortical-cognitive processes that interface more directly with the external world. When emotions are aroused, higher brain functions are often constricted into obsessive channels. Higher cognitive activities, on the other hand, can inhibit subcortical

emotional arousals, explaining the efficacy of cognitive behavioral therapies.

Such findings have had salutary effect on modern brain imaging, which is often more effective at highlighting higher rather than lower brain functions, since action potentials are typically much more rapid in upstairs than in the highly functionally overlapping areas found subcortically. Thus, when humans are confronted by emotional stimuli in scanners, special care must be taken to reduce cognitive-affective cross-talk. For instance, if people are presented with emotionally provocative stimuli, one should avoid asking people about their emotional experiences while being scanned. That forces them into a cognitive mode, with higher brain arousals, that may disrupt subcortical affective processing. One should analyze the feelings off-line, after scanning – after the brain changes in response to emotional stimuli have been harvested. When such procedures are used, one typically finds that affective change to various emotion-provoking stimuli typically correlates positively with very deep subcortical regions on the midline of the brain, while correlating negatively with arousals in higher neocortical regions.

Clearly, basic (primary-process) emotions are concentrated in deep subcortical regions we share with the other animals. The manner in which these ancestral voices of the genes are parsed by living in the world – learning (secondary processes) and thoughts (tertiary processes) – is regulated much more by higher regions of the brain that are much harder to study neuropsychologically in animal models.

See also: Animal Tests for Anxiety; Conscious and the Unconscious; Fear, Anxiety, and Defensive Behaviors in Animals; Human Fear and Anxiety; Motivation; Neural and Pharmacological Substrates of Aggression; Neural Substrates of Unconditioned Fear, Defense, and Anxiety; Neural Systems of Motivation; Offensive and Defensive Aggression; Pain and Addiction; Play Behavior; Rewarding Brain Stimulation; Stress and Emotionality; Subjective Experience and the Expression of Emotion in Man.

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Evolution of Emotions

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Glossary

Altruism – Behavior in which the actor incurs a cost (up to and including death) in order to benefit another individual.

Emotions – Essentially human constructs used as a shorthand to refer to the range of subjective feelings to which humans are exposed in their conscious actions (to a lesser or a greater degree).

Evolution – The view (usually credited to Charles Darwin) that new species (and their attributes) are derived by the forces of natural selection acting on pre-existing organisms. Essentially more organisms are produced than can flourish, so some of them do better as a consequence of their (often slight) variations. A change in the environment can alter which organisms (or attributes) benefit. It can be contrasted with the view that organisms are separately created.

Kin selection – Occurs when a gene is selected because it increases the reproductive success of relatives of the bearer of the gene. Genes can be selected in such circumstances because genetic relatives are more likely than chance to possess copies of the same gene. Kin selection gives rise to kin-directed altruism. In general, the higher the level of relatedness, the higher the level of altruism.

Starting with Darwin, evolutionary explanations have been proposed for a wide range of emotions. There are at least two ways to situate emotions with respect to evolutionary theory: they may be viewed as adaptations, or they may be viewed as by-products of adaptations. If an emotion is an adaptation, we can immediately say several things about it. To begin with, we can presume that its evolutionary function relates to the voluntary behavior it motivates. Thus, fear motivates avoidance or escape, whereas rage motivates some forms of aggression. In addition, we can say something about the emotion's evolutionary history. For instance, we can presume that, at each stage in its evolution, the resulting successful emotional disposition had behavioral effects that, on average and relative to other existing variants, enhanced its bearers' inclusive fitness (i.e., its ability to make genetic contributions to the next generation).

Probably not all emotions are Darwinian adaptations; some may be better construed as by-products of adaptations. Archer has argued, for example, that grief results from the maladaptive overstimulation of evolved attachment mechanisms, in particular, responses associated with separation from the object of attachment. These reactions usually lead to adaptive patterns of thought and behavior, such as a preoccupation with the lost person and a desire to locate them. However, when the person is deceased, these reactions cease to be adaptive, and constitute the phenomenon of grief. Thus, grief itself is not an adaptation, but can be traced to adaptive aspects of the mind.

It seems worth initially illustrating some of the varied emotions that have been postulated in our species and attempting to link them to physiology and to context.

The Nature of Emotions and Their Evolution

The Oxford English Dictionary defines emotion as "A mental 'feeling' or 'affection' (e.g., of pleasure or pain, desire or aversion, surprise, hope or fear), as distinguished from cognitive or volitional states of consciousness." There has been considerable debate about the actual nature of emotions. They have, at various times, been viewed as feelings accompanying primary instincts (e.g., flight), or even reflections of the internal, physiological changes (e.g., increased heart and respiratory rates) induced by an emotional stimulus (e.g., a predator). At the very least, what we call emotions can be linked to generally reliable patterns of internal changes, relatively precise and recognizable situational contexts, and accumulated experiences.

Anger

Anger or 'rage' is an important emotion that is linked to threat and attack (aggression), both of which are phenomena with wide-ranging and sometimes subtle emotional and biological consequences to our species. Aggression (whether physical or verbal) is actually a highly heterogeneous phenomenon. Attack can, in some cases, be a product of fear (e.g., defense by a cornered subordinate), rather than anger. In such cases of defensive aggression, there is activation of the sympathetic nervous system. Other forms of physical attack (e.g., by a predator on a prey) seem to be accompanied by minimal signs of internal physiological change or arousal (e.g., there is no activation of the sympathetic nervous system). It is also

clear that angry individuals can be socially inhibited from actually expressing this emotion. Conversely, individuals (e.g., in a military context) can be trained to kill or injure members of their own species without much involvement of anger as an emotion. Having said that, anger is an emotion that is relatively easy to recognize in our species and one with which we are all familiar. Conflict is also a characteristic of virtually every animal species that has been investigated. Indeed, it has been argued that competitive (as opposed to defensive) forms of intra-specific threat and attack, whether directly concerned with the acquisition of food, a mate, a territory, or enhanced social status, are all broadly designed to increase the organism's relative fitness.

Fear

Fear is another important emotion, and is associated with a variety of behavioral responses aimed at protecting the individual from threatening situations. These responses include escape, avoidance, warning displays, defensive aggression, submission, and immobility. In many cases, our fears appear to be tuned to specific threats that our ancestors faced. Research has shown that people are more likely to form some phobias than others (an example of the phenomenon of preparedness). These phobias relate to threats faced by our hunter-gatherer ancestors. Even people living in modern environments are more likely to develop phobias related to ancestral threats, such as snakes and spiders, than they are of developing phobias related to items much more dangerous to them in their modern environments, such as guns, electrical outlets, and fast cars. Similarly, there is ample evidence that laboratory rats and mice retain many of the behavioral responses of their wild progenitors.

Love

Natural selection may also account for the origin of other, more complex emotions. This includes the emotion of love. Like aggression, the word love covers a range of phenomena, including romantic love and the love found among kin (familial love). It is not difficult to imagine the possible significance of romantic love in reproduction. Fisher has suggested that the early stages of romantic love keep couples together for long enough to conceive, whereas attachment or companionate love (which involves deep affection but a lower level of sexual passion) keeps them together until weaning, at around 4 years. Relationships often end after this, as one or both partners may fall passionately in love with someone else. Other forms of love, such as that between parents and their genetic offspring, can be given plausible evolutionary rationales.

Jealousy

Another example of a complex emotion claimed to have an evolutionary origin is jealousy. The suggestion is that, in a monogamous species with high male parental investment, jealousy prompts behavior that lessens the chances that one's mate will engage in extrapair copulatory activity. A large body of research attests to the fact that men are more distressed than women about sexual infidelities. This may be an evolved tendency designed to motivate mate guarding behavior and thereby ensure paternity. Women, on the other hand, appear to be more distressed about emotional than sexual infidelities – that is, about their mate forming a close emotional bond or falling in love with another female. This may be because a male's emotional involvement, more than sex, signals a potential loss of investment in the women and her offspring. A wide variety of evidence demonstrates the sex difference in jealousy. One study, for example, found that men preferentially recall details of sexual infidelity, whereas women preferentially recall details of emotional infidelity. Such findings make good evolutionary sense.

Do Nonhuman Animals Have Emotions?

Some commentators argue that emotions are unique to *Homo sapiens* and that talking about them in other animals is, at best, an expression of anthropocentrism. Others see nothing wrong in assuming that nonhuman animals share many of these characteristics that humans have found generally beneficial to their individual survival and their ability to pass on their genes. Many find it easier to acknowledge emotions in animals with a high degree of resemblance to human beings than they do for species that are more distantly related to us. They often do this on the basis of linking emotions to phenomenological consciousness or sentience (defined as the ability of an entity to recognize something it is like, e.g., what it is like for a bat to be a bat, or for an octopus to be an octopus). The Stanford Encyclopedia of Philosophy notes that “there is broad commonsense agreement that phenomenological consciousness is more likely in mammals and birds than it is in invertebrates... while reptiles, amphibians and fish constitute an enormous grey area.”

Given the recent flurry of interest in whether fish have sentience and the capacity to suffer (as well as being part of that grey area), it seems instructive to pose the question “do fish show emotions?” The answer has relevance to our position for other vertebrate groups. A number of authorities conclude that the evidence suggests fish do indeed have sentience and the capacity to suffer. Chandroo and co-workers maintain that the “affective states of pain, fear and stress are likely to be experienced by fish in similar ways to tetrapods.” Others conclude that fish can

experience fear-like states and probably have the capacity for suffering.

There are various difficulties associated with this issue. Part of the problem is the word ‘sentient’. In some usages, it is simply a synonym for ‘animate’ or ‘responsive to sensory stimuli’. Fish are certainly not inanimate and respond to nociceptive (pain-related) and other stimuli. Others describe sentience as a state of elementary or undifferentiated consciousness. For example, Broom claims “a sentient being is one that has some ability to evaluate the actions of others in relation to itself and third parties, to remember some of its own actions and their consequences, to assess risk, to have some feelings and to have some degree of awareness.” This is very much like the definition of phenomenological consciousness.

In relation to fish (or any other nonhuman animal), one could adopt one of two positions. First, one could regard emotions as being conscious states. This would imply that animals lacking phenomenological consciousness could not have emotions. Conversely, if a particular animal possesses phenomenological consciousness, it could have emotions and the question then becomes, are the emotions experienced by the animal the same as ours? Second, one could take the view that emotions do not necessarily have to be conscious states, but can be defined purely in functional, behavioral, and/or physiological terms. This would imply that, whether or not an animal has phenomenological consciousness, it could still have emotions. The only question is, does it have the same emotions we have or a different set?

The question of consciousness in other animals is thorny, especially when it comes to animals very different than ourselves. For this reason and others, it is probably best to take the second approach. There are then two ways to tackle the question of emotions in other animals. One is to think: “Fear is what I feel in situation X and what I assume other humans feel in situation X. In comparable situations, do other animals experience the same thing as us?” But this is a very anthropocentric approach, and is thus not perhaps the best way to proceed. A second way to approach the question would be to take a comparative perspective maintaining that, in certain situations, humans and other animals act in a comparable manner in response to threatening stimuli. Their senses become fully engaged, they retreat and adopt a defensive posture and their autonomic nervous systems are activated. Although there are differences among species in the details of these actions, they are all clearly evolved solutions to the same general selective pressure, that is, dealing with certain kinds of threats to life and bodily integrity. Furthermore, with some animals (e.g., humans and chimpanzees), the behavioral elements are very similar, presumably because they result from the activation of modified versions of mechanisms inherited from a recent common ancestor. Thus, they have a shared evolutionary

function and, in some cases, a high level of shared evolutionary history. These are important commonalities, and therefore we should gather these behaviors under the same heading: fear. Certainly, there are differences in these behaviors across species. But what they have in common is more important. This way of looking at things treats human fear not as the prototypical case which other animals have or do not have, but rather as just one example of a more general phenomenon in nature.

Some Animal Models of Emotions

Clearly many scientists, who use laboratory species in areas such as psychopharmacology, do so because they regard the animals as having emotional experiences that are not too different from those found in human beings. Naturally, if emotions are, in any sense, evolved, this is a wholly appropriate way to screen potentially psychoactive drugs. Willner has proposed a useful list of features diagnostic of properly validated animal models of psychiatric disorders. In addition to predictive validity (the action of the drug in the model should generally correspond to that seen clinically), the model should have face validity (the diagnostic characteristics of the model and the psychiatric disorder should be similar) and construct validity (the theoretical rationale should be convincing). It should be noted (perhaps in support of the evolutionary emphasis) that there has been a recent move from traditional psychological to ethological models rooted in the animal’s naturalistic behavior.

Anger or Hostility

A rather heterogeneous battery of tests with rats and mice has been used in the laboratory to tap into the emotion of anger. Some tests assess competitive motivations, others defensive or even predatory motivations. Although this makes it difficult to clearly relate the threat and attack recorded to anger, it has been pointed out that some forms of clinically relevant human aggression are expressions of hyper-fearful emotion and psychopaths may show behavior with parallels to predation.

Anxiety

Rats and mice have traditionally been used in tests to assess potential anxiogenic and anxiolytic drugs using the open field, social interaction, two-chambered light-dark transition, and elevated plus-maze tests that all utilize these rodent’s apparent fears of novelty, strangers, bright lights, open spaces, and heights. More ethological tests have utilized the ultrasonic distress calls of mouse neonates and the mouse defense test battery where mice respond to cues from predatory rats.

Depression

Human depression is described as “a debilitating phenomenon with major depressive episodes lasting at least 2 weeks and with core symptoms of depressed mood and markedly suppressed interest and lack of reactivity to pleasurable stimuli.” Rats and mice have been used in a behavioral despair model where they are forced to swim in a confined space. Rodent models have also been developed where animals are subject to chronic unpredictable substantive (e.g., electric shocks, cold water immersion, and light-dark rhythm reversal) or chronic mild (e.g., cage tilt, wetting of cage bedding, and changes to lighting) stressors. Finally (and more ethologically), others have explored loss of social status in the rat as inducing depression-like symptoms. In some cases, the effects of depression-inducing models on anhedonia (a condition where rewards are ineffective), noted above as a symptom of human depression, are studied by looking at reductions in the rate of intracranial self-stimulation of reward centers in the animal’s brain.

Although some human emotions such as social phobia do not appear amenable to developing meaningful animal models, the above items suggest that some emotional constructs have utility, at least across the class *Mammalia*.

Relating Emotions to Evolution

It seems appropriate to consider the lines of evidence and debate that have led people to conclude that emotions are subject to evolutionary pressures. These are largely focused on the human species, and can be divided into a number of broad categories.

Sociobiological Theories

One line of evidence concerns the fact that certain aspects of emotional behavior are predictable from sociobiological theories.

Kin selection theory

Hamilton’s kin selection theory (KST) assumes that individuals will tend to favor others with whom they share a higher than average proportion of genes (i.e., relatives). KST helps to predict emotional responses to individuals of differing degrees of genetic relatedness. For example, greater relatedness is associated with greater emotional closeness. Similarly, it has been found that grief following the death of a loved one increases as a function of relatedness.

Parental investment theory

A number of sex differences in emotional expression make good sense in light of Trivers’s parental investment

theory. Littlefield and Rushton suggested that grief would be positively correlated with average levels of parental investment for a sex. Consistent with this prediction, they found that, on average, mothers grieved the death of a child more than fathers. Based on this work, Archer made the interesting observation that, in species with a low male parental investment, such as polygynous elephant seals, males would not be expected to grieve the death of offspring at all, whereas in monogamous species, males would be expected to grieve.

There are other examples. Campbell has argued that, due to differences in parental investment, females are more valuable to offspring than males, and this is one reason they have evolved greater restraint in regard to risk taking. Consistent with this view, she has found that, though females are just as susceptible to anger as males, they are less likely to engage in direct aggression. This appears to be because they are more fearful than males about the potential consequences of aggression.

Reciprocal altruism theory

Another theory that helps make sense of the suite of human emotions is Trivers’s reciprocal altruism theory (RAT). RAT suggests that people have evolved to engage in patterns of reciprocal exchange, and to avoid the problem of being cheated (i.e., helping someone who does not return the favor). Trivers suggests that, to enact this behavioral strategy, we are equipped with a set of emotional response patterns. Anger and dislike motivate us to withdraw help to cheaters, or to punish them. Gratitude and a sense of obligation motivate us to reciprocate help received from others. Guilt occurs when we fail to reciprocate help, and may be a way of avoiding reprisals. These and other emotion reactions make good sense in light of RAT, which helps bolster the case that they have an evolutionary origin.

Direct Evidence for Evolutionary Origin

There is also more direct evidence that emotions have an evolutionary origin. This includes Ekman’s research showing the apparent cross-cultural universality of many of the basic emotions. Many emotions are associated with facial expressions and displays that appear to be universal among human beings. These include happiness, anger, sadness, fear, disgust, and surprise. The case for the evolutionary origin of emotions is further bolstered by the fact that analogs of several facial emotional expressions are found in nonhuman animals, and are elicited by similar situations. There is therefore strong evidence that at least the basic repertoire of emotions has an evolutionary origin.

General Conclusions

Although behavior is not directly subjected to evolutionary forces, it seems likely that there is some commonality in the emotions displayed by a range of animal species. The commonalities suggest that nonhuman animal models of human emotions are relatively meaningful and that one should take into account these phenomena when considering animal welfare.

See also: Animal Tests for Anxiety; Animal Models of Sexual Function; Cooperation; Comorbidity – Depression; Communication of Emotions in Animals; Depression; Emotions; Evolutionary and Developmental Issues in Cognitive Neuroscience; Emotion–Cognition Interactions; Fear, Anxiety, and Defensive Behaviors in Animals; Fear Conditioning; Fear: Potentiation and Startle; Genes and Behavior: Animal Models; Human Evolutionary Genetics; Human Fear and Anxiety; Infant Bonding and Attachment; Motivation; Neural and Pharmacological Substrates of Aggression; Neuropsychological Aspects of Anxiety Disorders; Neural Bases of Defensive Aggression; Neurobiology of Offensive Aggression; Neural Substrates of Conditioned Fear and Anxiety; Neural Substrates of Unconditioned Fear, Defense, and Anxiety; Offensive and Defensive Aggression; Psychiatric and Substance Use Disorder Comorbidity; Physical and Emotional Pain; Subjective Experience and the Expression of Emotion in Man; Sex Hormones, Mood, and Cognition; Social Bonding and Attachment; Stress and Emotionality.

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Offensive and Defensive Aggression

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Glossary

Abnormal aggression – Aggression that is different from offense in that it is nonresponsive to important inhibitory factors such as the sex and age of a conspecific opponent, submissive signals of the opponent, or target sites for bites and blows.

Aggressive display – Actions that precede attack and may provide signals that an attack is imminent. Different displays accompany offensive versus defensive aggression, but both may involve tactics that facilitate the actual attack behaviors, rather than serving simply as displays or signals.

Defensive aggression – Defensive aggression is a response to high-level threat, particularly when an animate threatening stimulus is contacting or within contact distance to the subject and it is difficult for the latter to escape.

Offensive aggression (offense) – Offensive aggression is aggression in the context of resource control. It typically occurs between conspecifics and, especially in social species, is aimed at target sites that may hurt, but seldom seriously damage, the opponent.

Resource – An object or event such as access to an opposite sex conspecific in breeding condition that facilitates the survival of an animal's genes. Adaptive resources may vary for animals of different species, age, sex, and breeding condition.

Target site – A site on the body of a conspecific opponent where bites and blows are aimed during offensive attack. These sites typically enable the attacker to hurt the opponent and claim relevant resources, without seriously endangering a potentially related animal.

or internal events (reactive aggression; emotional aggression), or the result of deficient inhibition (impulsive aggression), as opposed to a behavior based on expected gain (instrumental aggression). Attempts to provide a biologically based parcelling of aggression that may apply to both human and nonhuman animals has tended to focus on the analysis of its functions. Two such functions have been identified: controlling biologically important resources (offensive aggression) and thwarting bodily attack (defensive aggression).

Offensive Aggression

Offensive aggression is a major mechanism of resource control. In offensive aggression, an individual attempts to defeat or intimidate its opponent into abandoning its claims to a resource, leaving it for the winner. Resources are objects or relationships that are important to the extended reproductive fitness of an individual, facilitating the enhanced representation of that individual's genes in the next generation. Control of such resources is highly adaptive, and behaviors that facilitate this control would be expected to evolve as features of the biobehavioral repertory of most complex animal species. Some important features of resources that make them valuable enough to be fought over are the degree to which they promote extended reproductive fitness, their sequesterability, and their scarcity.

Most offensive aggression is aimed at conspecifics; they are the animals most likely to live in the same habitats and utilize the same resources as the focal animal. Indeed, particular resources may sometimes be uniquely important, to a given species. However, the most direct determinant of an adult animal's reproductive, adaptive, success, its sexual access to an opposite-sex conspecific in breeding condition, is consistent across all sexually reproducing species. Interspecies hybrids are almost always sterile, so this core resource for a particular animal is of little value to nonconspecifics, sharply limiting the motivation for interspecies offensive attack. In fact, many species that are capable of interbreeding have evolved elaborate patterns of courtship activities and displays that serve to discourage breeding among nonconspecifics. The degree to which a resource can be sequestered is also especially high in the case of reproduction: a female in

Conceptualizing Aggression

Animal aggression has often been categorized in terms of specific antecedents (e.g., pain-elicited aggression; isolation-induced aggression) or on the types of animals involved (e.g., male–male fighting; maternal aggression). Human aggression has more often been divided into categories reflecting whether it is seen as a reaction to external

breeding can, in principle, be totally sequestered from competitors if sufficient time, muscle, energy, and skill are put into the effort.

The sequesterability of some resources may vary for different species. Food for a predator—the prey—is often a substantial item, whereas herbivores tend to eat small items like grass, leaves, or fruits. While predators or scavengers on larger prey frequently fight over food, ungulates and other grazers show little tendency to do so: it is simply unproductive to risk the perils and uncertainties of a fight over such resources. Notably, however, several primate species have been reported to fight over ripe fruits. This suggests that the magnitude of the individual food item, which is clearly related to its sequesterability, is the operative factor, rather than the distinction between predator and nonpredator species. If the resource cannot be sequestered, or if it is not limited in availability (scarce) the very real dangers inherent in aggression may make it unprofitable for an animal to show aggression over that resource.

Offensive Aggression: Mechanisms for Reducing the Cost of Aggression

Fighting is dangerous. Each aggressive episode has the potential to produce damage to the initiator as well as to its opponent. Mechanisms that can achieve the functional goals of aggression without risking its dangers are adaptive. Among these mechanisms are several that are seen in many animal species: dominance relationships and territoriality. These ‘super resources’ initially must be gained in the same manner as other resources, through competition and, usually, fighting. There appear to be some exceptions: in spotted hyenas and some primate groups, not to mention humans, inheritance of the mother’s status may establish dominance without significant fighting. Once a dominance hierarchy or a territory has been established, it tends to confer priority of access to important resources without additional fighting unless the resource holder is specifically challenged. Dominance relationships may be long-lasting, as in species with small social groups in which every individual is familiar with every other, or, it may be occasion-specific, as in the rutting seasons that precede breeding in seasonal breeding species.

Territoriality involves the territory holder(s) marking and patrolling a space to deny entry to same sex conspecifics or members of other groups. The resident-intruder model, in which an unfamiliar same-sex conspecific is introduced into the subject’s home cage, constitutes a commonly used example of aggression based on territoriality. As with dominance relationships, variants to this pattern are frequent, including tolerance of same-sex individuals until they are mature or intrude on the prerogatives of the dominant. Harem-holding species, in

which dominant males strongly monopolize the reproductive services of females and nondominant males are much less likely to breed at all, show strong sexual dimorphism: Males are larger than females, and the larger the male the more likely it is to be dominant. In contrast, monogamous species, in which male–male fighting plays a much smaller role in reproduction, tend to show little sexual size dimorphism.

Dominance relationships may be particularly advantageous in situations in which the presence of a cohesive group provides additional sentinels, alternative targets for predators, or group defense against predators. Thus, unlike strict territoriality, dominance hierarchies usually include tolerance of the presence of sexually mature same-sex individuals (male except for a few species, such as spotted hyenas, where females are dominant), so long as these animals do not present challenges to the dominant. However, strategies to circumvent the dominant’s priority of access to some particularly important resources have also evolved. For example, DNA analyses have made it clear that ‘sneak mating strategies’ by subordinate or strange males may result in a substantial portion of offspring in some territorial or dominance hierarchy species being sired by these animals.

Both dominance relationships and territoriality often involve a further emergent resource: control over the actions of conspecifics. This becomes clearer in primate groups and clearest of all in humans. Anecdotal reports from field studies in many primate groups indicate that one feature of the behavior of dominants or territory holders is control of the behaviors of others in their group or territory, even when there is no apparent reason to do so, making another animal move away from a spot that is then not further utilized, for example. The value of this may be that it emphasizes the dominance relationship and facilitates future obedience of the controlled animal. In species showing coalitional aggression with one group attacking another group, the issue of the dominant’s control over members of its own group may take on additional and specific importance. The number of species showing coalitional aggression is increasing, and now includes several primate species, canids (wolves), meerkats, at least one bird species, several species of more primitive vertebrates, and some invertebrates (some ant, wasp, and sea anemone species).

Offensive Aggression: Behaviors and Targets

Most of the studies aimed specifically at analysis of behaviors involved in offensive aggression have been done in rodents. Adult rats can be maintained in small, mixed sex groups, whereas mice and hamsters are more difficult, as lethal attacks may be common when both sexes are present. Dominant male rats show a highly organized form of offensive attack, with bites aimed at the back of the

attacked (usually male) animal, a site in which bites are unlikely to cause debility or death. Such bites provide a means of delivering pain to subordinate males and encouraging them to leave the group, but without much risk of killing or damaging the reproductive potential of an animal that is likely in nature to be one of the dominant's own maturing male offspring. The same mechanism, a painful but nonlethal bite or blow, is additionally useful in species where the continued presence of subordinate adult males in a group adds to numbers that may be important in functions such as defense against predators; group predation tactics; or attacks on neighboring conspecific groups. It is notable that canids and primates as well as rodents appear to show a targeting of attack bites and blows. An additional adaptation reducing the dangerousness of fights is found in the evolution of protective structures for sites that are the targets of bites or blows. These structures may be especially necessary in species that are equipped with very dangerous weaponry. Thus lions, the only large cats to live in social groups, have a mane over the back of the head and the neck that is not found in other large cats.

However, the most common behavioral defense against bites or blows aimed at specific target sites on the body of the opponent may be for the defending animal to conceal or remove these targets by body manipulations or contortions. In rats, and also in mice, although targeting of attack is somewhat less stringent in this less gregarious species, behavioral protection of target sites for attack involves a number of back-defense behaviors, including upright facing of the attacker, interposing the defender's face (which is protected by long vibrissae, another structural protection for an area that would otherwise be a target of attack); and ventrum (an area that is highly vulnerable and not attacked) between the attacker's jaws and the defender's own vulnerable dorsal (back) area. The attacker counters this defense by attempting to lunge around the defender to reach its target, whereupon the defender pivots in the same direction, to continue to face the attacker. When the attack becomes very pressing, the defender may slip backwards to lie on its back, additionally rolling toward the attacker if the latter attempts to dig under for a bite. These attack and defensive behaviors occur sequentially in the actions of attacking and defending rats or mice in interacting dyads, with the form of defense typically serving to drive the specific actions involved in the attack.

The tenet that during intraspecific offensive attack damaging behaviors such as bites and blows tend to be aimed at less vulnerable sites on the opponent, with this tendency higher in social species, is important to understanding a number of factors in aggression. First, it represents an important enabling factor for the adaptive value of offensive aggression to be realized while avoiding damage to conspecific opponents. Second, it provides an

explanation for the specific form of a number of attack (and defensive) behaviors seen during intraspecific fighting. Third, it is highly compatible with findings of structural adaptations for intraspecific fighting, including manes, ruffs, antlers, horns, and the like. Fourth, it provides a means of evaluating whether a specific harm-producing action represents offensive or defensive aggression. Notably, the target site strategy applies to play fighting as well, but the targets of play fights tend to be different from those in either offensive or defensive attack.

If target sites for attack provide an explanation for the specific behaviors involved in attack and defense in some species, then these behaviors should be regarded as functional. In contrast, the more traditional ethological interpretation of many of the behaviors involved in conspecific fighting is that they represent signals to the opposing combatant. This is not to deny that there may be signal components to such behaviors: Thus piloerection, erection of hairs to make the combatant appear larger, might well be regarded as sending a (false or exaggerated) signal of the animal's size. But, a signal-only view of such behaviors requires a relatively convincing analysis that these actions are not functional in attainment or denial of access to a target site.

There is a clear and important use of target sites for offensive versus nonoffensive forms of attack to determine, in a given instance, what pattern of aggression is being demonstrated. However, in practice, target site analyses are rare, potentially leaving uncertainty about whether offensive attack is involved in a particular instance of fighting. In 'maternal aggression,' for example, attack by a lactating female on a small male intruder into her nest site appears to be offensive, although attack on a larger male intruder may involve a mixture of offensive and defensive elements. In particular, there are highly damaging forms of attack that are associated with defensive rather than offensive behavior, as well as a number of examples of abnormal aggression from recent literature, that are different than normal offense patterns (see below), suggesting that at least some of the confusions and contradictions in the literature on the neurobiology of aggression may be due to a mixture of offensive and nonoffensive attack modes in studies attempting to determine neurobiological factors in aggression.

Offensive Interspecies Fighting

Offensive aggression between different species is coming to be recognized as a relatively common event with important effects on species ecology and distributions. Many fish that live in coral reefs, for example, are highly territorial and will attack both conspecifics and nonconspecifics that intrude into their territories. Among mammalian predators, interspecific competition over

food or territories is particularly common, often with lethal results. This has been particularly well documented among larger predators on the African plains, perhaps reflecting the degree to which these predators and their behaviors are visible, and the consequent high numbers of studies involving them. Lions, leopards, hyenas, cheetahs, and wild dogs have been observed in cross-species altercations over food (prey). Many of these interactions are asymmetrical, with participants of the weaker species rapidly retreating while the victor consumes the prey. However, when larger groups of smaller/weaker predators confront smaller groups of stronger predator species, an aggressive encounter of substantial duration and potentially lethal intensity may occur.

Strategies for minimizing interspecific aggression over resources include spatial and temporal differences in habitat usage, such that the weaker participant species may be forced to completely withdraw from a given habitat, or to utilize it at a time of day or a season, in which they are less likely to encounter the stronger predator species. These ecological shifts may have effects on prey as well, in that animals selectively targeted by the weaker of two sympatric prey species may benefit greatly by the relative absence of their specific predators due to pressures from stronger predators. In theory, interspecific offensive aggression should not be inhibited by the potentially lethal impact of offensive aggression on the opponent. This is not an issue that has received a great deal of attention, but there are a number of reports of lethal wounds resulting from aggression between predator species, consonant with a view that the lethality of such fighting is not modulated by the types of mechanisms found in intraspecific offensive attack.

Defensive Behaviors

Defensive behaviors are the immediate and direct behavioral response to threats to life and bodily safety. They have evolved because of the differential survival success they provide when used successfully. For a defensive behavior to be successful, it should constitute an appropriate, that is, likely to be successful, response to both the type of threat encountered, and the situation in which it is encountered. While, as in offensive aggression, there may be substantial learning of what constitutes an eliciting stimulus, at least in more complex animals, defensive behaviors reflect evolved neurobehavioral systems providing a range of behaviors that tend to occur as unconditioned responses to threat stimuli of biological significance.

Defensive Aggression and the Defense Pattern

Flight, avoidance, freezing, defensive threat, defensive attack, and risk assessment to threatening stimuli have

been characterized in a variety of species, and these responses appear to be relatively common to most mammalian species. This reflects that higher animals face threats that are relatively consistent, involving predators and attacking conspecifics, and that appropriate and effective responses to these stimuli have a great number of parallels from one species to another.

The circumstances that promote defenses other than defensive aggression are outlined in this encyclopedia elsewhere. Briefly, these include features such as a way out or a hiding place (promoting flight or hiding, respectively) and stimulus ambiguity, which promotes active investigation of the stimulus; risk assessment. In contrast, defensive aggression is most strongly elicited to present, animate threats that are closely approaching the subject. For wild rats, defensive threat vocalizations to an approaching human will begin at about 1 meter, and defensive attack begins to occur at about .5 meters. While defensive aggression may be more likely in situations that are relatively inescapable, it is clear that the major environmental factor involved is the distance between the subject and the latter's attacker. Even while fleeing a rodent is likely to turn and attack as the pursuing predator comes within contact distance. This is functional, as the existence of an environmental escape route is irrelevant to an animal that is in the process of being overtaken by its pursuer. Notably, the actions comprising both defensive threat and defensive attack are free to occur at any prey-predator, or conspecific attacker distance. That they do not do so provides evidence for situational control of defensive aggression. Other species may show rather different threat-subject defensive distances, but again, these tend to be consistent within species and types of attacker. Thus, prey species that are large and formidable relative to their predators may utilize defensive aggression as their major defense, showing defensive threat, and even attack, at longer prey-predator distances than do animals for which defensive aggression represents a last-ditch effort.

Animate threat stimuli are more likely to elicit defensive threat and attack. From an evolutionary perspective, this is very sensible, as inanimate threats are seldom deterred by being attacked. However, an inanimate threat that delivers pain on contact may also elicit an immediate attack reaction. In laboratory rats, a group for which defensive threat/attack has largely been bred out by husbandry practices of consistently removing animals showing defensive aggression from the breeding pool, pain is perhaps the most efficient method of restoring defensive aggression to an animal's repertory. One very efficient means of doing so is to present a predator or conspecific to a confined rat while simultaneously delivering a mild shock to its tail. The result is a strongly time-bound (within 1 s of the shock) bite, but at a different body site on the opponent than is common in offensive

attack. Such defensive bites are most easily, in terms of the magnitude of tail shock that elicits them, obtained toward the opponent's snout and head, in contrast to the back targets favored in offensive attack.

This targeting appears to be consistent to both predators and attacking conspecifics, providing a view that in defensive aggression, the identity of the threat stimulus may be less important than it is in offensive aggression, where such features as the sex and pubertal status of an opponent have a major impact on the probability of attack. Another important implication of the extreme reduction in defensive threat and attack by domesticated rats, particularly to humans, is that it stands in contrast to offensive attack, which is apparently little changed from wild to laboratory rats. Findings that genetic selection against defensive attack has all but eliminated such attack in laboratory rats, in the absence of pain, but without much influence on offensive attack, strongly supports a view that the two are independently influenced by at least some genetic factors.

Differentiating Offensive and Defensive Aggression

Defensive aggression is different from offense in terms of the four cardinal features that traditionally define a behavior: Antecedent or triggering stimuli; organismic (e.g., motivational and experiential) variables; response characteristics; and outcomes. In terms of antecedent conditions, defensive aggression is a response to the threat of bodily harm or death, rather than to challenge another over resources. The motivation associated with defensive attack is fear, whereas offensive attack is reduced by fear.

Both the signals associated with defense and the actual form of defensive attack are different from those in offensive aggression. Defensive threat signals include loud vocalizations (screams, in both rats and cats) in a range likely to be audible to predators; display of weapons such as teeth or claws and body orientations and distentions that enhance the animal's apparent size; all useful in indicating the defensive capabilities of the defending animal. These are highly salient signals, impossible to ignore. Except for exaggerating the size of its body, offensive displays are different. The offensive cat walks quietly, albeit on straight, stiff legs that make it appear as tall as possible, toward the opponent. Offensive threat vocalizations tend to be quieter. In rats there is no specific vocalization associated with offense, whereas offensive cats emit a low growl rather than the scream of the defensive cat. In addition, the contorted movements of the defensive cat that is ready to attack, lying half on its back with claws of both fore and back limbs extended, have no counterpart in the offensively attacking animal.

These bodily distortions, often interpreted as displays or signals, may actually be more than displays. In addition

to indicating (and possibly exaggerating) the capabilities and determination of the defensive animal, such actions also present weapon systems in a state of readiness, constituting preparation for the species-typical fighting techniques that will be used if the display itself is not effective in deterring attack. Thus, the lying-half-on-the-back cat is in an excellent position to seize its attacker with its forepaws and hold it while it uses its hind paws (and claws) to rip at the attacker's ventrum, potentially leading to lethal disembowelment if the attacker is a conspecific.

This brings up the important distinction that defensive attack is often aimed at particularly vulnerable sites such as the eyes and snout, whereas offensive attack involves targets where damage is less lethal or crippling. The defensive animal typically does not show defensive aggression except as a terminal behavior in a series of actions such as flight and freezing that have failed. Injury or death to the opponent is irrelevant to the adaptive status of this behavior, whereas in offensive aggression where the adaptive outcome is more subtle, death or reproductive debasement of a relative is an additional consideration.

Factors Reducing Attention to Defensive Aggression

There are a number of reasons why the offense–defense distinction has been somewhat neglected in recent years. Because of selective breeding, in laboratory rats defensive attack is typically elicited only by painful contact with an attacker. When the attacker is a conspecific, this usual occurs only during the second or so following a bite, and only if the target sites for defensive attack, the face and snout of the attacker, are available to be bitten during this time. Defensive attacks are therefore very rare in aggressive encounters involving laboratory rats. Moreover, as defensive aggression in antipredator behavior can be seen only when the predator is allowed to carry out an actual attack on a prey animal, an event that is seldom allowed in most contemporary laboratories, it too rarely occurs in laboratory settings. However, experienced laboratories may utilize specific components of the defensive threat pattern, such as defensive vocalizations, as markers for the pattern. That this very limited role for defensive attack is misleading may be seen in findings that wild-trapped and first-generation laboratory-bred wild *Rattus norvegicus* do show high (and effective!) levels of defensive threat and attack to even nonpainful human contact.

For these reasons, when defensive attack does appear, following manipulations such as brain stimulation or lesions, it is often interpreted as offensive aggression, or, more commonly, as simply aggression, with no distinction made between the two forms of attack. In a classic case,

the so-called ‘septal syndrome’ is frequently interpreted as a hyperaggressive response. Rats with electrolytic lesions of the septal area will readily attack and bite when being handled, and will attack conspecifics when placed together with them in a small chamber. However, if the lesioned rats are allowed to escape the stranger rather than attacking it, they will do so.

Abnormal Aggression

More recently, a number of reports of ‘abnormal aggression’ from animals exposed to early stressors, or, following adrenalectomy, or as a result of selective breeding, have raised again the issue of forms of aggression that do not correspond to offense. What defines this abnormal aggression appears to be its lack of reliance on the features that normally control (offensive) aggression. Thus, abnormal aggression makes little distinction between legitimate targets of attack, for example, adult male conspecifics, and animals such as females and prepubertal males that are normally not attacked. Opponents displaying submission signals are also attacked. Finally, the target sites for attack in abnormal aggression are not, in rats, the back and flanks where bites are made in normal offensive aggression, but the head/snout.

These factors suggest that abnormal aggression may be more related to defensive than to offensive attack. While, at present, the case for such identity is largely inferential, it would be of great interest to evaluate this potential relationship, perhaps by more intensive comparisons of behaviors across these models, and also in terms of brain regional activation patterns, using c-Fos or other activity markers. The point is not just academic, as early stress or abuse, in particular, are substantially related to patterns of bullying and other hyperaggressive response in juveniles and later in adults. If the abnormal aggression patterns seen following early stress, or in animals of ‘short attack latency’ lines, are linked to human aggression phenomena, it is clearly important to learn whether these may be related to an offensive or a defensive aggression pattern.

This situation is further complicated by the fact that offensive and defensive forms of aggression may occur in the same animal or person, in the same agonistic bout. In fact, every example of two animals, or persons, engaging freely in an agonistic bout would be expected to involve some offensive motivations on the part of both parties. As

one of the combatants gains the upper hand, however, it is predictable that the other should experience an enhancement of fear, along with a diminution of offense. Such mixed motives in a naturalistic situation, are common; for example, sexual and aggressive motivations or defensive and appetitive motivations. What they do emphasize is the importance of adequate analysis in order to (experimentally) tease apart the mixed motives and polarize both motivations and behavior patterns in order to gain an improved understanding of when and how aggression occurs in the real world.

See also: Fear, Anxiety, and Defensive Behaviors in Animals; Hormonal Contributions to Arousal and Motivation; Neural and Pharmacological Substrates of Aggression; Neural Bases of Defensive Aggression; Neurobiology of Offensive Aggression.

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Pleasure

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Glossary

Conditioned place preference – A learned preference for an environment previously paired with a hedonic stimulus.

Frustrative nonreward – Nonhedonic approach behavior despite either intermittent reward or omission (i.e., extinction) of reward.

Negative reinforcement – Increasing the likelihood of a behavior by omitting a negative affective punisher.

Positive emotions – A category of emotion that consists of appetitive prosocial emotions (e.g., happiness/joy) and consummatory sensory pleasures (e.g., gustatory pleasure).

Positive reinforcement – The ability of a positive emotional reward to increase the likelihood of the reward-eliciting behavior.

Sonogram – A frequency (y-axis) by duration (x-axis) by intensity (grayscale) visual representation of sound.

Ultrasonic vocalizations – Vocalizations with a peak frequency above the threshold of the human hearing range (20 kHz).

positive affective states are primarily related to positive prosocial interactions.

Experimental studies that elicit positive affective states generally use social positive affective stimuli (i.e., positive feedback, giving a small gift, or watching a video tape eliciting positive affective states). Positive affective states that are elicited in an experimental setting by these stimuli have been shown to increase gregariousness, optimism, and openness to new experiences. These phenomenon have been referred to by Barbara Fredrickson and colleagues as 'broaden and build'.

Consummatory Positive Affective States

Experimental studies of pleasure have primarily focused on the hedonic effects of food or thermal regulation. These studies show that the function of pleasure is to maintain behaviors that return the body to homeostasis. For example, a warm stimulus would be experienced as pleasurable by a cold individual, with the magnitude of the pleasure being proportional to the ability of the stimulus to return the body to homeostatic conditions. This emotionally driven change in sensation associated with a return to homeostasis is referred to by Michel Cabanac and colleagues as sensory alliesthesia.

Defining Positive Emotion

In humans, positive emotional states are positively valenced feeling states that are associated with the propensity toward approach and/or consummatory behavior. There are two main classes of positive emotions: appetitive positive emotional states (e.g., joy) are associated with approach behavior and pleasure is associated with consummatory behavior. The primary function of positive emotions is to elicit and maintain appetitive or consummatory behavior as well to produce positive reinforcement.

Positive Affective States in Humans

Appetitive Positive Affective States

Socializing with friends or romantic partners is the activity that most robustly elicits appetitive positive affective states. However, not all socializing is hedonic. The same studies show that interacting with supervisors or other family members is not consistently hedonic. Therefore,

Health Benefits of Positive Affective States

Positive affective states, as studied longitudinally in humans, confer resilience to depression and anxiety, and lead to an increase in overall health and a decrease in mortality from all causes. The psychological and physical health benefits of positive affective states are likely due to increases in resilience (continued global functioning despite the presence of stressors). For example, following a major life stressor, individuals exhibiting greater positive affect are less likely to develop psychological disorders such as anxiety and depression. Longitudinal studies also show that positive affective states precede the health benefit effect of positive affect. Therefore, positive affect is not simply a secondary consequence of overall good health. Conversely, individuals who have low levels of positive emotion are at greater risk of developing anxiety disorders, depression, and global health problems. Interventions that increase positive affective states have been shown to reduce levels of depression and anxiety.

Neurobiology of Positive Affective States in the Human Brain

The primary neuroanatomical underpinnings of positive emotion are associated with the ascending mesolimbic dopamine system, as described by brain imaging studies (i.e., functional magnetic resonance imaging or positron emission tomography), as well as the direct elicitation of positive affective states through drug administration or electrical brain stimulation. Brain imaging studies using recall of positive affective memories, listening to positive music, male orgasm, and positive anticipation of monetary reward have been shown to activate aspects of the ascending mesolimbic dopamine system (which includes the ventral tegmental area, nucleus accumbens, medial prefrontal and orbital frontal cortices). The euphoric effects of intravenous amphetamine have been shown to be directly related to dopamine activity in the nucleus accumbens. Direct electrical brain stimulation of the accumbens has been shown to elicit Duchenne laughter and self-report of positive affect. Patients given the opportunity to self-administer electrical stimulation to the nucleus accumbens or to an area at or near the ventral tegmental area repeatedly self-administered this stimulation and reported that the stimulation elicited a positive affective state.

The molecular underpinnings of positive affective states are still largely unknown. The endogenous molecules associated with the euphoric effects of drugs of abuse have received the most study. These consist of the endogenous opiates acting on μ -receptors (endorphins, met-enkephalin, and β -endorphin) and dopamine. μ -Opiate and dopamine levels in the mesolimbic positive affect circuit have been found to be positively correlated with the euphoric effect of exercise and amphetamine, respectively.

Intravenous administration of μ -opiate and dopamine agonists produced positive affective states in humans. μ -Opiate receptor antagonists have been shown to blunt the positive affective states elicited by exercise and alcohol. Dopamine receptor antagonists decreased positive affective states associated with psychostimulants. However, aversive stimuli also increased μ -opiate and dopamine levels in the nucleus accumbens. Therefore, the μ -opiate and dopamine systems are not completely specific to positive emotions.

Measuring Positive Affective States in Animals

In order to establish that an animal behavior reflects a positive affective state, at least two criteria must be met: (1) the behavior must serve as a positive reinforcer and (2)

in humans a homologous behavior must be associated with the self-report of a positive affective feeling state.

Is Reinforcement Sufficient for Positive Affective States?

Positive reinforcement is specific to positive affective states, whereas negative reinforcement is not. Negative reinforcement serves to reduce negative affective states, which is not the same as increasing positive emotion. Compulsive behaviors (e.g., checking or hand washing) and frustrative nonreward are examples of nonhedonic reinforcement.

Positive reinforcement as measured by conditioned place preference is a sensitive and specific index of positive emotional states in laboratory animals. Conditioned place preference can distinguish between positive and negative reinforcement. Stimuli that elicit positive affective states in humans increase conditioned place preference, whereas negative affective stimuli induce conditioned place aversion.

Operant responding alone is not a sufficient index of a positive emotional state. Rates of operant responding (i.e., bar pressing) can be increased both by positive affective stimuli (positive reinforcement) and the avoidance of aversive stimuli (negative reinforcement).

In humans, there is not a clear positive relationship between the frequency at which a behavior is performed and the level of positive affect associated with that behavior. In the study of positive affect in the everyday lives of Americans, watching television is only weakly hedonic but is engaged in more frequently than more hedonic activities such as prosocial behavior and exercise. In addition, working, cleaning, and commuting are high-frequency behaviors that are nonhedonic.

Taxonomy of Positive Affective States in Humans

The scientific study of positive affective states primarily relies on affective self-report. In these studies, the most consistent and robust elicitors of positive affect are prosocial interactions, eating, and exercise. Each of these behaviors has clear homologies in animals.

There are other behaviors that are hedonic in both humans and laboratory animals that do not occur frequently enough in everyday life to be captured by these self-report studies. Offensive aggression and predatory behavior are hedonic in laboratory animals, and are probably also hedonic in humans.

Some hedonic behaviors in humans have either not yet been adequately modeled in laboratory animals or modeled in animals. Relaxation is a major source of pleasure in humans, but has not yet been adequately modeled in laboratory animals.

Communicated Positive Affective States in Laboratory Animals

Some positive affective states are communicated by facial/vocal displays in both humans and laboratory animals; these displays can be used as unconditional indices of positive emotional states. Prosocial behavior elicits hedonic Duchenne smiling and laughter in humans. In laboratory rats, hedonic 50-kHz ultrasonic vocalizations (USVs) are a homolog of these behaviors. Hedonic food elicits hedonic oral facial behavior (e.g., lip smacking) in humans. In laboratory rats, hedonic taste reactivity responses are a homolog of these behaviors.

Hedonic 50-kHz USVs in Rats

Frequency-modulated 50-kHz USVs have been shown to reflect a positive affective state in rats. Positively reinforcing social interactions (i.e., mating and rough-and-tumble play), anticipation of food, and action of euphorogenic drugs of abuse increased number of emitted 50-kHz USVs, whereas aversive stimuli such as social defeat, frustrating nonrewarding situations, sickness-inducing doses of lithium chloride, and foot shock all decreased the number of 50-kHz USVs. The rewarding value of the stimuli eliciting positive affective states was positively correlated with the rates of 50-kHz USVs elicited by positive social, drug, and electrical brain stimulation reward. μ -Opiate and dopamine agonists, as well as electrical brain stimulation of the mesolimbic dopamine system, also increased rates of 50-kHz USVs in rats. The neuronal and pharmacological underpinnings of rat 50-kHz USVs appear to be consistent with those of human positive emotions.

Alternative nonhedonic interpretations of the emission of 50-kHz USVs are not supported by the available data. (1) 50-kHz USVs have been hypothesized to be an artifact of locomotor activity-induced thoracic compressions. However, additional studies found that only 10% of 50-kHz USVs were coincident with thoracic compressions, and 50-kHz USVs could be dissociated from locomotion. (2) 50-kHz USVs have been hypothesized to reflect a nonaffective contact call. More detailed studies found that flat 50-kHz calls appear to be a nonaffective contact call, occurring at the highest rates during nonpositive affective social interactions. However, frequency-modulated 50-kHz calls appeared to be selective for positive affective social interactions. (3) 50-kHz calls have been hypothesized to reflect a nonpositive affective wanting state. 50-kHz USVs are increased in the anticipation of delivered reward, which in humans has been shown to elicit a positive affective state. However, during extinction bursts or frustrating nonreward, such appetitive behavior decreased rates of 50-kHz calls and increased rates of aversive 22-kHz calls. Therefore, only positively

valenced appetitive behavior appears to elicit frequency-modulated 50-kHz USVs. (4) 50-kHz ultrasonic calls have been hypothesized to reflect a state of high arousal that is not specific to positive affective states. Highly arousing aversive stimuli such as predatory odor, foot shock, and bright light decrease rates of 50-kHz calls, whereas rewarding stimuli increase rates of 50-kHz calls.

Hedonic Taste Reactivity

Hedonic taste reactivity responses in rats, consisting of midline and lateral tongue protrusions and paw licking in response to oral infusions of tastants, have been shown to be homologous to positive affective states in humans. Hedonic sweet solutions (e.g., sucrose or saccharin), or salty (NaCl) solutions in salt-deprived animals, increase hedonic taste reactivity scores. Aversive solutions (e.g., quinine) and conditioned taste aversion produced by LiCl reduce rates of hedonic taste reactivity scores. Both sucrose and saccharin produce conditioned place preference, and LiCl produces conditioned place aversion in rats. μ -Opiates and cannabinoids increase positive affect in humans and increase hedonic taste reactivity responses when injected into the nucleus accumbens.

Noncommunicated Positive Affective States in Laboratory Animals

Not all positive affective states have a social communication component. The communication of positive affective states serves to both promote prosocial behavior and provide positive reinforcement in the receiver. During offensive aggression, the communication of positive affective states is inappropriate given that prosocial behavior and positive reinforcement in the attacked animal is not warranted. Exercise and predatory behavior do not necessarily involve conspecifics. Therefore, communication of a positive affective state during exercise and predation is also not warranted.

Offensive Aggression

Offensive male–male aggression serves as a positive reinforcer for the dominant animal in the conditioned place preference test, and therefore is considered to reflect a positive affective state. The rewarding and positive affective value of aggression is probably limited to only certain kinds of aggressive behaviors. Defensive male resident-intruder or maternal aggressive behavior may be motivated by negative reinforcement (i.e., avoid loss of sexual partner, offspring, and/or territory), and therefore not necessarily associated with a positive affective state. In contrast to the dominant animal, submissive animals have

been shown to exhibit diminished positive affective states and increased levels of depression and anxiety.

Predatory Behavior

Predatory behavior has also been shown to reflect a positive affective state in laboratory animals. In rats, animals that readily engage in mouse-killing behavior show conditioned place preference for environments paired with mice. In cats, electrode sites that support electrical brain stimulation-induced mouse killing also uniformly support self-stimulation.

Exercise

Rats will show place preference for environments paired with voluntary wheel-running behavior. In a similar manner as human positive affect, voluntary wheel running induces resilience to depression and increases the lifespan of the animal. It is unclear if forced exercise is hedonic in laboratory animals.

Drugs of Abuse

Rats will show place preference for drugs that robustly increase positive affect in humans. In contrast, aversive substances such as LiCl induce robust-conditioned place aversion. Therefore, the hedonic value of drugs of abuse can also be measured in laboratory animals.

Conclusions

The 50-kHz USVs and hedonic taste reactivity have been shown to reflect a positive affective state in rats.

Noncommunicated positive affective states can be measured by conditioned place preference. Because it is now possible to study positive emotional states in rats, the neuroanatomical basis and molecular mechanisms of these forms of positive affect can now be elucidated. These studies should lead to a deeper understanding of positive affect in humans and should lead to the development of novel therapeutics for the treatment of depression.

See also: Evolution of Emotions; Play Behavior; Subjective Experience and the Expression of Emotion in Man.

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Social Competition and Conflict Resolution

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Glossary

Affiliative interaction – Positive social interaction between individuals such as grooming, playing, sleeping in contact.

Anxiety response – Emotional reaction caused by aggression. This response can be objectified using the measurement of different behavioral indicators. In primates, for example, rates of scratching increase after a conflict both in the aggressor and the aggressee.

Conflict – The aggressive behavior of one individual (vocalization, stare, lunge, pursuit, slap, grab, and bite) and the response of the opponent (aggressive or submissive). It does not necessarily involve physical contact.

Dominance hierarchy – The network of dominance–subordination relationships in a social group. When the hierarchy is linear, individuals can be ranked from the most dominant animal to the most subordinate one. Individuals can be qualified as high ranking or low ranking.

Dominance relationships – In a pair of individuals which know each other, one (the dominant) regularly wins conflicts with the other (the subordinate). When a subordinate meets a dominant, it expresses its lower status by either avoiding the dominant or submitting to it.

Reconciliation – Positive contact between former opponents following a conflict. Reconciliation is presumed to be a positive factor in repairing the relationships jeopardized by the conflict.

Stress response – Adaptive physiological reaction to either an internal or external stimulus. The adrenal cortex secretes glucocorticoids in response to stress. Chronic stress is the result of repeated exposure to stressful situations and has deleterious consequences, due in particular to the oversecretion of glucocorticoids.

Submission – One individual acknowledges its lower status to a higher-ranking individual by displaying behaviors such as avoidance, crouching, presentation of hindquarters, or specific facial expressions and vocalizations.

Third-party intervention – One individual intervenes in a conflict between two opponents. The intervention may be peaceful or aggressive. In the latter case, aggressive support leads to a coalition of two opponents against a third one.

In group-living animals, conflicts of interest over limited resources may lead to open contests between group members. Aggression occurs in many circumstances of daily life. While it is potentially disrupting for societies, it can also structure relationships. In the following text, the term ‘aggression’ will be used to describe the whole phenomenon, whereas ‘conflict’ will specifically refer to social interactions between animals. This article focuses on intra-group competition. When aggression occurs, conflict escalation can be prevented by various management strategies, and its deleterious effects on individuals and social relationships can be mitigated through post-conflict resolution and social dominance. Considering the physiological underpinnings of aggression can lead to a better understanding of the consequences of aggression and dominance for individuals.

Conflict Management

The outcome of conflicts depends on the balance of power between opponents. When the aggressee flees or submits, the conflict is called unidirectional or asymmetrical. When it counter-attacks, the conflict is bidirectional or symmetrical. Counter-attacking in a conflict is not very common and only occurs if there is a low risk of injury. Their escalation is usually nonadaptive, since suffering injuries would decrease the fitness of opponents. However, during challenging periods for top-ranking positions, animals can fight to the death. In this context, group members should maintain the balance of power and solve conflicts through several mechanisms. To study aggression, we therefore have to consider not only agonistic behavior such as threats and attacks, but also the response to this aggression in forms such as escape, appeasement, submission, or redirection.

Appeasement and Submission

The first solution to conflict is the use of appeasing behaviors between opponents immediately after the conflict. Appeasement can therefore be considered to signal the end of the conflict. One or both opponents can display these behaviors, which include facial displays, contact calls or positive contacts such as grooming, mounting, or playing. There is no division of roles between opponents:

the aggressor can start appeasing the aggrieved, or the opposite. Aggression can also be stopped by the submission of one individual to its opponent. Submission can include behaviors such as displaying facial expressions, fearful vocalizations, or bending down. Subordinate individuals generally address submissive signals to higher-ranking opponents.

Redirection of Aggression

In some instances, one opponent, more often the aggressor, redirects aggression by threatening or attacking a third individual, thus transferring to the latter the attention of its aggressor and of other group members. The target of the redirection is generally a lower-ranking individual. Redirection is only an efficient strategy if the previous aggrieved is certain to win the new conflict, and is especially efficient in species such as rhesus macaques where counter-aggression is scarce. In species where counter-aggression is common like Sulawesi macaques, redirecting aggression toward another group member would lead to new conflicts and would be a quite unproductive strategy.

Revenge

The target of a redirection can sometimes be a relative of the aggressor (kin-oriented redirection) which is often less powerful and more vulnerable than the latter (as observed in Japanese and pig-tailed macaques). It has been shown that in such cases, the former aggressor rarely supports its relative, mainly because such redirection occurs during polyadic interactions where it would have to face several opponents. This kind of strategy could be a means to modify the aggressor's attitude toward the aggrieved on a long-term scale. Indeed, this aggressor will avoid attacking this specific individual again in order to avoid redirected aggression toward its relatives and thus protect them. Such social manipulation has been considered as a revenge system; it has been reported in macaques, baboons, and chimpanzees. However, the revenge system in chimpanzees has been described as a case of social reciprocity, since the former aggressor intervenes in favor of its attacked relative during redirection events. Such differences in the aggressor's behavior in a revenge system could be explained by the different degree of nepotism and despotism observed within the two forms of social organization. Besides kin-oriented redirection, a generalization of a former conflict to matri-lineal level has been observed, with an aggrieved's kin attacking an aggressor's kin. In baboons, aggrieved and aggressor's relatives do not need to witness the conflict: hearing vocal threats and/or protest of kin's fight is sufficient to induce a conflict between them.

Third-Party Intervention

Long-lasting social tension may affect foraging performances, defense against other groups, and antipredator vigilance, which all require some degree of social stability to be efficient. Therefore, besides having direct effects on opponents, conflicts also indirectly influence the behavior of others. Any disturbance of the social balance is of interest to all group members. Given the potential effects of escalated aggression on other group members, individuals unrelated to both opponents seek to influence events following a conflict between two group members and can directly intervene in the conflict.

Interventions in conflicts have mostly been described in nonhuman primates, but they have been recently reported in rooks. The third party generally supports one individual (the beneficiary) against its opponent (the recipient of the intervention). The third party can reinforce its relationship with the beneficiary, or increase its dominance over the recipient of its intervention. The motivations and benefits of interveners are competitive. The most frequent form of intervention is aggressive, in which the intervener forms a coalition with one opponent: they attack the other opponent together and win more often (**Figure 1**). Rank reversals often occur after repeated coalitions between partners. In several nonhuman primate species, nonaggressive means of intervention occur. In agonistic buffering, during male–male conflicts, a young individual is used by one challenger to appease the other one. This intervention has been reported for Barbary macaques and several baboon species and is frequently an involuntary participation for the juvenile: adults use infants and juveniles' immunity for their own interests. More rarely, the approach of a high-ranking individual toward opponents can be sufficient to stop



Figure 1 Coalition in white-faced capuchins (*Cebus capucinus*) at Lomas Barbudal Reserve, Costa Rica. Photograph by G. Prats.

the conflict. In other cases, a third individual unambiguously intervenes in conflicts by appeasing one opponent using signals such as affiliative facial expressions, touching, clasping, and mounting.

In Tonkean macaques, nonaggressive interventions are more efficient than aggressive ones to prevent conflict escalation. They have been qualified as peaceful interventions. Similar behaviors have been reported in gorillas and chimpanzees. In peaceful interventions, contrary to aggressive ones, the intervener does not make a choice against one opponent; it helps one partner and preserves its relationship with the other. On some occasions, the intervention is directed to both opponents. The intervener threatens both opponents or simultaneously appeases them. Peaceful interventions appear more effective than aggressive ones in reducing social stress and avoiding conflict escalation.

PostConflict Resolution

De Waal and van Roosmalen defined reconciliation as a positive contact occurring between previous opponents immediately after a conflict. The measurement of specific postconflict attraction between former opponents is used to demonstrate the occurrence of reconciliation. By comparing the postconflict period to baseline conditions, it is possible to demonstrate that friendly contacts between opponents occur earlier following conflicts. Thanks to this method, reconciliation has been observed in many species such as dolphins, hyenas, wolves, goats, and more particularly in nonhuman primates: baboons, macaques, capuchin monkeys, chimpanzees, etc. The form of reconciliation and the probability of reconciliation differ not only between species, but even within a same genus. Some species display low rates of conciliatory tendencies while others display high rates. Reconciliation appears to covary with the degree of tolerance between group members, especially in macaques. Indeed, in species characterized by a strict hierarchy and a high preference for relatives, reconciliation is less frequently observed than in species where dominant individuals are tolerant toward subordinates and in which good relationships exist between most group members, including nonrelatives. Strong conciliatory tendencies keep competition costs low and help preserve social relationships. Behaviors used in postconflict interactions are not specific to reconciliation. Animals display behavior patterns such as clasping, grooming, mounting, or social play.

One might think that kin-related individuals or individuals with good relationships rarely fight, and that conflicts occur mostly between nonaffiliated dyads.

Rather to the contrary, preferred partners are often close together, so they are more likely to interact aggressively. As a consequence, they need to repair their relationships, which often suffer through conflicts. In many species, reconciliation is more likely after conflict between valuable partners.

Reconciliation may reduce the occurrence of further aggression, reduce anxiety caused by the conflict, and restore the damaged bond to preconflict levels. The integrated hypothesis links the quality of the opponents' relationship and the level of postconflict anxiety. In primates, scratching is a stress-related behavior, an indicator of tension that has been used as a measure of postconflict anxiety. Scratching provides information about the animals' perception and evaluation of a certain event, and is a valuable tool to study the impact of conflicts and postconflict events on the participants. Conflicts lead to increased levels of scratching in both aggressors and aggrieved individuals in several species, such as chimpanzees, long-tailed macaques, and baboons. The disturbance of the relationship with the adversary may induce anxiety. Reconciliation reduces stress-related behaviors to control levels. Thus, one of the main functions of reconciliation might be stress alleviation.

Given the possible effects of aggression on the whole group, the aggrieved's kin, aggressor's kin, and unrelated individuals are all liable to take part in the postconflict situation. In addition to dyadic conflict resolution, triadic postconflict affiliation has been described in several species. This refers to affiliation between conflict participants and uninvolved bystanders called third parties. Such contacts may involve the aggressor or the aggrieved as well, and third parties may be kin to either one of the contestants, or unrelated to them.

When a former aggrieved individual affiliates with a third party, this is considered as a consolation. It has been proposed that such consolation would occur when reconciliation is not possible, and allows stress alleviation. In general, triadic postconflict resolution is not an alternative postconflict interaction, it does not alleviate stress levels in aggrieved individuals, and even aggressors may receive such postconflict affiliative contact. For example, in long-tailed macaques, female aggressors showed selectively increased affiliative contacts with kin of the aggrieved and an increase in contacts with other group members (own kin and unrelated individuals). Such behavior might benefit individuals which provide affiliation: in this way they avoid escalation of aggression to other group members. The hypothesis of such self-protection is supported by the fact that affiliation in chimpanzees is selectively given to those opponents showing further aggression to third parties, and this behavior actually decreases the risk of receiving aggression from contestants.

Social Dominance

Group members should avoid systematically entering conflicts concerning competition for food, water, or mates. To deal with social competition, animals generally establish stable and long-term social relationships based on dominance, which is an adaptive strategy to maintain social cohesion. Such relationships are established through aggressive encounters where one opponent wins and the other loses. Such an outcome refers to real dominance. The iteration of aggressive interactions between two partners leads to a stable dominance–subordination relationship, with one partner being dominant over the other. In subsequent encounters, the subordinate avoids the dominant partner or submits. This formal dominance is then based on directional signals formally acknowledging the relative status of partners. This relationship lasts as long as the subordinate accepts it.

A high dominance status confers many advantages such as priority access to best resting places, sexual partners, or preferred food. The network of dominance relationships for all group members constitutes the social hierarchy. When the hierarchy is linear, the top-ranking animal of the group (sometimes called the alpha individual) dominates all other group members, the second higher-ranking animal dominates all group members except the first, and so on. In some species, we observe triangular relationships that stem from nontransitive relations: A dominates B, which dominates C, which dominates A. In species such as horses and social carnivores (mongooses, wolves, and wild dogs), dominance rank strongly depends on age. Similarly, in gorillas, an age-graded hierarchy is reported among males. The fully mature male is recognizable by its silver back; silverback and top-ranking individual are synonyms in this species. Many species display a dominance of males over females, but in some primates, alliances between kin-related females can alter it.

Dominance-rank inheritance is reported in species such as macaques and baboons, where females are philopatric, that is, females stay in their natal group during their whole life. Mothers support their daughters in conflicts against other group members, and thus help them to reach a hierarchical status close to their own. As a result, all members of the same matrilineal clan – all kins descending from the same female – have similar dominance – ranks. Within a matrilineal clan, young daughters are supported by their mother in conflicts with their older sisters, and they often outrank them.

The social hierarchy can be more or less steep. In some species, low-ranking individuals may easily approach higher-ranking individuals, and they may have access to limited resources. In other species, higher-ranking ones systematically displace lower-ranking animals. The latter

stay in the periphery of the group most of the time, preventing the occurrence of positive interactions between group members of contrasted dominance ranks. These different societies are observed in macaques, where some species are considered as tolerant and others as intolerant. In the former, aggression intensity remains low and conflicts are mostly bidirectional because individuals can protest and counter-attack without risk. This contrasts with the relations observed in the more intolerant macaques where biting is not rare; in such species, it is more effective to flee when attacked.

Physiological Determinants

The response of individuals within the context of aggression and social dominance depends on their ability to react to stressful situations. In rodents, higher social status and aggressiveness are associated with high testosterone levels, whereas subordination and defeat of conflicts are associated with increased adrenal corticoid production. Nevertheless, there is no typical scheme over time for the physiology of aggression and dominance. It depends on stability versus instability observed over the studied period. Stable periods are characterized by clear-cut hierarchical relationships where subordinate animals spontaneously submit to higher-ranking individuals. During unstable periods, some low-ranking individuals – often adolescent ones, and more especially young males – challenge high-ranking animals. Whereas an immediate stress response is an adaptive physiological reaction which is both useful and healthy, a chronic response may have pathological consequences.

Aggression and Hormones

The neuroendocrine regulation of aggression has been well studied in birds, rodents, and primates. Testosterone is linked to aggressive male behavior in response to reproductive challenges; increased testosterone output corresponds to periods of increased aggression during territory defense or mate guarding. Increased glucocorticoid secretion is also an adaptive stress response to challenging situations.

In rats and mice, aggression is associated with high levels of glucocorticoids in both opponents, although the increase is observed over a shorter period of time in the winner. In nonhuman primates, both contestants show an increase in heart rate after an agonistic interaction, and increased rates of scratching that are associated with sympathetic activation. Being attacked is also associated with increased concentrations of glucocorticoids. In general, high rates of aggression tend to be positively correlated with elevated testosterone concentrations in primates. Causality is implied by typical decline in rates

of aggression following castration and testosterone-induced restoration. However, testosterone secretion is neither necessary nor sufficient for aggression to occur. Testosterone seems to have a modulatory rather than an activational effect on aggression.

The challenge hypothesis states that plasma testosterone levels and aggression are positively correlated during periods of social instability or challenge. During such periods, aggressive interactions are more frequent, leading to high testosterone levels. Glucocorticoids and testosterone show significant increases during both mating and birth seasons in male red fronted lemurs. This increase has also been observed in birds. In seasonally breeding birds, circulating sex steroids fluctuate, generally with high levels during the breeding season and basal levels during other periods of the annual cycle. In many avian species, territorial behavior shows a similar seasonal pattern. In a migratory population in a common North American songbird, plasma testosterone levels in males are high in early spring, when males are aggressively establishing territories.

During the nonbreeding season, aggression is independent of testosterone and other sex steroids. Male and female spotted antbirds show high levels of territorial aggression toward same-sex intruders in the nonbreeding season and show elevated concentrations of dehydroepiandrosterone (DHEA) and low (or untraceable) concentration of testosterone and oestradiol. DHEA appears to play the same role in the aggressive behavior of hamsters and humans.

Serotonin is involved in the determinism of aggression. Male rhesus macaques with low central serotonin levels early in life show social isolation, impulsive aggression and high levels of violence. This profile exposes these individuals to increased risk of traumatic injury and early death. Moreover, low concentrations of cerebrospinal fluid levels of serotonin metabolite (5-HIAA) are associated with negative life-history patterns, characterized by social instability and extreme aggression, and are also linked to positive patterns such as high dominance rank.

Dominance and Stress

Many studies have found that in stable hierarchies, low-ranking individuals have higher stress levels (measured in heart rate or cortisol) than high-ranking individuals. Stress levels may cause changes in individuals that affect a number of aspects of their biology. This includes factors such as their immune competence, coronary health, basal hypertension, and a slow activation of the cardiovascular stress response after conflict, together with delayed recovery when stressful pressure reduces. Animals that are more socially stressed by the dominance hierarchy show signs of hyperactivity of the glucocorticoid system. The adrenal cortex secretes glucocorticoids in response to

stress and these steroids are central to the adaptations that the individual must carry out to cope with physical stressors. Glucocorticoids increase cardiovascular tone and inhibit a variety of costly anabolic processes – including digestion, growth, reproduction, and immune and inflammatory responses – until after the stressor has decreased. These functions represent high catabolic process costs. Therefore, it is not surprising that the pathological consequences of chronic stress are largely mediated by overexposure to these same steroids.

Dominance rank was found to correlate with urinary cortisol in female long-tailed macaques: high-ranking individuals had lower rates than low-ranking individuals. In addition, high-ranking females attracted more, gave more agonistic support, and were bitten less frequently than low-ranking females, but they were not attacked less frequently than low-ranking ones. Similarly, in rhesus macaques, low-ranking animals were attacked more severely, but not at higher rates than high-ranking ones. A correlation between dominance rank and aggressiveness has been reported in the females of the latter species, high-ranking females being more aggressive than low-ranking ones. Moreover, a negative correlation was found between aggressiveness and cortisol. Thus, individuals near the top of the social hierarchy differ in physiology and behaviors from those occupying lower positions. Interestingly, in baboons, subordinates that initiate fights or redirect aggression have lower cortisol levels than subordinates that do not display these behaviors. From these results, no single physiological profile can be associated with either dominance or subordination; some studies found no relation between stress levels and position in the hierarchy.

Social instability may explain variations in the interrelations between endocrine function and dominance. During periods of social stability, basal testosterone concentrations are similar in high- and low-ranking males, although the former tend to be more resistant to stress-induced suppression. Indeed, they are relatively resistant to the inhibitory effects of stress, that is, they are more prone than others to maintain and even to increase concentrations of testosterone during stress. Studies of dominant male primates in stable hierarchies have shown their adrenocortical axes to possess rapid and high secretion with the onset of stress, with prompt termination at the end. In baboons, high-ranking males tend to have low resting cortisol secretion, an ability to rapidly increase secretion during stress, and an enhanced sensitivity to the feedback regulation that terminates secretion. Under stable conditions, dominant males also show metabolic, cardiovascular, and immune profiles that are potentially less pathogenic than those of subordinates. In contrast, during periods of instability, basal testosterone levels are considerably higher in high-ranking than in low-ranking males. High rank during times of social

instability is associated with both high rates of aggression and high concentrations of basal testosterone, as proposed by the challenge hypothesis. In challenging periods or during group formation in baboons, dominant males most frequently initiate aggressive interactions and also have the highest basal testosterone levels. On the contrary, a severe decline is observed in subordinates at the same period. Similar results have been reported in rhesus macaques and squirrel monkeys. Therefore, a positive correlation emerges between rank and testosterone, although it is not apparent during stable periods. In addition, during instabilities dominant males no longer show the lowest glucocorticoid concentrations. They no longer display the most spectacular increases in cortisol concentrations during stress. Differences between dominant and subordinate males in gonadal activity during stressful situations in stable groups are associated with altered regulation of pituitary–adrenal activity. These adaptive and dormant features of adrenocortical function, characteristic of high-ranking males during periods of social stability, are lost during periods of instability. It appears unlikely that high testosterone causes dominance. The association between dominance and high testosterone concentrations appears to be primarily due to an effect of behavior on the endocrine system; one would expect a decreased correlation as the relationship become more relaxed under stable conditions.

Reproductive Correlates

From an evolutionary perspective, being dominant appears more advantageous than being subordinate, at least during stable periods. Struggling for dominance is presumed to yield higher benefits at higher costs, whereas accepting subordination probably produces lower benefits but at lower costs. But no consensus exists as to whether dominant or subordinate animals are more physiologically stressed. When considering which ranks are more stressful, we must look at the dominance style of the species. In species where higher-ranking individuals have to repeatedly and physically reassert their dominance, they are the most stressed animals, reflecting the physical demands of frequent fighting. This applies to cooperative breeders where all group members rear young, whereas dominant individuals monopolize reproduction by harassing subordinates and those with temporary periods of rank instability. In contrast, in species where social hierarchy is stable and high rank is

maintained through nonphysical pressure rather than through aggression, this profile occurs among subordinates because they are more exposed to frequent social stressors.

Experiencing low rank can lead individuals to develop alternative strategies, less conspicuous than aggressive ones. In various species of monkeys where the reproductive skew is biased in favor of the top-ranking male, subordinates can increase their reproductive success using alternative strategies such as sneaking copulations. Females have some control over the partners they mate with in species like baboons or chimpanzees. They may choose low-ranking males with whom they have preferential relationships. These males not only increase their reproductive success but they show fewer physiological indices of stress than expected from their social rank.

To summarize, no clear pattern can be drawn concerning the link between aggression, stress, dominance, and reproductive success. Dominance might have advantageous correlates as long as subordinates accept their low social status.

See also: Animal Tests for Anxiety; Hormones and Memory; Mating Behavior.

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Stress and Emotionality

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Glossary

Anxiolytic/anxiogenic – The stimuli (chemical, drug, environment, etc.) that decrease anxiety; anxiogenic refers to stimuli that increase anxiety.

Corticotropin-releasing factor (CRF) – A stress hormone produced by the hypothalamus and other brain regions. It binds to the cognate receptors, CRF-R1 or CRF-R2 (also known as CRF1, CRF2).

Hypothalamic–pituitary–adrenal (HPA) axis – The hypothalamic–pituitary–adrenal axis is responsible for the neuroendocrine response to stress.

Phenotype – A detectable expression of a genotype.

Selective breeding – The breeding/mating of animals showing similar traits (behavioral, physical, or physiological) to enrich offspring with the same trait.

Stress reactivity – An individual response to a stress/stimuli expressed by behavioral and endocrinological measures.

pituitary corticotropes to stimulate the release of adrenocorticotropic hormone (ACTH). ACTH, in turn, increases the synthesis and release of glucocorticoids from the adrenal gland. Glucocorticoids direct the metabolic and energetic changes that allow the body to respond to the stressful stimulus and help to terminate the HPA-axis response through a negative-feedback loop to return the body to homeostasis.

The activation of the HPA axis can result from internal or external signals, often categorized as either processive or systemic stressors. Processive stressors are those that invoke higher cognitive functions such as restraint, novelty or novel environments, and social stress. Systemic stressors are elicited by sensory stimuli such as metabolic or immunologic disturbances; examples include hypoxia, pain, inflammation, and blood loss. Experience with any of these stressors results in increased release of CRF, ACTH, and glucocorticoids within the HPA axis; a variety of major neurotransmitters (i.e., norepinephrine and epinephrine, dopamine, and serotonin (5-HT)) or other neuropeptides (vasopressin, neuropeptide Y (NPY), etc.) are also released, often from non-HPA-axis circuitry.

Strikingly, the frequency (acute vs. chronic) and familiarity of a stressor have differential effects on both the physiological response as well as activation of non-HPA-axis neuronal circuits. Acute stressors reliably elicit an HPA-axis response, but the level of response is controlled in part by the type and intensity of the stressor. Chronic stress can be predictable, as in exposure to restraint stress for multiple sessions, or unpredictable, using a varied number of stressors administered in a random fashion, and the response of the HPA axis differs depending on the predictability. A repeatedly administered or encountered stressor – such as daily restraint stress (homotypic stress) – often leads to habituation, evidenced by decreased glucocorticoid release. In contrast, if multiple stressors are administered in a random order, even if repeated over several weeks (chronic unpredictable stress), little or no habituation is observed. While the duration, controllability, and intensity of the chronic stress episode influence the outcomes, a wide variety of behavioral effects (e.g., aggression and anxiety) and physiological responses (e.g., elevated corticosteroids (CORTs), enlarged adrenal, involuted thymus, and decreased body weight) have been observed. The inability to adapt to stress has been associated with changes in

Stress

Stress is often broadly defined as a disturbance in the homeostasis or well-being of an organism caused by a physical or psychological challenge (stressor). At the physiological level, stress is characterized by the activation of the hypothalamic–pituitary–adrenal (HPA) axis that results in an increase in circulating glucocorticoids (cortisol in humans, corticosterone in rodents, etc.). The acute stress response is highly beneficial to humans and animals since it facilitates the fight-or-flight response to endangerment and a return to homeostatic conditions. However, either the inability of the body to respond adequately to stress or prolonged exposure to stress can result in adverse consequences on physical and psychological well-being.

HPA Axis and Stress

The HPA axis is the direct pathway to the release of adrenal stress hormones into the circulation. In response to stressful stimuli, the corticotropin-releasing factor (CRF) produced in the paraventricular nucleus (PVN) of the hypothalamus is released from the median eminence into the portal blood, acting on anterior

CRF and glucocorticoid secretion and can lead to increased risk for affective disorders in humans. Sustained changes in glucocorticoid secretion have also been linked to cardiovascular disease, obesity, and type II diabetes.

Basic Neural Circuitry Underlying Stress Response

While all stress ultimately results in HPA-axis activation, the type of stressor strongly influences which other neural circuitry (e.g., limbic circuitry vs. brainstem) is activated. In general, systemic stressors activate the brain areas involved in the initial sensory processing of the stimulus – especially brainstem nuclei – and are notably independent of activation of limbic circuitry. Many brainstem nuclei send direct projections to the PVN, resulting in activation of the HPA axis; cognitive awareness is not necessary to activate a stress response since systemic stressors tend to acutely threaten homeostasis.

In contrast, processive stressors generally activate the limbic circuitry, including the amygdala and hippocampus. Limbic circuits appear to play a primary role in the conditioning that leads to the memory of a stress in the absence of the actual stimuli. Through the repeated pairing of a stress-inducing stimulus with a neutral one, the stress response can be induced by the once-neutral stimulus. For example, if a rodent is shocked in an environment that otherwise is not harmful, the rodent will release stress hormones in that environment and avoid it even in the absence of the shock. Both the amygdala and the hippocampus have been shown to be involved in the consolidation of these emotional memories. Cortical activation is also involved in processive stressors, adding to the cognitive dimension. As these stress-regulatory limbic sites (cortex, hippocampus, and amygdala) lack significant direct input to PVN, they relay their information through synapses in the bed nucleus of the stria terminalis (BNST), preoptic area, and hypothalamus – providing the opportunity for integration with other information on ongoing physiological status prior to the final signal to the PVN stress neurons. It is important to note that within each class of stressor, the same circuitry is not necessarily invoked, but any mono- or multisynaptic circuitry involved eventually innervates the PVN to activate the HPA axis.

Emotionality

Emotionality in humans refers to the experience of emotions (e.g., happiness, sadness, fear, anger, and anxiety) and includes the accompanying physiological changes, such as alterations in heart rate and sweating. Emotions are forms of communication between individuals and, as

in the case of fear, can be a useful life-saving defense mechanism. However, when the extremes of emotion are exhibited for extended periods of time, they can represent a more severe pathological state. Affective (mood and anxiety) disorders represent the dysregulation of emotional states. Mood disorders, as categorized in the *Diagnostic and Statistical Manual-IV* (DSM-IV), include major depressive and bipolar disorders, while anxiety disorders include generalized anxiety, panic, phobia, and posttraumatic stress disorder (PTSD). Understanding the etiology of these affective disorders is critical for developing appropriate treatments to reestablish personal well-being.

In animals, especially rodents, emotionality usually refers to fear and anxiety; some investigators also include depression-like behavior. Although animals may not experience the same constellation of emotions as humans, fear- and anxiety-related behaviors in particular represent negative emotions that are driven by species-specific, yet evolutionarily conserved, defense mechanisms. Fear and anxiety are distinguished by the reality of the harmful stimulus: a real, imminent threat results in fear, whereas the perception of a harmful threat, not necessarily present, results in anxiety. Further distinctions between the emotional states of fear and anxiety have been elucidated pharmacologically. In rodents, benzodiazepines (anti-anxiety drugs) reduce anxiety-related behaviors such as risk assessment, but do not affect fear responses to an imminent threat or danger.

Emotionality in rodents is measured by a behavioral response to a specific change in the animal's environment. Historically, one of the most commonly used responses was the locomotor activity observed after placement in a novel, aversive environment (open-field). Locomotor stimulation or depression was often accompanied by changes in autonomic responses such as heart rate, defecation, and urination. Together, activity and defecation were used to describe rodents as more emotional (decreased activity and increased defecation) or less emotional (increased activity and decreased defecation). Although this test is still commonly used, other behavioral assays have been developed to measure anxiety (elevated plus maze (EPM), light-dark box, etc.), fear (startle, conditioned fear, etc.), or depression-like behavior (forced swim, tail suspension, etc.). However, it must be emphasized that no single test can be used to definitively measure anxiety, fear, or depression-like behavior in rodents since these emotional states are complex behaviors, subject to individual variation. Thus, animal models are often tested on a battery of behaviors to better assess an anxious, fearful, or depression-like phenotype. Pharmacological treatments that affect outcomes in these behavioral tests have been used for the validation of these behavioral assays, and these behavioral tests continue to be used for the identification and development of novel therapeutics for the treatment of affective disorders.

Brain Circuits Mediating Fear and Anxiety

Our understanding of the circuitry underlying emotional states has been advanced by the use of imaging studies in human subjects. The ability to visualize the brain regions activated by fearful or anxiety-provoking stimuli has indicated that activation of the amygdalar nuclei is critical to anxiety and fear responses. Functional imaging studies have also identified the amygdala, hippocampus, and cortex as regions involved in anxiety disorders, including PTSD and phobia.

In rodents, the neural circuitry underlying fear (using the startle response and conditioned fear/fear-potentiated startle) and anxiety have been identified by lesion and pharmacological studies. Results from these studies have identified limbic regions, in particular the amygdala and BNST, in mediating fear- or anxiety-related behaviors. Using fear-potentiated startle as a model, the general flow of information within the extended amygdala has been identified with the basolateral nucleus of the amygdala (basolateral amygdala (BLA)) acting as the input center for fear or anxiety stimuli and the central amygdala (CeA) and BNST acting as the main output regions. Fear and anxiety have also been dissociated anatomically; the BLA and the CeA mediate responses to fear-related startle stimuli, whereas the BNST appears to mediate anxiety-related responses. For example, CeA lesions block fear-potentiated startle, whereas BNST lesions do not. In contrast, anxiogenic stimuli (e.g., bright light) enhance startle – an effect that is altered by BNST lesions, but not by CeA lesions. Thus, the dissociation of fear and anxiety can be seen not only at the level of the activating brain regions but also at the subsequent downstream neural circuits.

Stress and Emotionality

Many affective disorders are precipitated and/or exaggerated by stress. Assessing the interplay between stress and emotionality is, therefore, critical to understanding the development and progression of emotional psychopathology. Does stress reactivity predict anxiety/fear reactivity? Does increased anxiety predict altered stress reactivity? The answers to these questions are complex and require an understanding of the overlapping circuitry of these processes and a wide variety of *in vivo* studies using pharmacological, behavioral, and genetic approaches. In the sections below, we attempt to provide some insights into these areas using both human and animal model systems.

Behavioral Interactions of Stress and Anxiety

A review of the literature clearly demonstrates that stress and emotionality are closely interrelated in human and

rodent behavior. In humans, early-life stress can predispose vulnerable individuals to depression and/or anxiety disorders later in childhood or adult life. Children who lose a parent or are physically or sexually abused show a much higher risk for the development of affective disorders as adults. With these early-life stressors, the HPA axis often becomes hyperactive, resulting in altered glucocorticoid release and feedback – sensitizing the individual to subsequent stressors. Similarly, chronic stress during childhood or as an adult can result in altered emotional response and increased risk in susceptible individuals for mental disorders such as generalized anxiety, major depression, and bipolar disease. In addition, a single traumatic and intensely stressful event can result in PTSD.

Dysfunction of the HPA axis is observed in a wide variety of these affective disorders. In social phobia, an elevated HPA-axis response to social stressors is observed; the HPA-axis response is often dysregulated in PTSD as well. These results suggest that stress or stress-associated factors may contribute to the development of anxiety and mood disorders; however, it is possible that the HPA-axis dysfunction may develop subsequent to the psychopathology in some cases. Finally, the contributions of genetics and environment cannot be understated as the individual differences in response to similar stressors suggests that both genetics and environment contribute dramatically to an individual's coping skills and vulnerability to affective disorders. Recent data suggest that the negative effects of early-life stress can be moderated by both subsequent caregiving environment and by genetic factors – further emphasizing the important interactions between genes and environment in determining one's sensitivity or resilience to stressful stimuli.

In animal models, the interactions of stress and anxiety-like behavior have also been investigated. Not surprisingly, even a single exposure to an acute stressor can affect anxiety-like behavior, depending on the type and duration of the stressor, time of testing, and stress-sensitivity of the rodent. A single, intense stressor such as footshock can decrease the time spent in the open arms of the EPM, increase acoustic startle, or decrease social interaction – all indicative of increased anxiety-like behavior. Animals exposed to unpredictable chronic mild stress paradigms have been widely used as models for depression-like behavior, and the effects of these chronic (usually 2–4 weeks) treatments on anxiety-like behaviors have also been investigated. While these chronic mild stress paradigms have significant effects on depression-like behaviors, they often show lesser effects on anxiety-like behavior, with great variation in results depending on the specific stress paradigm utilized. In general, while acute stress and chronic stress both produce a reliable HPA-axis response, the effects on anxiety-like behavior

can be quite varied, suggesting activation of both similar and distinct neural pathways. Finally, it should be noted that some animal models with increased or decreased anxiety-like behavior show relatively normal HPA-axis responses to stress, again suggesting that stress reactivity and anxiety-like behaviors can overlap or dissociate, depending on the stimuli, neural pathways, and genetic and environmental status of the animal.

Neurocircuitry Connecting Stress and Emotionality

The limbic regions that are activated by processive-type stress stimuli provide the connection between stress and emotionality. As described above, these processive stressors activate the amygdala, BNST, hippocampus, and cortex – all regions that also process fear- and anxiety-provoking stimuli. Neurons in the CeA and BNST project to the lateral hypothalamus, midbrain, and brainstem nuclei (e.g., ventral tegmentum, locus ceruleus, and dorsal raphe nucleus), and hypothalamic PVN, to name a few. Hence, integrated information in these limbic regions can alter HPA-axis and autonomic stress responses as well as fear- and anxiety-related behaviors. Recent studies further suggest that chronic stress can lead to dendritic remodeling in the amygdala and BNST, with resultant changes in BNST volume as well. These structural changes suggest a potential mechanism for the functional modulation of emotional behavior by chronic stress.

Stress Hormones and Emotionality

The relationship of stress hormones – especially CRF and CORT – to emotionality has been an area of significant interest due to the expression of CRF, CRF receptors, and CORT receptors in the limbic structures. As noted earlier, alterations in HPA-axis activity are clearly associated with susceptibility to various affective disorders including depression and anxiety, and CRF and CORT have been widely associated with fear- and anxiety-related behaviors as well. CRF binds to two receptor subtypes in the brain, CRF-R1 and CRF-R2, and these receptors are expressed not only in the HPA axis (e.g., in the PVN and pituitary), but also in numerous limbic regions including the amygdala and BNST, key areas involved in fear and anxiety, and brainstem nuclei such as the locus ceruleus and the dorsal raphe, key sites of interaction between the CRF and noradrenergic and serotonergic systems, respectively. A CRF-binding protein (CRF-BP) distinct from the CRF receptors is also widely expressed in the brain and pituitary where it is thought to bind CRF and reduce CRF receptor activation. Finally, the brain receptors for glucocorticoids, the low-affinity glucocorticoid receptor (GR) and the high-affinity mineralocorticoid receptor (MR), are also highly expressed in

the limbic system, making the hippocampus and amygdala particularly sensitive to circulating CORT levels. Specific roles for each receptor type/subtype have been investigated in terms of stress and emotionality responses.

CRF is generally found to be anxiogenic in tests of anxiety and fear (EPM, social interaction, fear-induced freezing, etc.), although familiarity with an environment can elicit anxiolytic behavioral responses to CRF. Studies from many groups suggest that activation of the amygdalar CRF system, particularly via CRF-R1, may contribute to the stress-evoked alterations in fear/anxiety-related behaviors. If nonselective CRF receptor antagonists are injected into the amygdala (CeA or BLA), an anxiolytic effect is observed. As mentioned previously, CRF and CRF-R1 expression in the PVN are also central to the HPA-axis modulation of stress responsivity. Hence, it is not surprising that total pharmacological blockade of brain CRF-R1 receptors can diminish both stress responses and anxiety-like behaviors.

CORT also play very important roles not only in stress, but also in fear and anxiety. However, the effects of CORT on anxiety-related behaviors are highly dependent on the levels of CORT (low levels activate only MRs, while high levels activate both MRs and GRs), the timing of CORT exposure relative to the stressor, and whether the stressor is unconditioned or conditioned (i.e., based on memory). CORT levels are elevated in rodents after exposure to the EPM, and elevated CORT levels have been correlated to risk assessment behaviors (stretch-attend postures). Interestingly, CORT administration enhances CRF gene expression in the CeA (in contrast to decreasing expression in the PVN), suggesting that CORT may increase anxiety in part by increasing CRF expression in CeA. Finally, chronic-stress-mediated changes in CORT levels may also increase CRF in the CeA and BNST, contributing to the maladaptive, higher anxiety state that may predispose to other pathophysiology.

Animal Models of Stress and Emotionality

It is generally accepted that affective disorders have a genetic component that – when combined with environmental factors – results in greater susceptibility. Identification of the risk factors, both genetic and environmental, can be a daunting task. While early-life stresses have been shown to increase risk for pathophysiology in later adolescence or adulthood, pharmacological or behavioral therapeutics/treatments have not yet been developed for these environmental challenges. Genetic linkage and genome-wide association studies have led to the identification of candidate genes for a number of affective disorders, but further testing the role of those genes is not feasible in human populations.

However, there are several types of animal models that have been used to probe the interrelationships between stress and emotionality and the potential contributions of specific genes or sets of genes to these behavioral responses. The simplest models rely on the variable expression of stress and emotionality traits in large, general populations. Using this phenotypic approach, the relationship between stress reactivity and anxiety-like behaviors has been examined. If rats are phenotypically selected for low- or high-anxiety-like behavior, no differences are seen in stress responsivity. However, if rats are selected for high or low activity in a novel environment, the high-activity rats generally show a greater stress response, but often exhibit either no change or decreased anxiety-like behavior (greater risk takers). While this phenotypic approach is very useful for behavioral correlations, it is limited in its ability to provide information with regard to common genetic mechanisms. To address this issue, the use of selective breeding in animals has allowed for the determination of (1) genetic (heritable) factors underlying the selected trait (e.g., stress or behavioral response) and (2) genetic correlations with other (nonselected) traits. For example, rat lines bred for differential locomotor response to a novel environment can be tested for HPA-axis response to restraint stress to ascertain the extent to which common genetic factors underlie both traits. Inbred rat and mouse strain studies are additional genetic models that provide useful information with regard to the genetic relationship between different behavioral traits.

While selective breeding moves closer to identifying common genetic factors underlying stress reactivity and emotionality than phenotypic studies, this technique is still not capable of identifying or testing the role of specific individual candidate genes that may be involved. Genetic engineering has provided important tools for generating animals with targeted disruption or over- or underexpression of specific genes to determine their effects on specific behaviors. While some of these methods are being used in rats, most of the genetically modified models have been created in mice.

Not surprisingly then, numerous genetically modified mouse models have been examined to address the role of specific stress-related molecules on emotionality. While one might predict that a single-gene effect would not have a large effect on quantitative traits such as anxiety or fear, many of these genetically modified mice showed changes in anxiety-like behaviors, suggesting that many genes may contribute in significant ways to emotionality. For example, the data from knock-out (KO) mice involving CRF receptors support pharmacological data indicating an important role for CRF receptors in anxiety-related behaviors. Mice lacking CRF-R1 showed less anxiety than wild-type mice, suggesting a role for CRF-R1 in mediating anxiety-like behavior. As predicted,

CRF-R1 KO mice show impaired ACTH and CORT responses to stress as well. The role of CRF-R2 is more variable, but data suggest it is important for restraining the stress (HPA-axis) response and anxiety-like behavior, as CRF-R2 KO mice appear to be more stress-sensitive. CRF-BP KO mice show increased anxiety, consistent with higher than normal levels of free active CRF. Paradoxically, CRF KO mice show a dramatically impaired stress response, but do not show altered anxiety-like behaviors, likely due to compensation by other CRF-related molecules. Finally, overexpression of CRF in mice leads to increased basal circulating glucocorticoids, characteristic of Cushing's disease, with reports of either no change or increased anxiety-like behavior, consistent with the predicted roles of CRF.

Glucocorticoid manipulations have also been shown to affect stress-, anxiety-, and fear-related behavior. Mice lacking functional brain GRs demonstrate increased basal corticosterone, but decreased anxiety. However, mice lacking GRs only in the forebrain show an altered HPA-axis response and an increase in stress-induced locomotion, but no change in anxiety-like behavior – indicating the importance of subcortical regions in modulating anxiety-related behavior. Transgenic mice with an overexpression of forebrain GRs demonstrate increased anxiety and depression-like behavior. In contrast to overexpression of GRs, transgenic mice that overexpress MRs in the forebrain exhibit decreased anxiety with normal basal HPA-axis function. While alterations in GR and MR levels would be predicted to alter HPA-axis function in stressed and basal states, the changes in anxiety-like behavior or stress-reactivity are also quite striking, likely due to the important roles of these receptors in fear/anxiety pathways as well.

As stress and the CRF system also regulate the serotonergic system, it is not surprising that many mouse models with alterations in 5-HT signaling also show altered phenotypes relevant to stress and emotionality. The neurotransmitter 5-HT is released during stress and anxiety and has been widely implicated in mood disorders due to the effectiveness of selective serotonin reuptake inhibitors (SSRIs) as antidepressant therapeutics. Among the many 5-HT receptors, 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} receptor subtypes have been targeted for roles in stress and anxiety responses. In contrast with pharmacological studies, the deletion of 5-HT_{1A} receptors resulted in an increase in fear and anxiety-like behavior in a number of tests, suggesting an effect of this receptor in decreasing anxiety behavior. Interestingly, using a tissue-specific, conditional 5-HT_{1A} KO mouse, deletion of this receptor in embryonic and early postnatal development – but not during adulthood – resulted in increased anxiety-like behavior, suggesting an important developmental period for the expression of 5-HT_{1A}. Consistent with the role of 5-HT_{1A} in anxiety-like behavior, the

overexpression of 5-HT1A in the brain resulted in decreased anxiety-like behavior. 5-HT1B receptor KO mice tend to show hyperactivity, with no change or a decrease in anxiety; 5-HT2A receptor-deficient mice show decreased anxiety-like behavior in the open-field, EPM, or light-dark tests, but show no alterations in fear conditioning or HPA-axis function. Finally, 5-HT transporter (5-HTT) KO mice exhibit significant increases in anxiety and stress-related HPA-axis function, consistent with the linkage of 5-HTT-gene-linked polymorphic region (5-HTTLPR) with anxiety- and depression-related personality traits. Clearly, the role of the 5-HT system in mediating anxiety, fear, and stress responses is complex, depending on specific brain circuits and thus the receptor subtypes expressed in those circuits.

Together, the results from all of these animal models support the critical roles of the CRF, CORT, and 5-HT receptor systems in the pathophysiology of anxiety disorders and depression. The specific genetic linkages that contribute to vulnerability to affective disorder remain a key area of analysis.

Emerging Areas of Interests

Genetics and environment are key factors related to stress-reactivity and the development of anxiety and mood disorders. While genetics is a key area of study in both humans and animal models at the present time, the long-term effects of environmental challenges are less well understood. Studies with human subjects and nonhuman primates have shown that early-life stress can have long-lasting psychological effects, increasing the risk for emotional disorders like depression and anxiety. In rodents, early postnatal stress caused by maternal separation can result in increased stress responses and higher anxiety in these animals as adults. Studies suggest that prenatal stress can have significant impact on the pups as well. Recent work by several groups suggests a model in which environmental conditions result in epigenetic alterations in expression of key stress-related molecules. The role of these epigenetic changes is an exciting new area of study that is key to elucidating the long-term effects of stress and anxiety across development. Finally, male/female differences in stress and emotionality should

not be ignored as sex differences are quite apparent in many affective disorders. The interactions of gonadal hormones and stress hormones during critical periods of development may impact the maturation of stress-sensitive brain areas, including the limbic-forebrain areas, affecting long-term sex differences in responses to stress and anxiety. Additional studies in all of these areas will enhance our understanding of the impact of sex, genes, and environment on our ability to cope with stress and anxiety in adaptive versus maladaptive ways and may lead to new therapeutic strategies.

See also: Animal Tests for Anxiety; Effects of Stress on Learning and Memory; Human Fear and Anxiety; Measuring Stress; Neural Substrates of Conditioned Fear and Anxiety; Neural Substrates of Unconditioned Fear, Defense, and Anxiety; Regulation of the HPA Axis by Acute and Chronic Stress; Stress and Brain Morphology.

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Subjective Experience and the Expression of Emotion in Man

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Glossary

Cultural display rules – Rules learned early in life that dictate the management and modification of emotional displays depending on social circumstances.

Culture – A meaning and information system, shared by a group of individuals and communicated from one generation to the next.

Emotion – A transient, bio-psycho-social reaction designed to aid individuals in adapting to, and coping with, events that have implications for survival and well-being.

Microexpressions – A fleeting, transient facial expression of emotion that is often a form of nonverbal leakage. They are signs of concealed emotions, and are characterized by their speed, subtlety, and often fragmentary nature.

Schadenfreude – A German word referring to the pleasure one feels in the misfortunes of others.

Moreover, affect and emotion are aspects of psychology that all humans have a lifetime of access to, and a lifetime of contemplating the proper words to describe nuances of an inner physiological state or sensation. Thus, it is difficult to arrive at a consensual definition of emotion that encompasses all the possible types of emotion, and at the same time differentiates it from affect. Nevertheless, it is important to adopt a working definition of emotion so that readers can understand what part of the affective world we call emotion.

We define emotion as “transient, bio-psycho-social reactions designed to aid individuals in adapting to and coping with events that have implications for survival and well-being.” They are biological because they involve bodily responses; they are psychological because they involve specific mental processes required for elicitation and regulation of response, as well as affect; and they are social because they are often elicited in interactions, and have meaning to those interactions. (We use the word ‘social’ here in the broadest sense in relation to our evolutionary history, which includes interactions not only with other humans, but also other living beings, such as snakes, bears, wild pigs, etc.)

Emotion, therefore, is a special class of affective phenomena. They are unique, evolved, higher-order information-processing systems that allow the human to regulate and modulate all the other evolved cognitive, motivational, and motor-behavioral systems in order for the human to survive. When emotions occur, they turn-on some systems and turn-off others, so that the human is not overloaded with sensory and cognitive stimuli when dealing with events that have import and require a response. Emotions prevent internal chaos, and provide the human with a rapid, unconscious, and automatic system of processing information to facilitate goals, and a mechanism to deal with events, ultimately promoting survival. They evolved to help us cope with events and situations that have consequences for our ‘immediate’ welfare. If the human did not have emotions, we would not know when to attack, defend, flee, care for others, reject food, or approach something useful, all of which were helpful in our evolutionary histories (as they are today). Thus, emotions helped the human adapt to immediate needs in their environments, and were instrumental in our survival as a species.

Affect and Emotion

In psychological science, affect refers to the various feeling states the human, and perhaps other animals, experience. Affect plays an important role in our lives, serving as a mechanism of self-information about one’s internal states, events that occur, and our relationship with others and our environment. The human experiences a wide range of affective phenomena, such as being tired, bored, sleepy, excited, hungry, angry, afraid, sad, ashamed, proud, embarrassed, happy, or jealous. Affect motivates our behavior, directs our goals, changes our ways of thinking, and colors all of life’s experiences in meaningful, relevant, and important ways. Feeling hungry, for instance, motivates us to find food, just as feeling tired motivates us to rest.

One of the problems that plagues our understanding of the subjective experience and communication of emotion is that emotion is often equated with affect, and this should not necessarily be the case. Indeed, the universe of affective phenomena includes all feeling states, which includes emotion, but also moods, affect-related personality traits, some psychopathologies, and well-being. Our affective world also encompasses physiological need states – hunger, thirst, tiredness, sleepiness, and boredom.

How Are Emotions Elicited?

Emotions are elicited when we perceive the occurrence of an event that is important to us and requires a behavioral response. This system originally developed to deal with species-constant problems that could occur in interactions with nature or other beings, and are related to survival in a time-tested, predictable, and automatic fashion. The emotion system is hard-wired, fairly impermeable to modification by experience, and relatively unchanged throughout the life span.

When sensory information is perceived, it is converted to schemas – mental representations of the situations or events being perceived. These schemas may consist of two components – one referring to the physical characteristics of the sensory information associated with the perceived event trigger, the other referring to psychological meanings or themes associated with the event trigger. In other words, perceived schemas may describe what the events are, and/or what they mean.

Then, the created schemas are evaluated in an appraisal process, which is immediate, unbidden, opaque, unconscious, and automatic. In that process, perceived schemas must be compared to a known set of emotionally relevant schemas, that is, schemas that when matched should initiate an emotional response. These latter schemas are held in an ‘emotion-schema database.’ For example, the perception of a coiled, cylindrical object that is hissing may match the schema of a snake in the emotion-schema database, triggering the emotion of fear. The perception of the smell of feces may match the schema of contamination in the emotion-schema database, triggering the emotion of disgust.

The schemas in the emotion-schema database have been learned in our evolutionary history and are hard-wired into the emotion system, so that individuals do not need to re-learn them in their lifetime. The learning has already occurred, and one of the beauties of the emotion system is that events associated with these hard-wired schemas will trigger emotional responses with no learning, thus allowing individuals to rapidly respond to the event triggers automatically and unconsciously. Indeed, if the human had to learn about schemas for fear and then consciously think about the appropriate responses, we may not be alive today. However, because we have learned them in our evolutionary past, or because these schemas remain through a process of natural selection, we do not have to relearn them in each and every new lifetime, which is a considerable survival advantage.

If the perceived schemas do not match those in the emotion-schema database, no emotion is elicited and the individual continues to scan the environment. A match, however, initiates a group of responses, which are coordinated, integrated, and organized, and constitute what is

known as an emotion. The term ‘emotion’ is a metaphor that refers to this group of coordinated responses.

The Emotion Response System

Emotions aid in adaptation because they recruit programs that coordinate and orchestrate other evolved systems, such as perception, attention, inference, learning, memory, goal choice, motivational priorities, physiological reactions, motor behaviors, and behavioral decision making. We group these responses into four categories – cognitions, physiology, expressive behavior, and subjective experience. Their engagement allows for the simultaneous activation of certain evolved systems and deactivation of others, in order to prevent the chaos of multiple, competing systems being activated at the same time, allowing for coordinated, orchestrated responses to environmental stimuli. Thus, anger prepares the body to fight, and fear prepares for flight. To be sure, not everyone who is angry actually does fight, nor does everyone who is afraid actually flee. In these cases, anger and fear ‘prepare’ the individual to do so; engaging in such motor behaviors, however, depends on a host of other factors, both cultural and individual.

The Subjective Experience of Emotion

One of the major components of emotional responses involves affect. The subjective experience of emotion refers to the affective feeling states associated with the arousal or elicitation of emotion. These experiences are most likely heavily influenced by the physiological reactions that occur when emotions are aroused. Research has demonstrated that emotions such as anger, disgust, fear, joy, sadness, and surprise are associated with distinct physiological signatures both in the autonomic and central nervous systems. These physiological changes help prepare individuals to respond to the eliciting stimulus immediately and effectively by initiating and maintaining whole-body activity. Anger, for instance, produces vaso-dilation, pupil constriction, foaming, and piloerection, each of which prepares the individual to fight. Fear, however, produces vasoconstriction, pupil dilation, and bulging eyes, preparing the individual to flee. The same physiological signatures have been found in people of very different cultures, and thus are strongly suggestive of a biologically innate, universal program for emotional responding that is unique for each emotion.

Because these physiological changes occur, they produce sensations that are felt as part of the subjective experience of emotion. Thus, anger produces feelings of pressure and heat in a container, while disgust produces feelings of revulsion in the mouth. Fear feels cold and

constricted, sadness brings about loss of muscle tone and aching in the eyes, and joy brings about tingling. All of these sensations are experienced precisely because of the physiological changes that occur when emotions are aroused.

Emotions also recruit a host of cognitive processes that support the action-preparedness of the individual. Two types of basic-level cognitive processes are associated with this arousal. One is the perceptual/attentional system, which maximizes attention to the elicitor and minimizes attention to distractors. The other is the gating of higher mental processes, which limits the novelty of the response and aids in the accessing of memories and other knowledge stores helpful in determining the appropriate subsequent behavioral response. This is why when one is angry, it is easier to think about aggressive thoughts, and to remember previous anger-eliciting episodes. One's senses are heightened to perceive anger in others. All of these cognitive reactions are part and parcel of emotional responses, and contribute to emotional experiences.

Because each emotion recruits a different physiological and cognitive response, each emotion is associated with a unique subjective experience; thus, anger feels qualitatively different than disgust or fear or jealousy. Sadness feels different than shame or guilt. Even positive emotions such as pride, amusement, awe, gratitude, excitement, relief, wonder, or *schadenfreude* feel different from each other. Moreover, each of these emotions is elicited by different types of events, turns-on different physiological and cognitive responses, and leads to different potential behavioral outcomes.

Like all affective responses, the subjective experience of emotion serves a function. Feelings of anger, joy, jealousy, or disappointment tell us important information about our internal states, and whether we are cognitively or physiologically prepared to run, fight, jump with joy, or just sit and recuperate. Subjective feelings tell us about our relationships with others or the environment. The thrill of victory and the agony of defeat would not occur if competition and its associated outcomes were not important to us, just as are many other matters, such as birth, battle, seduction, and just plain getting along. In addition, our subjective experiences motivate our future behavior. Success breeds success, or more pointedly, the joy associated with success leads one to want more, just as the disgust that occurs when tasting spoilt milk assures that we do not want to drink it again in the future.

It is useful to distinguish among the affective component of emotion, conscious awareness of it, and the labeling of it. Although affect is aroused as part of the emotion response system, sometimes individuals are not consciously aware that an emotion has been elicited, and that their bodies and minds are reacting. Thus, it is possible to have an emotion but not be aware of it. Even when one is consciously aware of one's feelings, one may

label them in a variety of ways, or attribute their causes to different things. Because emotions and their response components are universal, there are great degrees of similarity around the world in how emotional reactions are labeled and interpreted. However, both labeling and the causal attributions about emotions can be dependent on cultural and individual differences. Thus, it is also entirely possible for anger to be elicited in two people with very different backgrounds, and for one to label and interpret their reactions as anger, and for the other to label and interpret reactions differently, even though the reactions themselves are quite similar.

The Communication of Emotion

Darwin suggested that expressive behaviors associated with emotion are the residual actions of more complete behavioral responses. Facial and vocal expressions are part of those actions, and occur in combination with other bodily responses – postural, gestural, skeletal muscle movements, and physiological responses. This perspective suggests that different emotions are associated with specific, unique facial configurations, and that each facial configuration occurs because it complements the behavioral response associated with the aroused emotion. Thus, we express anger in our faces by furrowing the brow and tightening the lips with teeth displayed because these actions are part of an attack response; we express disgust with an open mouth, nose-wrinkle, and tongue-protrusion as part of a vomiting response. Facial expressions are part of the coordinated response involving multiple systems.

There is strong evidence for the universality of facial expressions of seven emotions. One such line of evidence comes from judgment studies, in which observers in different cultures are shown various facial expressions and are asked to judge which emotion is being portrayed. Observers around the world universally recognize the facial expressions of anger, contempt, disgust, fear, joy, sadness, and surprise across different stimulus sets, investigators, expressor ethnicities and sex, and response formats.

Even stronger evidence, however, comes from production studies, in which emotions are elicited and the resulting facial behaviors are measured. The most well known of these is Ekman's classic study of Americans and Japanese college students. However since then, there have been many other studies that measured facial behaviors that occurred in reaction to emotionally evocative situations, and reported that the facial configurations originally posited by Darwin, and verified and somewhat modified by Ekman, actually occur. These studies have involved a variety of emotion-elicitation methodologies, and participants from many different countries and cultures,



Figure 1 The seven universal facial expressions of emotion.

demonstrating the universality of facial expressions of emotion. Examples of the universal facial expressions of emotion are depicted in Figure 1.

Moreover, the available evidence strongly suggests an evolved, biological basis of universal facial expressions of emotion. The universal facial expressions among the human have been observed by ethologists in nonhuman primates for years. Most recently, methods to measure the facial expressions of nonhuman primates have been developed, and studies show that nonhuman primates have the same facial musculature for emotion signaling as does the human, and that the same expressions occur in the same types of emotionally evocative situations as with the human. In addition, congenitally blind individuals spontaneously produce the same facial expressions as sighted individuals when emotion is aroused, and their expressions are more concordant with their kin than with strangers. Interestingly, congenitally blind individuals have difficulty posing expressions voluntarily when asked to do so; but when emotions are aroused spontaneously, they produce the same facial muscle movements as do sighted individuals. Similarly, facial expressions of emotion are more concordant among monozygotic twin pairs than dizygotic twins. Each of these lines of evidence points to an evolved, biological source of universal facial expressions of emotion that does not have to be learned or recreated in ontogeny, because the learning and selection has already occurred in our phylogeny.

Although an evolutionary perspective suggests that vocal expressions of emotion are also universal, the evidence for the universality of vocal expressions of emotion is equivocal. There is some evidence to suggest that vocal expressions associated with anger, fear, and joy may be reliable and universal. Anger produces voices with harsher, louder sounds, and with an edge to them; fear produces higher pitches, and joy produces enthusiastic and excited sounds. Evidence for the universality of

vocal expressions of disgust, sadness, and surprise is weaker, but suggests that disgust is associated with yuck sounds, sadness with softer, labored sounds with longer pauses, and surprise with higher pitches and inhalations. It is no wonder that the face and voice are tied together, as there are some connections between the neural control of the facial musculature and the vocal chords. That is why one can hear another person smiling while talking on the phone, or why it is easier to speak in lower, harsher tones when one's brows are lowered.

Because emotions evolved in order to aid the human by preparing us to engage in some action, the responses associated with emotion – physiology, expressive behavior, cognitions, and subjective experience – need to be organized and coordinated. This notion is referred to as ‘response system coherence,’ and has garnered empirical support over the last decade, especially in studies that use facial expressions as markers to signal when an emotion is occurring; that is, facial expressions of specific emotions are correlated with feelings of the same emotion, but not other emotions; thus, facial expressions of anger are correlated with the feelings of anger but not other emotions. Likewise, facial expressions of disgust, fear, joy, or sadness are associated with the subjective experiences of disgust, fear, joy, or sadness, respectively, but not with other emotions. These linkages are universal across cultures.

Cultural Influences on Universal Facial Expressions of Emotion

There are cultural differences in how universal facial expressions of emotion are displayed. Many years ago, psychologist Paul Ekman and his colleague Wallace Friesen coined the term ‘cultural display rules’ to describe the rules that people learn, from early in their childhood and throughout their lives, to manage and modify their

emotional expressions based on social circumstances. These rules are cultural norms that provide guidelines for what is appropriate to display and what is not, depending on the context.

Display rules can act to moderate expressions in many ways. When experiencing emotion, for instance, individuals can express emotions as they feel them with no modification. However, they can also amplify their expressions, displaying more than they truly feel (e.g., laughing at your boss's bad jokes); deamplify them, displaying less than they truly feel (downplaying your anger toward your children's misbehavior); neutralize them, showing nothing when in reality something is felt (the poker face); qualify them, showing emotions in combinations with other emotions or signals that comment on the original feeling (smiling at the same time showing that one is miserable); or mask them (smiling instead of showing one is miserable). Display rules, therefore, allow for a considerable range of individual and cultural variation.

In the first study to demonstrate the existence of cultural display rules conducted almost 40 years ago, American and Japanese participants viewed highly stressful films, initially alone and then in the presence of a higher-status experimenter. When alone, both the Americans and Japanese displayed the same facial

expressions of disgust, anger, fear, and sadness to the films, highlighting the universal nature of the expressions. In the presence of the experimenter, however, cultural differences emerged. While the Americans generally continued to display their negative feelings, the Japanese were much more likely to smile to hide their feelings. Interestingly, these were the very same participants who, just moments before in the first condition of the experiment displayed the very same expressions as did the Americans. These differences in expression were interpreted to have occurred because of a Japanese display rule to mask their negative feelings with smiles in the presence of a higher-status individual. Figure 2 provides examples from this experiment. The two photos to the left show the Americans and Japanese displaying the same expressions of disgust when alone; the two photos on the right, however, depict their expressions when with the experimenter.

Another example of cultural display rules comes from a study of medalists of the Olympic Games. Athletes all around the world at this level of competition are under intense pressure to 'be a good loser' or 'be a good winner'; that is, they learn display rules of managing their emotional reactions after winning or losing matches. Figure 3 depicts three expressions shown in sequence by an athlete

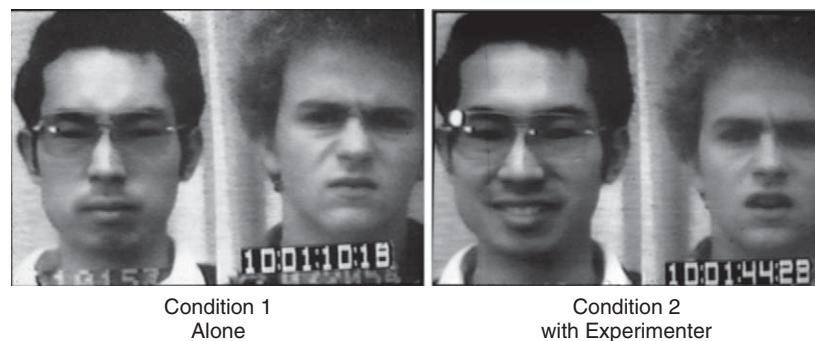


Figure 2 Examples of cultural display rules.

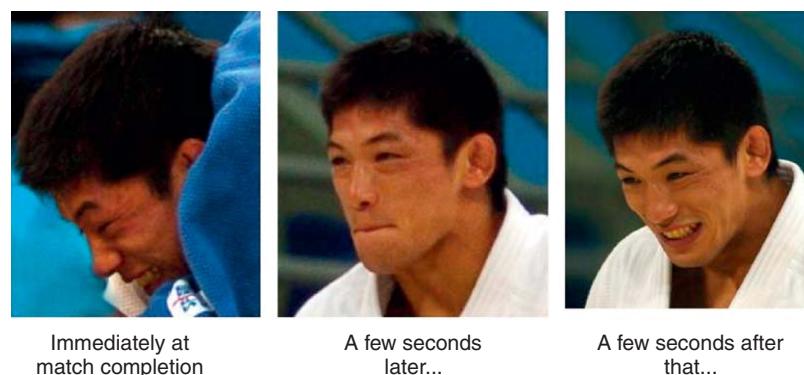


Figure 3 Example from the 2004 Athens Olympic games. Courtesy of David Matsumoto.

who just won the gold medal at the 2004 Athens Olympic Games. The first photograph was taken precisely when the match was over and the outcome was known. Here you can see a broad, strong expression of intense joy from having just won the gold medal. In fact, when observers around the world were shown this photograph and asked to make judgments about it, most people judged this person to be happy and to have won the match.

A few seconds later, however, the athlete displayed the second photograph, which shows him controlling his expression by rolling his lips in and pressing them together as he is smiling. Interestingly, when observers around the world are shown this photograph, most people judged this person to not be happy, and to have lost the match.

A few seconds after that, the athlete was unable to control his joy, and the smile once again burst onto his face in the third photograph. Once again, most observers around the world judged him to be happy, and to have won the match.

These photos are interesting because they highlight the temporally dynamic nature of facial expressions of emotion. Universal facial expressions of emotion were displayed immediately at the end of the match, when outcomes were known. These expressions were universal, and there were no cultural differences in them. A few seconds later, however, cultural influences kick in, and expressions are modified according to culturally learned rules. Thus, facial expressions of emotion are both universal and culturally dependent. This example also highlights why we think cultural differences are so pervasive; when emotionally evocative events occur, we often focus our attention on the event, and not on the expressions of the people involved in the event. When we turn our attention to the people involved even a few

seconds later, however, cultural display rules may be kicking in, and what we see are culturally modulated expressions; that is, we may have missed biologically based, universal expressions that occur immediately, automatically, and unconsciously, because we often do not look at people when they occur.

Macro versus Microexpressions

When individuals experience an emotion and there is no reason to modify or conceal their feelings, the facial expressions that occur have certain characteristics. They are likely to have smooth onsets and offsets, last between 0.5 and 4 s, and be symmetrically displayed on the face (with the exception of contempt, which is depicted by a unilateral or one-sided tightening of the lip corners, giving the impression of a smirk). These kinds of expressions occur in normal discourse, are easily seen, and form the basis of emotional sharing that occurs among friends, colleagues, and many interactants. These are known as ‘macro-expressions.’

There is a special case of facial expressions of emotion, however, called ‘microexpressions.’ The major characteristic of these microexpressions is their speed; they can occur as quickly as for 1/15th or even 1/30th of a second. Because they are so fast, it is easy to miss them. Indeed, if you blink you will not see them.

Microexpressions are signs of concealed emotions, that is, when the expresser does not wish for his true feelings to be known to others (or him or herself). They were first observed by psychologist Paul Ekman 40 years ago, who analyzed footage of depressed inpatients who were lying to obtain a weekend pass in order to commit suicide. When asked if the patient had any plans to hurt herself,

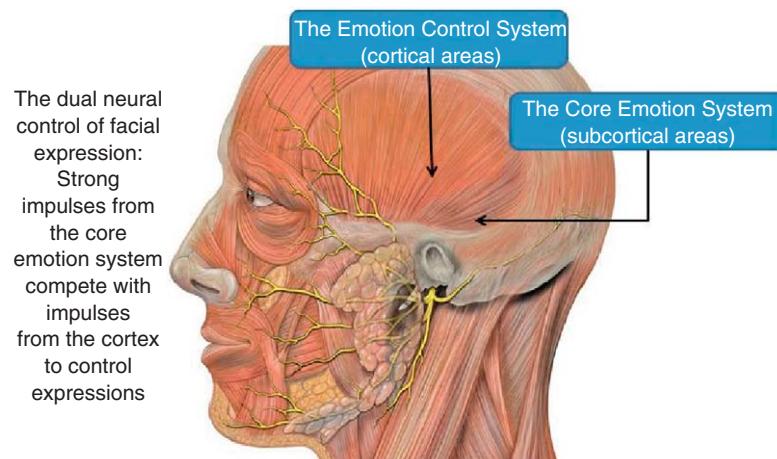


Figure 4 Why do microexpressions occur? By Patrick J. Lynch and C. CarlJaffe, MD. Reprinted by permission (c) Paul Ekman Group 2008.

the patient displayed a microexpression of sadness when she replied "no." Since then, Ekman and his colleagues have created valid and reliable methods of assessing the ability to read microexpressions, as well as training tools to improve people's ability to read them. Research has also demonstrated that microexpressions are often (but not always) a reliable sign of deception.

Microexpressions of emotion occur because of the dual neural control of facial expressions of emotion. The processing centers of emotion are widely believed to exist in certain subcortical areas. When emotions are elicited, neural impulses emanating from these areas trigger the emotion response system, turning on the physiological, cognitive, and expressive systems. Direct impulses from these areas to the facial nerve tell the face to fire the facial muscles in the configurations associated with the emotion aroused. At the same time, however, display rules and other culturally learned norms are widely believed to be stored in the cortex, and the cortical areas of the brain control voluntary behavior. Thus, if an individual experiences an intense emotion but at the same time is in a context in which he or she does not wish to display his or her feelings, the facial nerve will receive dual, competing impulses – one from the subcortical areas saying to fire, the other from the cortex saying to neutralize the expression (Figure 4). The result is what is known as emotional or nonverbal 'leakage' in the form of a microexpression.

See also: Communication of Emotions in Animals; Emotions; Emotion–Cognition Interactions; Evolution of Emotions.

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Relevant Websites

- <http://www.davidmatsumoto.com> – David Matsumoto's personal site.
<http://www.paulekman.com> – Personal site of Dr. Paul Ekman.

Behavior Adaptation and Selection

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Glossary

Genetic drift – Changes in the frequency of alleles in a population which are due to chance events or stochastic effects.

Heritability – Proportion of the interindividual variation observed in a phenotypic trait that is due to additive genetic variance.

Natural selection – A process of sorting phenotypic variants on the basis of their differential ability to survive and reproduce. Natural selection works as soon as

- (1) there is phenotypic variation in a population,
- (2) this variation is correlated with fitness differences, and
- (3) is heritable. The response to selection is observed in the resulting change in allelic frequency occurring in the population between two consecutive generations.

Phylogeny – The connections between all groups of living organisms as understood by ancestor/descendant relationships.

Sexual selection – A particular case of natural selection in which sorting of phenotypic variants depends on their ability to secure access to gametes of the opposing sex.

The evolutionary approach to behavior is concerned with the ultimate causes of behavior, that is, its evolutionary origin and adaptive function. As any observable trait, the behavior of an organism is, to some extent, the final product of an evolutionary process, during which both natural selection and genetic drift may have exerted some influence. However, because genetic drift is essentially a random process, only natural selection can promote a better fit between an organism's features and its environment.

Although adaptation is a central concept in evolutionary biology, its significance remains somewhat ambiguous. Strictly speaking, the term 'adaptation' refers to the endproduct of an episode of natural selection acting on a trait – at which stage there is no more additive genetic variance on the trait in question. Under the assumption of environmental stability, stabilizing selection is then supposed to maintain the trait at its optimal value, through eliminating deleterious mutations. However, the term adaptation is also used to refer to

natural selection in progress. In that sense, any trait for which certain values confer higher phenotypic fitness (i.e., a superior ability to survive and reproduce) can be regarded as 'adaptive.' The distinction is important as the two acceptations of the term 'adaptation' generate contrasting research programs.

The Cost–Benefit Approach to Behavior

One way to test hypotheses about the current adaptive value of a behavioral trait is to examine the current benefits and costs associated with the trait. Consider the 'broken-wing' display, a form of distraction display used by nesting waders and plovers and some dove species to attract the attention of an approaching ground predator away from the nest or young. Typically, incubating or brooding birds walk away from the nest with one wing hanging low and dragging on the ground, thus appearing to be an injured, easy prey for a predator. Through doing their best to distract the predator's attention from their progeny, the parents expose themselves to the risk of being wounded or killed. To decide whether the broken-wing display is adaptive, one has to establish whether the costs of attracting the predator's attention are outweighed by the benefits in terms of survival of the eggs or young. Such a question is typical of the cost–benefit approach by which behavioral ecologists assess to what extent costly traits can be favored by natural selection. In the case of the broken-wing display, one would have to measure the frequency with which the display is used in the face of an approaching danger, its efficiency in actually driving out the predator away from the progeny, and the frequency with which adults performing the display are predated upon or injured by the predator. The same logic has, for instance, been applied to food hoarding. Various bird and mammal species hoard food that they retrieve and consume after a certain period of time. The evolutionary logic of hoarding behavior is that it increases food availability during periods of food scarcity. In the Eurasian red squirrel (*Sciurus vulgaris*), for instance, it has been observed that females that spent more time caching seeds had higher survival and tended to wean more young in their lifetime than those that spent less time recovering hoards, suggesting that food hoarding is an adaptive foraging strategy.

Optimization

A more refined version of the cost–benefit approach consists in the use of optimization modeling to assess the adaptive value of behavior. The behavior of an animal is then described as a sequence of decisions, without explicit assumptions about the cognitive abilities involved in the decision-making process. Basically, the approach considers that any animal regularly faces several alternatives of which one must be favored or chosen. For instance, an animal may have to decide where to establish its breeding site among different possible locations, choose a reproductive partner from several potential mates, or decide whether to attack a prey or not upon encountering it. The logic of the use of optimization models in investigating animal behavior is based on an analogy with human economics. Animals, through their decision, maximize a certain value, more or less closely linked to phenotypic fitness. This axiom implies that natural selection acted in the past to design the decision rules used by animals in such a way that they now behave efficiently in terms of maximizing survival and reproductive output. However, resorting to optimization models to study animal behavior does not imply that animals are considered to be perfectly adapted to their current environment. Simply, the tenants of the optimization approach consider that the gap between what the animal maximizes through its decision and the value the animal should maximize if it were perfectly adapted to the environment is not too large.

Classically, optimization models include three main components: decision assumptions, currency assumptions, and constraint assumptions. Consider the case of an animal foraging for prey. The decision assumptions deal with the nature of the problem the animal is facing. For instance, the predator may have the opportunity to consume several prey types (be generalist) or focus on a single type of prey (being specialist). Or the forager may have to decide how much time it should spend in one particular patch before leaving for another one. The currency assumptions define which criteria must be used to compare among themselves the outcomes of different alternatives. Ideally, one should be able to measure the fitness consequences of the decision made by the animal in terms of lifetime reproductive success (LRS). However, this is often not an achievable goal in practice, and one has to consider a proxy for fitness such as the net rate of energy obtained over a given time period, or the ability to survive over a certain period of time. The constraint assumptions include all the factors that affect the relationship between the currency and the decision variables. These may include both intrinsic constraints (e.g., those related to the cognitive abilities of animals or their tolerance to various external factors such as temperature and humidity) and extrinsic ones (e.g., duration of daylight for a diurnal forager). Based on the different assumptions, a

model is developed which allows one to predict which option confers the greater fitness to the animal. The performance of the animal (observed in the field, or, preferentially, in a controlled environment) is then compared to the predictions issued from the model. Any discrepancy between the observed and predicted performances leads to a reconsideration of the assumptions, and, eventually, the formulation of new predictions. Through this process, the researcher can progressively identify the major factors influencing the behavior under scrutiny.

Optimization models have been widely and successfully used in behavioral ecology, especially in the field of foraging behavior. A large amount of empirical evidence, encompassing a large array of species, shows, for instance, that animals often tend to behave optimally (i.e., maximize their long-term food intake) when selecting prey, or during patch exploitation.

Game Theory

A different situation emerges when the costs and benefits associated with any behavioral option depend on the frequency with which it is used at the population level. Consider, for instance, the case of N foragers that have to make a choice of exploiting two food patches a and b , in which there is continuous input of food at rates k_a and k_b (defining the profitability of each patch), respectively. Let us further consider that each food item appears at the food patch and then disappears if not consumed, and that each forager has the same ability to catch a food item (a case of equal competitive abilities). Clearly, all possible distributions of the N foragers over the two patches are not equivalent in terms of individual gains. If all foragers gather in patch a , then all the prey in patch b will disappear without being eaten, and vice versa. Now consider that N_a and N_b foragers exploit patch a and patch b , respectively. The average gain of any individual foraging during a time T is $P_a = (k_a T)/N_a$ in patch a , and $P_b = (k_b T)/N_b$ in patch b . There is only one distribution at which no individual can increase its gain through switching between patches. This equilibrium distribution can be found by solving the equation $P_a = P_b$, which gives $N_a/N_b = k_a/k_b$. For the distribution to be stable, the ratio of foragers at each patch must match the ratio of profitabilities of the two patches, a rule known as the ‘input matching rule.’ More complex solutions exist when foragers differ in competitive ability, or when the relative payoffs change across patches.

Behavioral ecologists rely on game theory (a branch of economic theory) to deal with similar situations of frequency-dependent selection. Game theory has particularly been applied to the study of social behavior. Central to its application is the concept of evolutionary stable strategy (ESS). A strategy is an ESS if, when adopted by a large majority of individuals in a population,

it cannot be invaded by any rare alternative strategy. An important aspect of ESS is that it represents a competitive optimum that differs from what can be named a simple optimum, when benefits are not frequency dependent. Whereas the simple optimum provides the highest possible fitness gain, the competitive optimum is only a point of equilibrium and is not the point at which individual fitness is at its highest. A simple game, known as the producer–scrounger game, provides a perfect illustration of the phenomenon. It refers to a situation where individuals in a group, foraging for food that is patchily distributed, have a choice between two strategies. They either can search for their own food, and ignore what other individuals around them are doing (the producer strategy), or they can monitor the behavior of their conspecifics and join any individual who has discovered a food patch to share its content (the scrounger strategy). The payoff of each strategy is frequency dependent. When the majority of individuals play the producer strategy, it pays more to play the scrounger one and vice versa. Obviously, on the one hand, the scrounger strategy cannot be a stable strategy, because if no one looks for food all animals will end up dying of starvation. On the other hand, the producer strategy is not a stable strategy either, because when played by all individuals in a population it is particularly vulnerable to invasion by a few rare mutants playing the scrounger strategy. **Figure 1** shows the payoffs of the two strategies as a function of the number of scroungers, and the equilibrium point at which the benefit of a scrounger is equal to that of a producer. Predictions from this model have been verified in a large number of experiments, often using small passerine birds feeding in small flocks. As can be seen on the graph, at the equilibrium point, each individual receives a

lower payoff than if all individuals were playing the producer strategy. This is because natural selection does not act at the level of the group, but at that of the individual. Natural selection can only favor behavioral strategies which are evolutionary stable.

Phenotypic Engineering

The study of adaptive design (i.e., the estimation of the relationship between phenotype and fitness) in natural populations is constrained by the distribution of phenotypes available for selection to act upon. Strictly speaking, if a behavioral trait represents an adaptation, its heritability should be null (because the alleles coding for the optimal value of the trait have been fixed in the population), and, consequently, its expression should vary very little among individuals (beyond the variation attributable to environmental effects). It is then often impossible to use natural variation to ask questions about adaptive design because such variation is weak or does not even exist. It is, however, possible to create artificial variation to assess whether the mean value of a particular trait observed in the population corresponds effectively to an optimum. Through artificially broadening the distribution of the trait, phenotypic engineering provides a way to increase the statistical power of detecting relationships between traits and fitness.

Phenotypic engineering has been used, for example, to understand what limits the intensity of aggression in territorial species. In many species, the ability to survive and reproduce critically depends on the possession of a territory. Once they hold territory, individuals must regularly fight-off intruders to maintain their ownership. It is, however, difficult to evaluate to what extent the observed

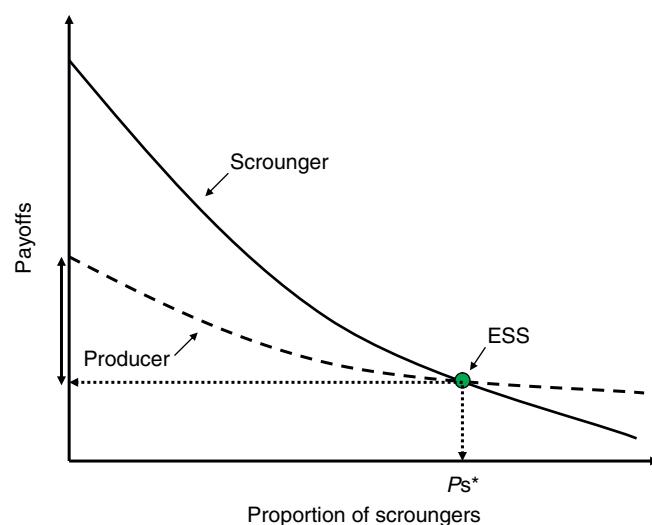


Figure 1 The payoffs of the producer and scrounger strategies as a function of the proportion of scroungers in the population. At the ESS, the payoffs of the two strategies are equal. Note, however, that at this stage, individuals do worse than if the producer strategy was played by all of them (see the bidirectional arrow on the left-hand side of the graph).

levels of aggressiveness have been shaped by natural selection. One way to tackle the question is to artificially increase the amount of territorial defence through manipulating the hormonal profile of individuals. For instance, increasing the level of testosterone (the hormone implicated in the regulation of aggressiveness) did increase territorial defence in male mountain spiny lizards (*Sceloporus jarroo*). However, increasing testosterone level reduced survival, essentially through increasing energy expenditure, while standard metabolic rate remained unaffected. Thus, survival costs may limit the level of aggressiveness in mountain spiny lizards.

Phenotypic engineering is also popular among researchers working on sexual selection. Ever since Darwin, the role of female choice in the evolution of male ornaments has been controversial. According to some models, the extravagance of some male traits such as the peacock tail could result from the existence of open-ended preferences for larger values of the trait in females. At some point in evolution, a stable equilibrium must be reached at which the costs of having an extravagant ornament are compensated by the increased attractiveness of their bearers. However, it is expected that females would prefer males with larger ornaments if only they were present in the population. Several studies have checked the validity of this prediction through manipulating the size of ornaments in males, extending the range of values available to females beyond that offered by natural variation. One famous study concerns widow birds (*Euplectes progne*) – a polygynous bird species characterized by extensive sexual size and color dimorphism. In particular, males have a very long tail (measuring up to 50 cm) that is used during displays. The researchers increased or decreased the tail length of males, while control males simply had their tails cut and restored. Males with experimentally elongated tails attracted more females in their territories than both males that had their tails shortened and control ones, thus demonstrating the preference of females for males with larger ornaments. However, experimental manipulation of tail length in other species suggests that increased tail length is likely to reduce flight manoeuvrability as well as impose fitness costs. Tail length observed in nature may then often be a feature under stabilizing selection.

The Comparative Approach

Another way to assess to what extent behavioral traits can be regarded as adaptations is to rely on the comparative approach. Making comparisons between species to identify behavioral adaptations is a recurrent approach in the study of animal behavior. For instance, the famous ethologist and Nobel prize winner Niko Tinbergen and his colleagues adopted a comparative approach to study the

adaptive function of eggshell removal in several species of gulls. Black-headed gulls (*Larus ridibundus*) produce eggs with a cryptic external color that make them inconspicuous in an environment made of sand and vegetation, whereas once the eggs have hatched, the white interior of the eggshell contrasts markedly with the background. However, soon after hatching, the parents carefully remove pieces of eggshell and deposit them at some distance from the nest. This particular behavior is not observed in the closely related black-legged kittiwake (*Rissa tridactyla*): whereas the black-headed gull nests on the ground in the vegetation, nests of the black-legged kittiwake are placed on cliff ledges; this makes a big difference in terms of exposure of eggs and young to predators. Ground predators can easily access the nests of black-headed gulls but cannot reach those of kittiwakes. Hence, the assumption that the removal of eggshell observed in black-headed gulls is an antipredator adaptation. Through a series of experiments, Tinbergen and his colleagues checked that the presence of eggshell close to the nest of a black-headed gull did indeed increase its chance of being predated. However, it was not enough to prove that eggshell removal does constitute an adaptation. Further evidence comes from the observation that the behavior is systematically present in ground-nesting gull species and absent in cliff-nesting ones.

Comparison between species is indeed a powerful tool to assess whether a behavioral trait is an adaptation. However, two main reasons can explain why a similar behavior is present in two distinct species. The behavior may have been inherited from a common ancestor (a case of homology) or the similarity can correspond to evolutionary convergence (a case of analogy). In the latter case, the two species facing the same problem come about with the same solution over evolutionary times, independently of each other. It is then crucial, in order to identify behavioral adaptations, to distinguish between analogy and homology. To that end, behavioral ecologists rely on statistical methods taking phylogenetic relationships between species into account. The evolution of jumping performance in Tropidurinae lizards provides a good example to illustrate the use of the comparative method. In Brazil, eight species belonging to this subfamily have colonized two contrasting habitats. One is a semiarid area characterized by a sandy substrate, while the other is a savannah-like habitat with dense vegetation and rocky outcrops. Because locomotion on sand imposes high energetic costs, one would expect that lizard species in the first habitat have a lower tendency to jump and have a lower level of jumping performance than lizards in the second one. Phylogenetic analyses confirm this prediction, through demonstrating a large difference in jumping capacity between sister species from the two habitats. Thus, the observed differences in jumping performance appear to be independent of

phylogeny, and, most probably, reflect behavioral adaptations to contrasted habitat pressures.

Genetic Tools to Study Behavior Adaptation and Evolution

Natural selection is most often a slow, gradual process. Studies quantifying selection in the wild generally report weak, albeit significant, selection coefficients acting on phenotypic traits. In addition, the pace of evolution strongly depends on generation time, such that evolutionary changes in behavior are difficult to detect in the wild, especially in long-lived species. Another problem lies with the difficulty in quantifying behavior, in contrast with, for instance, morphological traits. However, sudden environmental changes may provide opportunities to observe natural selection acting on behavior over short periods of time. For example, recent studies have shown that the adaptation of migratory behavior in birds in response to climate change involves changes in the genetic composition of populations. However, antagonistic correlations between a particular behavior and other life-history traits may slow down adaptive evolution and make it difficult to document in the wild. For a long time, then, behavioral ecologists have ignored genetic analyses in their approach to behavioral adaptation. The situation has changed, though, and genetic tools are increasingly used to answer questions about the adaptive significance and evolution of behavioral traits.

Artificial Selection

One simple way to reveal the existence of genetic variation in natural populations on which natural selection can act is to perform artificial selection experiments. The method has been used widely to assess to what extent a given behavior can be shaped by natural selection. Death-feigning, for instance, is characterized by a state of immobility in response to external stimuli. This antipredator behavior is observed in both vertebrate and invertebrate species. Through performing two-way artificial selection for the duration of death-feigning in the red flour beetle (*Tribolium castaneum*) across 10 generations, researchers obtained two strains characterized by either long-duration or short-duration death-feigning, thus demonstrating heritable variation of death-feigning behavior. In addition, individuals from the short-duration strain were more vulnerable to predation by a female Adanson Jumper spider (*Hasarius adansoni*) than those from the long-duration strain. Taken together, these results strongly suggest that death-feigning behavior may have evolved under natural selection.

Artificial selection might also be useful to visualize correlated or indirect selection on behavior. The

phenomenon may arise from the existence of genetic correlations between different behavioral characters, or between behavioral characters and other traits, or because two traits compete for the same resources during development. For instance, it has been shown that artificial selection for the ability to cope with limited nutritional conditions results in reduced learning ability in the fruit fly *Drosophila melanogaster*. Reduced learning ability appears, in that case, to be a correlated response, whereas higher survival, faster development, and reduced adult size were the direct responses to selection.

Genetic Dissection of Behavior

One major question in behavioral ecology is to understand how genetic diversity and alternative strategies can be maintained in natural populations. Genetic dissection of behavior complements nicely phenotypic approaches based on density- and frequency-dependent selection or environmental variation, although its use remains so far limited to a few model organisms. For instance, it has been shown that both the solitary versus gregarious polymorphism in the nematode *Caenorhabditis elegans* and the rover versus sitter polymorphism in *D. melanogaster* (two polymorphisms that influence how the animals forage in the wild) depend on allelic differences at a single gene. Furthermore, it has been shown that the foraging polymorphism in *D. melanogaster* is maintained by both negative frequency-dependent selection and density-dependent selection. When food is scarce, the fitness of each strategy is inversely proportional to its frequency, whereas when food is abundant, the sitter strategy shows higher fitness. This contributes to explain why natural populations of *D. melanogaster* generally consist of 70% sitters and 30% rovers. In *C. elegans*, the solitary strategy has a higher fitness than the gregarious one when food is clumped. However, no difference in fitness is observed when food has a patchy distribution. Thus, variation in the distribution of food may contribute to maintain the polymorphism in nature.

Reconstructing Recent Evolutionary Changes

Quantitative genetics can be a particularly useful tool to document past evolutionary changes, as shown by a study of the behavior of the waterflea *Daphnia* in relation to changes in predation pressure. This study took advantage of a peculiar characteristic of waterfleas: they produce long-lived (>100 years) dormant propagules, under the form of a stratified egg-bank accumulating in the lacustrine sediments. This gives the possibility to reconstruct recent evolutionary changes in behavior through a quantitative genetic analysis of resurrected populations. The study compared the rate of evolutionary rates of change in an adaptive antipredator behavior (e.g., orientation to

light, related to diel vertical migration) with those in selectively neutral markers (DNA microsatellites). The study population had experienced variable and well-documented levels of fish predation over the last 30 years. Genetic changes in orientation to light correlated with episodes of high predation pressure by fish. In addition, genetic differentiation for the studied behavioral trait was significantly higher than that observed for neutral molecular markers. Taken together, these results provide strong evidence that evolutionary changes in orientation to light were driven by natural selection.

The Role of Behavior in Evolution

Despite ample evidence that natural selection can shape behavioral traits, the role of behavior in evolution is still debated. As a matter of fact, behavior can be both subject to selection and a major agent of selection. It has thus been argued that animals would not necessarily be passive in the presence of selective pressures. According to this constructivist approach, animals, though their ability to select and perturb their environment, could minimize exposure to new selective pressures. Ultimately, behavioral change could prevent the operation of natural selection following perturbation of the environment. However, the relevance of this hypothesis remains elusive, as it has seldom been tested rigorously. One noticeable exception, however, concerns a study of predator-induced behavior shifts in *Anolis* lizards. This field-experimental study consisted in comparing changes in antipredator and natural selection on morphological traits in a small lizard species *Anolis sagrei*, on several small islands in the Bahamas, following the experimental introduction of the larger, predatory lizard *Leiocephalus carinatus*. Populations of comparable small islands where the predator had not been introduced were used as controls. Because sprinting ability is largely influenced by body size and relative limb length, it was expected that larger and long-legged individuals should be favored by selection on islands where the predator had been introduced. On the other hand, rapid behavioral adjustments may allow *A. sagrei* individuals to find refuge in arboreal vegetation, and then escape from the mainly terrestrial *L. carinatus*. Indeed, the study convincingly demonstrated rapid changes in antipredator behavior in *A. sagrei* following the introduction of the predator species. After 6 months, only 12% of *A. sagrei* individuals were seen on the ground on islands where the predator had been introduced as compared to 34% on control islands, a statistically significant difference. In addition, perch-

height did also increase over 6 months in the populations exposed to predation. However, patterns of natural selection on body size and limb length differed significantly between experimental and control islands. Natural selection significantly favored larger and long-legged individuals on experimental islands, whereas no consistent pattern was observed on control islands. This particularly valuable experiment shows that although behavioral shifts can occur following environmental perturbation, they are not necessarily sufficient to counter or cancel natural selection.

See also: Cooperation; Genes and Behavior: Animal Models.

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Cooperation

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Glossary

Altruism – In biology, altruism is defined on the basis of the outcome of interactions expressed in terms of the effect on the fitness of the individuals involved. An altruistic act, seen in isolation, is detrimental to the actor and beneficial to the recipient.

Biological market model – It belongs to the partner-choice models. This paradigm stresses the selective effect of partner choice in cooperative interactions and of the effect of changes in supply and demand on the exchange rates of goods or services that are traded between cooperating agents. Agents often belong to distinct classes of traders, such as plants and pollinators or breeders and helpers.

Collective actions – The production of a public good by means of cooperation by several, usually many, agents. The problem of collective actions is that the contribution of individual agents is difficult to control, as is the exploitation of the public good that results from the collective action. An example is the construction and exploitation of a communal irrigation system.

Cooperation – The term cooperation stands both for interactions that are simultaneously beneficial to all its participants and for the behavior of the participants in such interactions.

Cooperative investment – An investment, in another agent, that aims at obtaining a net benefit from the interaction with that agent, but bears the risk of resulting in a net loss.

Cultural evolution – Evolution resulting from selection of traits that are culturally transmitted, as opposed to those genetically transmitted. Group selection is much more prominent under cultural selection than under natural selection.

Group selection – A trait is group selected if the fitness of the members of the group depends on the success of the group in competition with other groups (of the same species). Group selection is generally a much weaker force than individual selection. The term ‘multilevel selection’ is used to describe the combined effect of selection at the individual level, the group level, and eventual further levels.

Kin selection – An explanation for the evolution of altruism among closely related individuals proposed by W. D. Hamilton on the basis of an idea originating from J. B. Haldane. A trait is kin selected when it penetrates in

future generations as a result of investments in the improvement of the reproductive success of close relatives, rather than as a result of investments of the actors in their own reproduction.

Mutualism – Used by ecologists to describe series of discrete cooperative interactions between members of different species. Outside ecology sometimes used for intraspecific interactions to describe mutually beneficial interactions, as opposed to altruistic interactions.

Partner-choice models – Models explaining the evolution and/or stability of evolution that concentrate on the effect of partner preference upon the outcome of cooperative interactions. These models resemble models of sexual selection, but apply to cooperation rather than reproductive behavior.

Partner-control models – Models explaining the evolution and/or stability of evolution that concentrate on the problem of controlling the investment of the partner(s).

Public goods – Goods that can be accessed by a, usually large, pool of actors (also known as common-pool resources). Examples include public infrastructure, fish in the ocean, and the global climate. Public goods can give rise to social dilemmas, because individual agents can exploit the public good in their private interest and thereby lower the value of the public good for all other members of the pool. The best-known description of the public-goods problem is known as the tragedy-of-the-commons: the overgrazing of communal meadows caused by farmers adding cows after the carrying capacity of the meadow has been reached.

Reciprocal altruism – An explanation proposed by R. Trivers for the evolution of altruism among unrelated agents. The idea is that, by taking turns, a pair of unrelated individuals can gain a net benefit from a series of altruistic acts as long as (1) they take turns in playing the roles of altruist and beneficiary and (2) the benefit of receiving altruism is, on average, higher than the cost of performing an altruistic act.

Sexual selection – A trait is under sexual selection, as opposed to natural selection, when it enhances individual fitness through its effect on the access to sexual partners. The access to sexual partners largely depends on the success of competition with same-sex competitors, either in direct competition or in indirect competition as a result of the preferences of the other

sex for certain characteristics. Examples of sexually selected traits include the peacock's tail and antlers.

Social dilemmas – Social dilemmas are situations in which the interests of individual agents collide with the interest of the community to which the agents belong. Each agent is better off following a selfish strategy, but when all agents use such a strategy everybody is worse off than when all agents follow a strategy that maximizes the total payoff. Social dilemmas are often split into two categories, depending on whether the dilemma is caused by the production or the exploitation of a common resource.

Symbiosis – Used by ecologists to describe long-lasting, mutually beneficial relationships between members of different species.

history, replicator chemistry, etc.). Each discipline uses its own terminology and, even more confusing, different disciplines use the same terms but for different phenomena. The word mutualism, for example, is used by anthropologists, behavioral ecologists, ethologists, and economists for interactions that lead to an immediate net benefit to the agents involved, but stands for cooperation between members of different species in community and ecosystems ecology, as opposed to ‘cooperation,’ which refers to within-species interactions.

Taking an evolutionary perspective implies that definitions should refer to the actions of agents rather than outcomes of interactions, since natural selection acts on the former rather than the latter. To give a simple example: hunting lionesses may show the same cooperative behavior during several hunts independent of the outcome of each hunt. Natural selection has resulted in the evolution of cooperative behavior in lions, while this behavior led to net benefits to individuals showing it, averaged over individuals and long series of such interactions. Both successful and unsuccessful attempts have determined which form of cooperative behavior was finally selected. Cooperative hunting has evolved in lions, because on average the returns have been higher than the investments for those individuals that took the risk to invest time and energy in hunting with others.

In this article, specific jargon will be avoided by using generic terms, for example, ‘cooperative investment’ instead of ‘altruism’ or ‘cooperation.’ Altruism is the term traditionally used in evolutionary biology to describe interactions that, seen in isolation, are costly to the actor and beneficial to the receiver. How such behavior can evolve in spite of being detrimental to the actor is the enigma that has kept evolutionary biologists busy for decades. Cooperation is used for both an interaction in which all participants gain a net benefit and the behavior of those participants in such an interaction. The crucial characteristic of both altruism and cooperation is that actors take the risk to invest in other individuals with no guarantee of returns high enough to reap a net benefit. The investment risk becomes higher the lower the degree of control of the actor over the receiver is and the longer it takes to receive compensation.

Multiple Forms of Cooperation: Different Ways to Cut the Cake

The first dichotomy to be made is that between natural forms of cooperation, which result largely from natural selection, and human forms of cooperation, which are often under cultural selection. This dichotomy is reminiscent of splitting a bimodal frequency distribution rather than distinguishing two mutually exclusive categories, as is the case for most other classifications used in

Cooperation: A Crucial Element in the Evolution of Life

Loosely defined, cooperation is an interaction between entities that results in a net benefit to each of them. The entities, henceforth called ‘agents,’ can be molecules, cells, individuals, groups, firms, nations, and so forth. Defined along the same lines, an agent is called ‘altruistic’ if an interaction results in a net loss to himself and a net benefit to his partner. These are functional definitions as used in biology and economics in which no prior assumptions are made about the mechanisms and motives underlying the agents’ behavior. Functionally, altruism boils down to taking the risk of investing in a partner without having the guarantee of obtaining net returns. The evolution through natural selection of evermore complex agents, such as molecules, chromosomes, cells, multicellular organisms, and groups, would not have been possible without cooperation among agents forming the preceding layer. A major scientific challenge is to understand the mechanisms that lead to the origin and maintenance of cooperation in each layer of complexity. Most forms of cooperation are only possible due to mechanisms that help to surmount the inherent instability that characterizes most forms of cooperation. More often than not, one or more agents have the option to reap immediate benefits by not cooperating, which they may prefer over future benefits produced by cooperation, even if these are likely to be higher.

Confusing Semantics

Cooperation is studied in many different major disciplines and subdisciplines (biology, anthropology, economics, political sciences, psychology, sociology,

this article. The evolutionary mechanism that is diagnostic for the split is group selection, which tends to be a weak force under natural selection and a strong one under cultural selection. Natural selection can act at multiple levels, such as single genes, individuals (i.e., packages of genes), groups, populations, and species. The individual level is generally considered to be the most important by far. However, the rules of the game are considerably different under cultural selection, which acts on information multiplied by communication rather than genetic information multiplied by sexual reproduction. Under group-selection, the fitness, or utility gain in economic terms, of an individual depends more strongly on the fate of the group to which the individual belongs, relative to other groups competing over the same resources, than on the individual's own success in competition with other individuals within the group. Selection can take place at multiple levels simultaneously, however, and both natural and cultural selection can act in tandem.

Historically, the enigma of the evolution of altruism has played a pivotal role in putting evolutionary biology back on a sure footing with selection at the individual level prevailing over group selection. Darwin's original insights were largely correct in this respect, but later generations of biologists have had a tendency of explaining the evolution of altruism rather sloppily – using concepts like group selection and deme selection in an intuitive manner. The general public probably understood natural selection as a force acting at the level of species in those days and this vision is still reflected in the popular press, at times, even today. A sharp critique of group selection by G. C. Williams in a book published in 1966 has been an eye-opener for many an evolutionary biologist and has helped to develop the new discipline of sociobiology, which was later relabeled as behavioral ecology. As so often the pendulum of science had a tendency to overshoot and the almost dogmatic rejection of group selection that followed has hampered the recognition of the impact of this form of selection under certain circumstances.

Natural Forms of Cooperation

The traditional way of classifying phenomena in biology is to describe observable phenomena. This implies that interactions are in most cases classified as cooperative on the basis of outcome and the easiest way to describe cooperation between individuals is to use the jargon of ecology. Mutualism and symbiosis are two forms of interaction between members belonging to different species. The main difference is in the duration: mutualistic relationships consists of one or more discrete interactions that are short relative to the life span of the species involved, while symbiotic interactions last a major part or even the entire life span of the individuals involved. Examples of

the former include ant–aphid interactions, pollination, and seed-dispersal networks. Lichens, a combination of fungi and algae, and mycorrhiza, which consist of tissue formed by plant roots and fungi, are two examples of symbiosis. Mutualism and symbiosis, taken together, represent the lion's share of natural forms of cooperation. A third category is formed by cooperation among members of the same species. Within this category, the most important form is probably cooperation between closely related individuals, with examples ranging from parents and offspring and colony members in eusocial insects to biofilms formed by bacterial clones.

A relatively small category contains various forms of cooperation among conspecifics in which relatedness plays no role. The label 'cooperation among unrelated conspecifics' will be used as a shorthand here. It makes no difference from a theoretical point of view whether an interaction takes place between members of different species or between unrelated members of the same species, at least as long as individual selection is assumed. Hence, the term 'cooperation' used in a theoretical context often applies both to mutualisms between members of different species and cooperation between unrelated conspecifics.

There are important forms of cooperation that take place at the subindividual level and without which life as we know it would not exist. To name a few examples: the cooperation between replicator molecules that resulted in chromosomes and genomes, the symbiosis between unicellular organisms that gave rise to the eukaryotic cell, the cooperation between cells that preceded the evolution of multicellular organisms, etc. However, these forms of subindividual cooperation fall beyond the scope of this article.

Uniquely Human Forms of Cooperation

Humans show many forms of cooperation the equivalent of which can also be found among nonhuman organisms, for example, cooperative hunting, cooperative breeding, and the direct exchange of resources between unrelated individuals. However, humans also engage in other forms of cooperation that are unheard of outside our species. One is the simultaneous cooperative investment by large numbers of unrelated individuals, called collective actions – for example, building an irrigation system, raiding a neighboring tribe or organizing a strike. Closely related are the interactions of large numbers of individuals that have an interest in a communally used resource, known as a common good – such as an irrigation system, once it is constructed by collective action, fish stocks in a lake, and unpolluted air. The common label used for interactions involving communal actions and common goods, social dilemmas, hints at the highly unstable nature of these forms of cooperation, discussed

in more detail below in the section titled ‘Cooperation models.’ The fact that humans can solve at least some social dilemmas of this kind sets our species apart from all other species. The ability to solve social dilemmas is, of course, closely linked to several other defining characteristics of the human species, notably language and other cognitive highlights, such as planning deeply into the future and theorizing about processes in the minds of others.

Cooperation by a sizeable number of unrelated individuals is rare among nonhuman organisms. Animal aggregations might be seen as an example of such cooperation. A safety-in-numbers strategy is found in many species. Considerable risk-reduction occurs due to the dilution effect that results from the proximity to other potential prey. Staying together poses a coordination problem but lacks the risky-investment element of cooperation, however, because being near a conspecific inevitably leads to a dilution effect. Cooperation between entities consisting of multiple agents that in turn cooperate at a lower level, thus forming a hierarchical structure of cooperating entities – such as found in armies, government coalitions, and nation-states – is perhaps not uniquely human either, but certainly much more common among humans than anywhere else. A nonhuman example is the formation of associations by groups of different species. Such ‘polyspecific associations’ are common in forest-living primates and usually serve to augment the protection against predation without increasing the food competition as much as would be the case when monospecific groups of the same size would have formed. As in the case of aggregations, the groups of different species coordinate rather than cooperate. At least one group has to invest by diverting from its most profitable foraging pattern; but that investment carries no risk, because the returns are instantaneous.

Cooperation Theory

No Conflict of Interest

Not all forms of cooperation and altruism require mechanisms to solve conflicts of interest. Often enough, an individual can profit from the presence or the actions of another organism without having any influence, positive or negative, on that organism. Cattle egrets profiting from large herbivores that flush insects from the grass by their movements form an example of such ‘byproduct altruism.’ A closely related concept is ‘pseudoreciprocity’: the members of one species have been selected to provide a resource, for example, shelter, that is used by members of another species, for example, ants, which in turn provide a benefit, protection against herbivores, to the plant as a byproduct of their presence.

Conflicts of Interest

Virtually all forms of cooperation imply some degree of conflict of interest between the agents involved, most frequently about the height of the investment and the division of the gains due to cooperation. The degree of conflict varies considerably from case to case. Individual interests can become diametrically opposed after two partners obtain an indivisible resource at a high price. Male baboons forming a coalition against a high-ranking male to obtain access to a receptive female provide an example. The male pulling the short straw may face the wrath of the high-ranking male alone, while his partner consorts the female away from the scene. The potential for conflict is virtually absent when individual interests coincide, and no options for free-riding or exploitation of the partner exists. Think of a rowboat on a lake with two rowers each handling one oar. As long as they agree about a common goal and that goal can be reached following a straight line, they will have to pull equally hard. Endless variation in the degree of conflict of interest also exists in forms of cooperation with multiple partners. A famous example of a strong conflict of interest between individual agents and the rest of the community is known as the tragedy-of-the-commons. A commons is a communal meadow on which multiple farmers can graze their cows. There is little cause for conflict as long as the number of cows remains below the carrying capacity of the meadow. However, any cow added to the herd above the carrying capacity puts its owner on collision course with the other farmers. The owner is the only one to profit from the extra cow, but the costs of overgrazing are carried by all.

Partner Control, Trust, and Partner Choice

The most prominent problem agents need to solve, notably when the conflict of interest is strong, is to make sure not to be exploited by the partner(s). Most models of cooperation concentrate on this problem of partner control. The opposite side of that coin comprises gaining trust. When mistrust reigns but cooperation is profitable, agents have an interest in gaining the other’s trust as well as in judging correctly when the other can be trusted. However, attempting to understand cooperation by only concentrating on the problem of partner control is a bit like trying to understand mating markets by only paying attention to the interaction within couples. Another important aspect, therefore, is partner choice: selection of partner(s) from multiple candidates, switching from less profitable to more profitable partners, and selectively ending relationships with the least profitable of multiple partners.

Cooperation Models

The past half a century has seen an endless stream of theoretical papers on cooperation. Some proposed radically new models, but most suggested refinements of existing models. One way of classifying these models in different families is using a number of dichotomies: the agents are closely related or not; the agents interact only once or repeatedly; two or more than two agents are involved, and stress is laid on partner control or on partner choice. The scope of this article allows only a sketch of the outlines.

The best strategy of an agent confronted with the dilemma between maximizing benefit from cooperation and minimizing the costs of being exploited depends, in a dynamic way, on the strategies played by the other agents involved. The most effective tool for analyzing such situations is game theory: a mathematical branch of economics developed during World War II by J. von Neumann and O. Morgenstern and reinvented a few decades later by the evolutionary biologist J. Maynard-Smith.

Related Agents: Kin Selection

W.D. Hamilton's seminal paper of 1964 went a long way in explaining altruism and cooperation among closely related individuals. He introduced the concept of 'inclusive fitness' of an inheritable trait – in this case, the propensity to act altruistically toward familiar individuals. This is the sum of direct fitness (the chance that the trait is inherited by an offspring) and indirect fitness (the chance that the trait penetrates into future generations via a relative). Genetic information coding for a trait is considered to be identical when both sets of information are copies of genetic material carried by a common ancestor, hence the term identical by descent.

Hamilton proposed the inequality ' $rb > c$ ' to describe the conditions under which agents could increase their inclusive fitness by assisting a relative. The benefit to the recipient (b) and the cost of an act to the altruist (c) are expressed in terms of the increase and decrease, respectively, of fitness due to an act of altruism. The coefficient of relatedness (r) between two agents can be calculated using the formula $r = P(0.5)^L$ in which P is the number of pathways, which depends on the number of common relatives, and L is the number of generation links. For example, cousins have two grandparents in common, so there are two pathways ($P=2$), one via grandparent A and one via grandparent B. It takes four steps, or generation links, to go from one cousin to the other (cousin A-parent A-grandparent A-parent B-cousin B), so $L=4$. Hence, the coefficient r for cousins is $2(0.5)^4=0.125$. An agent could, therefore, augment his

inclusive fitness by helping his cousin if the benefit to the cousin is at least 8 times the cost of the agent's altruistic act. In other words, if the act would prevent the agent from producing a single offspring, the cousin should be able to produce eight extra offspring as an immediate result of the agent's altruistic act. This is an extreme example to illustrate why this form of altruism quickly becomes unlikely with increasing genealogical distance. However, the costs of altruistic acts are rarely that high. An individual that has no chance to breed in a certain year has relatively little to lose by helping relatives. For example, in many bird and mammal species young adults, with little chance to find a breeding slot, act as helpers raising the next batch of offspring from their parents. This explanation, which has become known as the 'habitat saturation' hypothesis for cooperative breeding, also applies to some human societies.

Kin selection implies kin discrimination and, thus, mechanisms of kin recognition. Often enough, these mechanisms will be based on rules of thumb that work in most cases, such as for birds feeding chicks as long as these are in the nest built or chosen by themselves. However, using rules of thumb make actors vulnerable to exploitation, for example, by cuckoos. In that case, we talk about (brood) parasitism by the cuckoo rather than altruism by the host. In principle, the evolution of altruism can be promoted in the absence of genealogical kinship as long as the altruistic trait is invariably connected with a perceptible marker – a so-called 'green beard' and altruism is aimed at carriers of green beards only. This theoretically interesting concept is probably hard to find in nature, but does exist, for example, in some flocculating yeasts.

Two Unrelated Agents

The discussion of the sixties of the last century that led to the demise of group selection in favor of individual selection centered on the question of explaining altruism. The hard nut to crack, assuming individual selection, was explaining how altruists escaped exploitation by free-loaders and otherwise selfishly acting individuals. Successful exploitation would result in higher fitness of selfish individuals, relative to altruistic ones, and thus lead to the extinction of altruism. The pivotal question asked was: 'How can altruistic behavior lead to net benefits in interactions with unrelated individuals?' Starting from this question one almost naturally arrives at the search of mechanisms of partner control in dyadic interactions. In the heat of the debate another crucial question was overlooked: 'How are dyads of cooperating individuals formed in the first place?'

The first to offer a plausible scenario for the evolution of altruism toward unrelated individuals was R. Trivers in 1971. He pointed out that two agents could both gain a net

benefit if they would engage in a series of interactions in which they would take turns in playing the role of altruist and beneficiary, if the costs of acting altruistically would be on average lower than the benefits received. The stumbling block of Trivers' 'reciprocal altruism (RA)' is, of course, that each agent may be tempted to reap short-term benefits at any time by breaking off the series after benefiting from an altruistic act. Following a suggestion by W. D. Hamilton, Trivers proposed to analyze this problem by using the 'Iterated Prisoners' Dilemma (IPD) as a paradigm. The IPD is the repeated version of a famous one-shot matrix game with the payoff distribution depicted in **Figure 1(a)**. The one-shot version has one Nash-equilibrium in which both players defect. The choice combination D–D is a Nash equilibrium, because both players are better of playing D, independent of the choice of the other player. However, the repeated version with an unpredictable number of rounds has no Evolutionary Stable Strategy (ESS; the equivalent of the Nash-equilibrium in evolutionary game theory) and it is, therefore, much less straightforward to find winning strategies. In 1981, Axelrod and Hamilton published the outcome of a round robin computer tournament in which they had different strategies playing against each other and which brought the winning strategy,

'TIT-FOR-TAT' (TFT), to fame. A TFT player always starts by choosing C and then copies its partner's choice in the previous round. Two TFT players will, therefore, always play C, as long as none of them makes a mistake or is unable to fulfill the requirements needed to play C. TFT is not an ESS, in spite of being the winner of the tournament, but so simple that agents with limited or no cognitive abilities can plausibly play it. A population of TFT players could be invaded by several other strategies, among which players always cooperate (ALL-C), but the resulting populations can always be invaded by other strategies in turn.

Strictly speaking, the IPD with its simultaneous choice by the two players cannot be a model of reciprocal altruism in which the players as per definition take turns in choosing an option. However, the flavor of the two is the same, and the IPD can be adapted to suit the assumptions of RA.

The IPD can by no means be used as the universal paradigm for all instances of cooperation involving two agents. There may be a conflict of interest, but the potential to exploit the partner is not always that strong. Many more matrix games have, therefore, been proposed as paradigms for cooperation, of which a few are discussed here. Described here are the one-shot versions; however, all games can also be played in an iterated fashion.

		<i>Player 2</i>	
		COOPERATE	DEFECT
Player 1	COOPERATE	1, 1	-1, 2
	DEFECT	2, -1	0, 0

		<i>Driver 2</i>	
		SHOVEL	DEFECT
Driver 1	SHOVEL	1, 1	0, 2
	DEFECT	2, 0	-1, -1

		<i>Husband</i>	
		BALLET	SOCER
Wife	BALLET	3, 2	1, 1
	SOCER	0, 0	2, 3

		<i>Hunter 2</i>	
		STAG	HARE
Hunter 1	STAG	3, 3	0, 1
	HARE	1, 0	1, 1

Figure 1 (a) The one-shot Prisoner's dilemma. Both players choose one of two options simultaneously and without communication. Each cell contains the pair of payoffs resulting from a pair of strategic choices made by the two players. Left: payoff of player 1; right payoff of player 2. (b) The Snow Drift game. (c) The Battle-of-the-Sexes. (d) The Stag Hunt.

A matrix game that resembles the PD, but differs in one crucial aspect is the 'Snow Drift' game. The story is as follows: two drivers approach a snowdrift that blocks their road from opposite directions. They have two options, stay in their car (or make a U-turn) or shovel the snow out of the way. As in the PD, it is best to defect when the other cooperates, that is, shovels; however, in contrast to the PD, it is better to shovel when the other defects, that is, stays in the car. The snow-shoveling story is used to stress the cooperative aspects, but the game is in fact the same as the 'Game of Chicken,' well known to economists and fans of James Dean in his role in the classic movie *Rebel Without a Cause*. Evolutionary biologists know the same game as the 'Hawk–Dove game,' so far as in the latter game the costs of escalation are higher than the value of the resource at stake. The game's ESS, well known from analyses under its name Hawk–Dove game, is a mixed strategy, meaning that one choice is made with a chance p and the other with a chance $1 - p$, whereby p depends on the relative costs and benefits of the two possible actions.

Sometimes, a cooperative dilemma boils down to a coordination problem rather than a conflict of interest. An example is given by a game called the 'Battle-of-the-Sexes' (**Figure 1(c)**). The story is that of a husband and wife that like to spend the evening together, but the wife would rather be doing that by going to a ballet performance together, while the husband would like to see his wife accompany him to a soccer match. The payoff matrix, depicted in **Figure 1(c)**, reveals that the game

has two Nash-equilibria (both BALLET and both SOCCER), but that does not mean it has a clear solution. A solution in this game is easily found, however, if one of the two players has the opportunity to choose first (technically turning the matrix game into a game in extensive form). For example, if the ballet starts an hour later than the soccer match, the wife can stick it out and wait and the husband has no rational choice other than staying with her till it is too late to go to the soccer match.

Yet another useful paradigm is known as the ‘Stag Hunt’ (**Figure 1(d)**). The story – which goes back to Rousseau – is one of two people going on a deer hunt, which can only succeed if they work together. Both hunters are tempted to go after a passing hare, however. A hare is worth less than half a deer, but can be bagged by a single hunter. Once one of the hunters goes after a hare, the chances of slaying the stag become quite dim. The game has two Nash-equilibria (both STAG and both HARE), but here one of those clearly yields a higher payoff than the other. This turns the problem not only into one of coordination, but also into one of trust. When the two players trust each other sufficiently, they can coordinate and stay on the deer hunt.

All such games serve as analytic models meant to reflect the bare-bones characteristics of naturally occurring cooperative interactions. Several of these games can also be used as experimental games to compare different classes of players and so forth. In ‘experimental economics,’ for example, different games are used to study the effect of gender, personality, cultural norms, and several other factors on human strategic behavior. In most such experiments, subjects play with anonymous partners via computer screens. One large study used two games – the ultimatum and the dictator game – to reveal the effect of different cultural norms in 15, mostly preindustrial, societies. In the ‘Ultimatum game,’ one player – the proposer – can decide to give a certain sum of money to the responder, for example, any amount between 0 and 10 euros in steps of €1. The responder can, in turn, decide to accept or reject the offer. If he accepts, he can keep the money offered to him and the proposer keeps the rest. If the responder rejects, both get nothing. Rational proposers, belonging to the illusive species *Homo economicus*, would offer €1, because rational responders would never choose lower payoff over higher payoff. However, real humans reacts rather differently and may reject offers of €4 while showing strong indignation. The ‘Dictator game’ resembles the Ultimatum game, but the responder is replaced by a player in a passive role with no option to punish the proposer by rejecting the offer. In theory, a proposer could get away with an offer of zero, keeping 10, but in practice most people make higher offers. However, the offers are usually lower than in the Ultimatum game, showing the effect of fear for direct punishment.

These games and several others, such as the Stag Hunt, can be used to measure a personality trait called trust. Trust is central to many cooperative interactions that could not take place when the agents would not trust each other at all. This trait is studied more specifically using the ‘Trust’ game. One of the many varieties goes as follows: player 1 has the choice between giving €4 to player 2 (trust) or keeping €2 and giving €2 away (no trust). Player 2 can then show greed, resulting in €4 for himself and only €1 for player 1, or altruism, which leads to a payoff of €3 each.

Partner-control mechanisms

Changing a partner’s behavior to one’s benefit can be advantageous, notably when future encounters with the same partner under the same circumstances are likely. A simple form of punishing a partner is by refusing further interaction, but obviously this leads to a loss of future benefit from cooperation by the actor too. Force can be used to change the partner’s behavior in an ongoing interaction. Such harassment may not only change the momentary, but also the future, behavior of the partner. Force can also be used after a dissatisfying interaction particularly to specifically alter the future behavior of the partner. This strategy is sometimes labeled punishment, but this term is also used in several other ways. A related term is sanction, which is most frequently used in the context of mutualisms in which a single, usually large, member of one species, for example, a tree interacts with multiple, usually small, members of another species, such as bacteria of the Rhizobium group. Sanctioning by the large partner is fatal for the small one. This strong form of partner choice does not change the partner’s behavior within the lifetime of that partner, but changes the behavior of a population of partners in evolutionary time by its selective effect.

Risk reduction in two-player IPDs

Partner control is the archetypical problem of agents confronted with IPD-like situations. Certain strategies can be used, however, to keep the agent out of an IPD (parceling) or reduce the risk of being short-changed (raising-the-stakes). A parceling agent makes such small investments in each round that the payoff distribution per round is no longer that of a two-payer PD. Individuals can also arrive at a state in which they exchange larger investments per round by starting small and slowly building-up trust.

Multiple Agents, Dyadic Interactions

A different class of models is used to investigate the effect of outside options, notably the possibility of partner choice and switching, on the interaction within dyads of

cooperating agents. The ‘biological markets’ approach has a lot in common with sexual selection theory: what happens between potential cooperation partners depends on the options each of them has to reach their goals by forming other dyads, just as what happens in reproducing couples largely depends on the male-to-female ratio on the mating market. The partner-switching option leads to potential partners being played-off against each other, which in turn can lead to bidding wars. Biological market theory aims at explaining two phenomena: traits selected as a result of partner choice at an evolutionary timescale and explaining the dynamics of cooperation in reaction to changes in supply and demand within the lifetime of agents. Partner choice does not exclude that dyadic partner-control models apply once dyads are formed, but the payoff matrix of, say a two-player IPD, looks rather different when players have only two options, COOPERATE or DEFECT, or when each players can turn to other partners as well.

The option of partner choice and partner switching have been added to several existing partner-control models as well. Yet other models define the constraints within which agents can interact with others by using ‘spatial models,’ such as single-layered or multiple-layered grids, or social networks.

The partner choice option also opens up a different opportunity for agents to control their partners. Agents can threaten to damage the reputation of their partner by revealing their uncooperative behavior to third parties. Obviously, sophisticated communication systems, such as human language, are needed to make reputation-based mechanisms work. Related to this concept is ‘indirect reciprocity’ – the idea that prior to making a choice, agents can learn about potential partners by observing their interactions with others. The latter option is also open to agents without access to language and has, indeed, been observed in the cleaner fish-client fish mutualism.

Multiple Agents Cooperating Simultaneously

Humans frequently forms large cooperating groups that consist of unrelated or only distantly related individuals. These forms of cooperation are inherently unstable, hence the common label social dilemmas. In 1968, Hardin drew attention to this phenomenon by presenting a verbal model, the tragedy-of-the-commons. Theoretical models of social dilemmas are often based on the ‘n-players Prisoners’ Dilemma (NPD).’ A large number of games with the fundamental structure of the NPD have been used in experimental economics, usually under the label Public Goods games.

Mechanisms used to solve human social dilemmas

Social dilemmas are so typically human that it is legitimate to ask whether we are equipped with specific mechanisms that help us to cope with such situations. This would imply that we would differ in crucial ways from nonhuman agents that do not succeed in cooperating under NPD-like circumstances. Traits could either have evolved under natural selection, for example, neuroendocrinological reactions after being short-changed, or evolved under cultural selection, for example, mechanisms that help to ostracize free-riders. Cultural evolution gained considerable momentum after the evolution of language. Our language skills, as such, may also have evolved as a result of natural selection in individuals that found themselves frequently confronted with social dilemmas, of course. Indeed, it is not unlikely that several feedback loops have simultaneously driven the evolution of language and the evolution of social dilemma-related skills under both natural and cultural selection.

A large number of mechanisms have been mentioned in this context, several of which are also known to occur in nonhuman agents and probably have roots deep in our phylogeny. The first set assures that we recognize specific agents and attribute the consequences of their actions to them, at least those that concern ourselves. Crucial skills include individual recognition and deciphering the intentions of others. Our ability to form a picture of what others are thinking (possessing a theory-of-mind) is indispensable in this context. A second set determines our attitude toward a specific individual by combining memories from past interactions with momentary information. Most frequently mentioned are selective adjustments of the titers of neurotransmitters and neurohormones, such as oxytocin, vasopressin, serotonin, and dopamine. These change the likelihood that we trust a person, feel disgusted by an act, value a possible benefit of cooperation, etc.. Research on the effect of these substances provides inroads to the study of the genetic basis of cooperative behavior.

Human societies brim with a large variety of institutions that, in one way or the other, stabilize NPDs and this solves social dilemmas: police, justice systems, governments, laws, religions, etc. Their main function is to police the behavior of agents involved in social dilemmas and correct their selfish behavior by punishment. Punishment does not necessarily need to come from an institution external to the cooperating group, however. Experiments have shown that human agents have a strong tendency to strong reciprocity, that is, to punish free-loaders and other selfishly acting agents within their group even at a cost to themselves. Some players in experimental games have even been found

to punish those that fail to punish free-riders. In order to determine whether or not another agent is acting selfishly, his behavior needs to be compared to some norm. These norms of cooperative behavior can differ considerably from society to society and thus appear to be socially learned to a substantial degree. A further strong mechanism to stabilize cooperation within a group is a conflict with an out-group. Experimental work shows that we have a strong tendency to trust members of our own group more than members of another group, even if the criterion for group membership is arbitrarily determined by the experimenter and groups are formed by a random process. This tendency for ‘parochial altruism’ is seen as the basis for a less fortunate form of human cooperation: warfare.

Cooperation Models and Empirical Observations

Both, the idea that reciprocal altruism can explain altruistic behavior among unrelated agents and that the IPD catches the essence of cooperation, gave rise to an enormous literature, notably in the theoretical domain. However, after three decades of frantic activity a good part of the cooperation community has come to the awkward conclusion that the theoretical emperor had no empirical clothes. Few, if any, examples of reciprocal altruism under natural conditions exist and few situations that resemble a two-person IPD also show cooperation through strategies like TIT-FOR-TAT or similar. Many such situations may exist, but if they do the agents apparently play an ALWAYS DEFECT strategy. Reciprocal altruism and IPD-like situations can be created under laboratory conditions, but only by taking away the option of partner choice and by reducing strategic options by using a highly artificial apparatus.

More realistic models, which were often inspired by empirical observation in the first place, fare much better. Both parceling and raising-the-stakes can be seen in nature and many examples of biological markets have been reported. The n-player models that specifically apply to human societies have, however, been developed by a healthy interaction between theoretical advances and empirical study.

See also: Behavior Adaptation and Selection; Brain Evolution in Vertebrates; Cognition: Learning and Memory: Pavlovian; Evolution of Emotions; Genes and Behavior: Animal Models; Human Evolutionary Genetics; Language and Communication – Brain Substrate; Mating Behavior; Neurobiology of Opioid Addiction; Primate Origins of Human Behavior; Psychoneuroendocrinology of Stress; Social Bonding and Attachment; Social Cognition: From Behavior-Reading to Mind-Reading; Social Communication; Social Relationships and Social Knowledge.

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Mammalian Parental Behavior and Neurohormonal Determinants

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Glossary

Amniotic fluid – The watery fluid in the amnion, in which the embryo and, later, the fetus are suspended.

Canopy – The cover formed by the leafy upper branches of the trees in a forest.

Catecholamines – Any of a group of amines produced by the brain and by the adrenal glands which includes epinephrine, norepinephrine, and dopamine.

Catecholamines have important physiological effects as neurotransmitters and hormones in situations of stress but are also known to modulate attention, memory processes and social behavior.

Endorphins – Endogenous opioid polypeptides produced by the central nervous system in vertebrates during strenuous exercise, excitement, pain, and orgasm. They resemble the opiates in their abilities to produce analgesia and a sense of well-being. Endorphins work as natural pain relievers and are known to be involved in social attachment.

Lagomorphs – Any member of the order Lagomorpha, comprising the hares, rabbits, and pikas, resembling the rodents but having two pairs of upper incisors.

Oxytocin – A mammalian hormone that also acts as a neurotransmitter in the brain and best known for its roles in female reproduction: it is released in large amounts after distension of the cervix and vagina during labor, and after stimulation of the nipples, facilitating birth and breastfeeding, respectively. Recent studies have begun to investigate the role of oxytocin in various behaviors, including social recognition, bonding, anxiety, trust, and maternal behavior.

Parental investment – In evolutionary biology, parental investment is any parental expenditure (time, energy, etc.) that benefits one offspring at a cost to parents' ability to invest in other components of fitness.

Components of fitness include the well-being of existing offspring, parents' future reproduction.

Prolactin – A polypeptide hormone produced by the anterior pituitary gland in mammals. Increased serum concentrations of prolactin during pregnancy cause enlargement of the mammary glands, increase the production of milk, and facilitate the onset of maternal behavior in some species. Sucking by the young on the nipple then promotes further prolactin release, maintaining the ability to lactate.

Prosimians – Species belonging or pertaining to the primate suborder Prosimii, characterized by nocturnal habits, a long face with a moist snout, prominent whiskers, large mobile ears, and large, slightly sideways-facing eyes, comprising the lemur, loris, potto, bush baby, and aye-aye.

Somatosensory system – A diverse sensory system comprising the receptors and processing centers to produce the sensory modalities such as touch, temperature, proprioception (body position), and nociception (pain). The sensory receptors cover the skin and epithelia, skeletal muscles, bones and joints, internal organs including the uterus, and the cardiovascular system.

Suids – Any member of the family Suidae, hooved mammals, order Artiodactyla, including the wild and domestic pigs. Suids are stout animals with small eyes and coarse, sometimes sparse, hair. All have muzzles ending in a rounded cartilage disk used to dig for food and some species have tusks.

Behaviors associated with the birth and care of young are essential for the survival of mammals. For the mother, they represent a means of investing in her reproductive fitness; for the young, it is a question of individual survival, as the mother is the only source of food, at least in the early stages of development. In some cases, the father may assist the female in care of the young but among mammalian species it is more an exception than a rule. The pattern of mother–young interactions varies according to the developmental status of the neonate and the litter size, and mammals differ in the extent to which physiological factors contribute to the postpartum expression of their caring behavior. However, in all species that have been studied so far (with the exception of some primates), maternal behavior has retained its close relationship to the endocrine and neuroendocrine processes that govern reproduction. Its appearance is synchronized with the end of pregnancy when the profound changes induced in the neuroendocrine balance regulate the synchronized onset of parturition, lactation, and parental behavior. Hormones act on the reproductive tract to initiate parturition, and the combination of hormonal and somatosensory stimuli activate brain structures involved in the initiation of

maternal care and bonding. Males are not stimulated hormonally to exhibit parental behavior; nevertheless, in the species concerned, they exhibit such behavior either at the birth of their young (rodents) or later (canids).

Characteristics of Mammalian Parental Care

Variety of Parental Behaviors

The variety of forms of parental care depends very much on the maturity of the young at birth. Mammalian neonates are subdivided into three classes: altricial (immature), precocial (fully developed), and intermediate species. These primary parameters interact with other factors, including postnatal growth rate, litter size, and social structure to determine the nature of care provided.

Species with altricial young

Altricial young are very immature at birth; they are blind, deaf, and have limited locomotor ability. Because they are born without fur, they are also unable to regulate their body temperature. Parental care usually consists of a nesting pattern after the mother has selected a site in which she may build a nest and gives birth to numerous young. This can be underground, as in many rodents and in rabbits, at ground level, as among carnivores, or in the canopy, as in squirrels and prosimians. The nest is then the central element around which maternal care is organized (Figure 1). Mothers nurse their young in the nest and stimulate them to urinate or defecate by licking their anogenital area. In many species, they retrieve them to the nest. Marsupials are an extreme case in this group as their young are very altricial and carried by their mother. At birth, the embryonic young crawl into the mother's pouch until they emerge as fully developed, mobile animals.

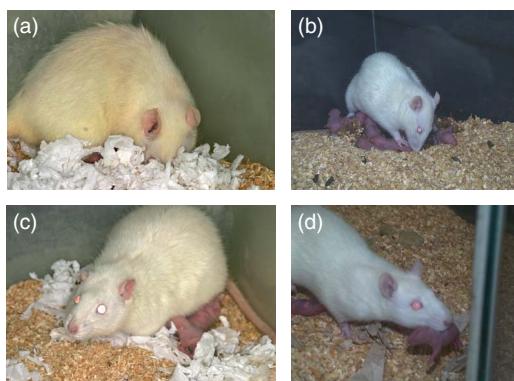


Figure 1 Maternal behavior in the rat. (a) Nest-building behavior; (b) licking behavior; (c) nursing posture; and (d) retrieving behavior. Photos courtesy of E. Jeanpierre (a,c) and A. Flemming (b,d).

Species with precocial young

A second major pattern is characterized by species giving birth to one or two precocial young (most ungulates). These newborns have fully functional hearing and vision, rapid locomotive development, and the ability to achieve thermoregulation. The young are able to stand up and engage in sucking by approaching the mother within minutes of being born (Figure 2). Mothers engage in a pattern of parental behavior that is called leading-following. Species of this type are usually herd animals, the mother leading the offspring which follow her as she moves with the conspecifics of the group (sheep, horse, etc.). In a few species, however, this pattern includes a brief postpartum period where the newborn is hidden (goat, deer, etc.). During this hiding phase, the mother returns periodically to feed her offspring. This system serves to protect the young from predators at times when the mother is foraging and while the young is still too weak to escape from predators.

Species with intermediate young

The third major pattern is expressed by many primates including the human, as well as by suids. In these species the young are intermediate at birth (semialtricial or semi-precocial). Hearing and vision are functional, but neonates have limited locomotor and thermoregulatory autonomy. The number of young among primates is small (mostly singletons, twins in tamarins, etc.) and they are carried by their mother for thermal comfort and transportation. Human mothers carry their infants in their arms, but among subhuman primates the infants must cling to the mother's fur and often ride on her back,



Figure 2 Maternal behavior in the ewe. (a) Two ewes licking indiscriminately two lambs immediately after parturition; cleaning of the neonate and consumption of amniotic fluids are typical traits of maternal motivation. (b) Mother sniffing her neonate while it is suckling in parallel-inverse position; olfactory recognition of the young by its dam leads to acceptance at udder. (c) Ewe displaying aggressive behavior (butting) toward an alien young. (d) Lamb suckling its own mother in parallel-inverse position while an alien young is suckling from behind; suckling from behind prevents olfactory identification by the ewe and reduces rejection behavior. Photos courtesy of R. Nowak (a,c,d) and P. Poindron (b).

side, or chest. In suids such as pigs, the mother gives birth to large litters which remain in a den or a nest for the first 2 weeks.

Maternal Behavior

Social isolation

Females show a tendency to isolate themselves from their social group just before giving birth, a behavior well described in ungulates. In these animals, nonmaternal females show very strong behavioral responses when isolated from conspecifics indicative of agitation and stress. Around parturition, such distress reactions disappear. Isolation is an important preliminary step in the formation of the mother–young bond as it protects the offspring from disturbances by congeners and predators, and facilitates early interactions. However, not all species seek isolation at the approach of parturition. African buffalos or the wildebeest, for example, give birth within the herd – a behavioral adaptation which may have been shaped by the communal defense against predators observed in the former species, and the tendency of the latter to congregate in huge herds during their annual migration.

Parental aggression

The physiological changes associated with pregnancy, parturition, and lactation bring about profound changes in the aggressive behavior of the female and these have to be considered as intrinsic components of the behavioral profile of motherhood. Aggression toward adult conspecifics, especially males, is a common feature in various rodents. The vulnerability of young to predators has shaped various behaviors considered to be antipredator strategies in adults. In suids, mothers are very reactive to the squeals of piglets. In case of danger, the young gather around the mother who may defend them with success. Domestic sheep have been observed to remain undisturbed in the presence of foxes but can repel ravens, and it is reported that mountain ewes are much less protective of their young than the large mountain goats with their lethal horns. In some species, active defense of the young is displayed by other members of the group as well. In African buffalo, an entire herd of adults may react to a calf's distress call, and the muskoxen are known for their successful use of social defense against wolves.

Nest building

The main characteristic of mammals that give birth to altricial young is to provide shelter and thermal protection to their offspring. In rats and hamsters, burrowing increases in the pregnant female but nest building is initiated at the very end of pregnancy (Figure 1). Carnivores also use nest sites, usually in the form of a den. But the most striking example of nest building is no doubt displayed by rabbits. A few days before giving birth,

the female digs several shallow burrows and collects straw and grass. The day before parturition, she selects one of the burrows and lines it with hair pulled from her own body, a behavioral feature unique in mammals, which results in an elaborate structure. Weakening of the ventral hair follicles precedes the initiation of nest building only by a short time. Among ungulates the wild sow, which gives birth to semiprecocial young, builds a nest of grass and small branches in an undisturbed place away from other adults. Even though domestic sows kept under intensive rearing conditions do not have the opportunity to perform such activities in their farrowing pens, free-ranging females display prepartum behavior similar to that of their wild counterparts. Although goats do not build a nest, they do select a site for kidding, a behavioral trait that is not as common in sheep.

Postpartum behavior

Cleaning of the neonate and consumption of amniotic fluids and placenta is one of the first behaviors displayed by most mammals. In sheep and goats, attraction to amniotic fluids and fetal membranes begins during the last stage of parturition, but complete consumption of the placenta is occasional. In ewes and less clearly in cows, the birth fluids are normally repulsive but become temporarily attractive around parturition (Figure 2). This attraction highlights the primary importance of olfaction for the establishment of maternal behavior. Suppression of olfactory cues coming from amniotic fluids leads to an absence of licking, refusal to nurse, and aggressive behavior in primiparous ewes. In contrast, species giving birth to altricial young lick their offspring and systematically ingest the placentas before engaging in nursing. Placentophagia can also be displayed by males as in biparental hamsters. Placentophagia and amniotic fluid ingestion serves several functions such as facilitating expulsion of the next fetuses, having an analgesic effect, regulating lactation, and, in species which build a nest, maintaining the birth-site clean and minimizing bacterial infection.

During grooming, many species of mammals emit characteristic vocalizations of low amplitude and frequency. These vocalizations are mainly emitted around parturition and, later on, during nursing. In precocial species, these calls are very attractive to neonates and have soothing properties. Parturient mothers are also very reactive to calls emitted by their young. In altricial rodents, ultrasonic vocalizations emitted by pups are potent activators of retrieving behavior in the mother. In ruminants, grooming and mother–young vocalizations contribute to early bonding enabling the dam to learn the odor and the voice of her offspring.

Nursing is undoubtedly the most common pattern of maternal behavior in mammals. Irrespective of the species, it usually occurs within an hour or two of parturition.

In altricial species, the first nursing may even take place before the whole litter is delivered. While nursing, mothers adopt a typical posture: female rats arch their back (**Figure 1**), ewes adopt a parallel-inverse position with their young (**Figure 2**), and sows lie on their side. The nursing posture is triggered by the young while exploring the abdomen of the mother. Locating and reaching the udder or the nipple depends on various sensory cues provided by the mother (odors, temperature, and texture of the body). In the first stage of their development, young are allowed to suckle at any time and for as long as they wish. Later on, the mother restricts the frequency and duration of the nursing episode, generally by walking away from the offspring if it attempts to suckle.

Parental Investment and Recognition of Young

The evolutionary-genetic consequence of reproduction is the direct transmission of the parents' allele to the next generation. Individuals which produce a relatively large number of surviving, reproductively capable young thereby pass on more of their genetic material than do individuals with lower reproductive success. Effective reproduction entails considerably more than fertilization, gestation, and birth. Neonates are entirely dependent upon the care and resources provided by their mother (as well as paternal care in some species). Care of the offspring is associated with substantial cost to the mother, including the expenditure of metabolic energy for milk production and thermal regulation, and the increased risk of predation resulting from the presence of conspicuous neonates and limited mobility. Because the mother's resources and reproductive life span are limited, postnatal investment in developing young through weaning ultimately reduces her opportunity to produce additional offspring. Therefore, it follows that natural selection should favor the evolution of mechanisms to ensure that the caregivers' own offspring are the beneficiaries of such parental investment. When parents' own young are likely to intermingle with unrelated neonates – as commonly occurs in a variety of gregarious mammals – mothers rapidly develop the ability to recognize their individual offspring (e.g., ruminants; **Figure 2**). In this context, parents that respond indiscriminately to their own and alien young jeopardize their own reproductive success, since their offspring are deprived of necessary resources, which are wasted on unrelated young. In contrast, when young are born in isolation from other neonates and remain in a den or a nest (most rodents and lagomorphs), there is little need for the mother to recognize her offspring during that stage of development. Rather, by providing care to all neonates present in the den or the nest, the mother effectively restricts her investment to her own offspring alone. Functionally, this parental strategy is equivalent to

one based on discrimination between parents' own and alien young since mothers are usually very territorial and will defend the entrance to their nest or den against all intruders making the presence of alien young in it very unlikely.

Regulation of Parental Behavior

Endocrine and Somatosensory Factors

Hormones associated with late pregnancy and parturition that act on brain receptors account for the rapid activation of maternal responsiveness seen at parturition; these include the steroid hormones estradiol and progesterone which are synthesized by the ovaries and released into the circulatory system, as well as the protein hormones prolactin and oxytocin which are released within the brain and from cells or nerve terminals within the pituitary gland during parturition (**Figure 3**). Other neuropeptides and neurotransmitters, such as β -endorphins and catecholamines, also show important variations at parturition and have both direct and interactive effects on maternal behavior. It is the mechanical stimulation of the genital tract (uterus, cervix, vagina, etc.) caused by the expulsion of the fetus that ensures the immediate onset of maternal behavior.

Estradiol and progesterone

As a rule, late pregnancy and parturition are characterized by a sharp decrease in plasma progesterone levels, followed by an increase in estradiol. This shift in the estradiol/progesterone ratio occurs in most mammals and plays a critical role in the hormonal induction of maternal behavior (**Figure 3**). In virgin rats, a progesterone and estradiol treatment for 12 days, followed by progesterone withdrawal, induces maternal response after exposure to pups. Estradiol is essential to stimulate maternal care and progesterone has two functions. First, a prolonged exposure to progesterone primes the female to respond to the rise of estradiol occurring prior to parturition. Second, the decline in progesterone secretion synchronizes the onset of maternal behavior with parturition. In rabbits, steroids influence the expression of nest building. After surgical removal of the ovaries, females given estradiol benzoate plus progesterone display digging while those given only estradiol benzoate do not. This treatment also induces straw-carrying, but only after progesterone withdrawal. On the other hand, progesterone withdrawal in estradiol benzoate/progesterone-treated females induces hair-pulling in no more than 40% of the animals. Additional factors are required to promote the loosening of fur and the participation of androgens is reported. In mice, nest building is under the influence both of high progesterone and low estradiol levels. In contrast to rats, rabbits, and mice, nest building

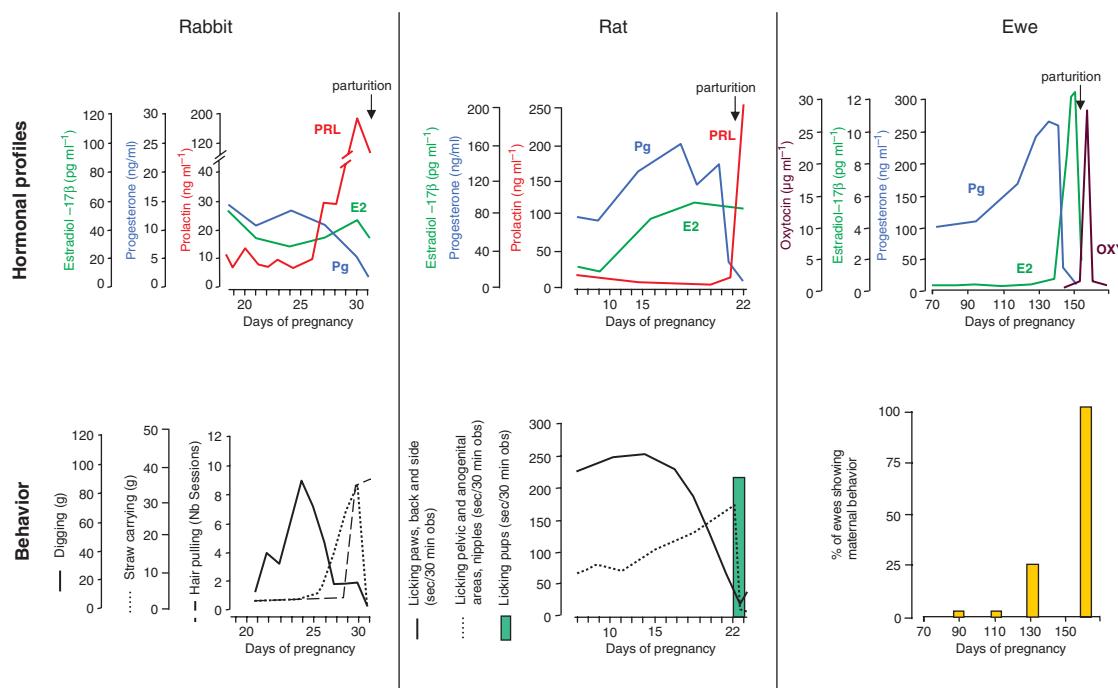


Figure 3 Changes in the plasma concentration of various hormones during pregnancy in the rabbit doe, the female rat, and the ewe (upper panel) and their concomitant maternal responsiveness (lower panel). The elements represented for each species are: (a) for the rabbit doe: digging, straw carrying, and hair pulling; (b) for the female rat: licking paws, back and side (noncritical regions for maternal care), licking pelvic region, anogenital area, and nipples (critical regions), and licking pups; (c) for the ewe acceptance of young. In rabbits, digging is stimulated by estradiol and progesterone, while straw carrying and hair pulling is under the control of prolactin. In the rat, the decline in progesterone and the increase in estradiol levels lead to a shift in licking behavior; maternal responsiveness is enhanced by vaginocervical stimulation, while maintenance of parental care is under the control of prolactin. In the ewe, maternal behavior (licking, low-pitched bleats, and nursing) is triggered by changes in progesterone/estradiol ratio, vaginocervical stimulation, and the concomitant release of oxytocin.

in hamsters begins in mid-pregnancy when levels of both steroids are elevated but they are not sufficient to induce maternal responses to pups. Likewise, in sheep, steroids alone are not sufficient to induce maternal responsiveness: high doses of oestradiol are required in nonpregnant females and only a few characteristics of parental care are displayed. In nonhuman primates, results are rather contradictory: evidence against a role for estradiol or progesterone has been reported in a number of species. In gorillas, no significant association was found between prepartum urinary steroid levels and maternal behavior. Similarly, in rhesus macaques, whether mothers were accepting or abusive was found to be unrelated to estradiol and progesterone plasma levels. In contrast, sex steroid profiles and maternal behavior at parturition are positively correlated in red-bellied tamarins and pigtail macaques. In common marmosets, operant studies have provided evidence indicating a close relation between circulating levels of estradiol and progesterone and motivation to have access to infant stimuli. In the human, although few correlations between steroids and maternal feeling were described during pregnancy, mothers who exhibit a smaller pre-to-postpartum decline in the estradiol/progesterone ratio are found to express greater

feelings of attachment to their infants during the 4 weeks following childbirth.

For some species (sheep, hamster, etc.), ovarian steroids are not able, in themselves, to stimulate maternal care, while for others (rat) they facilitate its onset but without triggering the immediate response normally shown at parturition. Therefore, steroids have a priming effect allowing other factors to trigger such behavior. These factors include additional hormones, such as prolactin and oxytocin, and somatosensory stimulation that are associated with parturition.

Somatosensory stimulation of the genital tract

Expulsion of the fetus also facilitates the onset of maternal behavior through somatosensory stimulation of the genital tract. This can be demonstrated experimentally with local mechanical stimulation. In rats, nonpregnant, intact, multiparous females receiving vaginal stimulation show immediate maternal behavior. However, this effect is synergistic with that of steroids as vaginal stimulation is ineffective in females that had their ovaries removed surgically. It is in sheep that vaginal stimulation plays a dominant role. In nonpregnant, steroid-primed females, not only does it increase the number of ewes displaying

maternal care but vaginal stimulation induces the full complement of maternal responsiveness, licking, low-pitched bleats, acceptance at the udder, and even attraction toward amniotic fluids. Similar to rats, vaginal stimulation without a steroid pretreatment is not effective. Inversely, epidural anesthesia prior to parturition inhibits parental care in primiparous ewes. In rhesus monkeys, cesarean delivery impairs acceptance of the neonate. However, vaginal stimulation is not a prerequisite for maternal care since, in the cesarean-sectioned mother, swabbing the newborn with amniotic fluids and vaginal secretions leads progressively to its acceptance. In the human, the impact of cesarean delivery or epidural anesthesia is poorly documented and whether such procedures disturb the initial mother–infant interactions or, on the contrary, are beneficial as a result of the reduced pain remains a question of debate.

Prolactin

Robust demonstrations of prolactin effects on maternal care have been reported in rodents and lagomorphs. In rats, suppression of endogenous prolactin delays the expression of maternal behavior by days – affecting mainly retrieving and crouching; these effects are restored by a concurrent administration of prolactin. Prolactin can cross the blood–brain barrier and exerts an influence on parental care via the medial preoptic area (MPOA). In mice, prolactin further enhances the levels of maternal behavior shown by virgin females, and prolactin-receptor knockout mice show major deficits in the onset of maternal behavior. In rabbits, prolactin modulates the expression of nest building. It specifically induces straw-carrying and hair-plucking, while at the same time inhibiting digging. Prolactin, together with estradiol and progesterone, also stimulates the emission of an olfactory signal from the nipple, ‘the nipple-search pheromone’ that allows the pup to locate it. In contrast, prolactin is not essential for maternal care in sheep.

Oxytocin

The neural mechanisms by which vaginal stimulation initiates the onset of maternal responsiveness primarily involve activation of oxytocin release within the brain. In rats and sheep, whereas oxytocin is a key factor for the onset of maternal behavior at parturition, it does not seem to play a major role in the maintenance of parental care once fully established. Administration of oxytocin within the brain facilitates the onset of maternal behavior in female rats and sheep, but is only effective in estradiol-pretreated animals. Inversely, administration of an oxytocin antagonist disrupts the onset of maternal behavior in steroid-primed or naturally parturient female rats. Steroids exert positive

effects on maternal behavior via central oxytocin by stimulating the synthesis of the neuropeptide and its receptors. One site of oxytocin synthesis, the paraventricular nucleus of the hypothalamus (PVN), and several sites of oxytocin release during parturition – including the MPOA, the ventral tegmental area (VTA), the nucleus accumbens (NAcc), and the olfactory bulb (OB) – seem responsible for triggering maternal behavior. Microinjections of an oxytocin receptor antagonist into these regions disrupt the onset of maternal behavior at parturition while microinjections of oxytocin in estradiol-primed females stimulate it. The effective action of oxytocin is the result of a positive feedback effect on PVN oxytocin-producing neurons which, in turn, stimulates the coordinated release of oxytocin into multiple target sites. In the human, oxytocin may be of importance during the neonatal period since levels of oxytocin are related to the mother’s level of calmness, interest in social interaction, and duration of breast-feeding.

Opioids

Three major endogenous opioid systems in the brain (β -endorphin, enkephalins, and dynorphins) coexist with three classes of opioid receptors (μ , δ , and κ). This makes opioid regulation of maternal behavior extremely complex. Different effects are observed based on the type of receptors, the brain regions, and the species. β -endorphin acting on μ -receptors in the MPOA inhibits the onset of active maternal responses in rats, while a nonspecific agonist, morphine, infusion in the VTA stimulates them. In addition, opioids binding to μ -receptors disrupt ongoing maternal care: pup grouping and retrieval are impaired in lactating females. The periaqueductal gray (PAG) is another site for an inhibitory action of opioids on maternal behavior. Finally, dynorphin levels increase in the lumbar spinal cord during late pregnancy, and by acting on κ - and δ -receptors in the spinal cord they produce an analgesic effect which might reduce the pain associated with parturition. Ingestion of amniotic fluids by parturient rats enhances this late-pregnancy-induced analgesia without disrupting maternal behavior. With respect to other species, there is evidence for a positive role of opioids in maternal behavior in sheep, while research on primates has provided evidence for both stimulatory and inhibitory effects.

Given the complexity of maternal behavior, it is not surprising that the underlying neural circuitry, where hormones act to induce the onset of maternal behavior, involves structures at all levels of the nervous system, some being primary, and others being an interface with this primary system.

Neural Control

Onset of Maternal Care

In rats, there are at least two antagonistic neural systems governing the expression of maternal behavior (Figure 4). One is excitatory and deals with the activation of maternal responses toward pups; the other is inhibitory and regulates avoidance and aversive responses to pups or pup-related stimuli. The excitatory system primarily includes the MPOA and ventral part of the bed nucleus of the stria terminalis (vBNST). Lesions of these regions or knife-cuts transecting their lateral projections abolish maternal behavior completely. Hormones that activate maternal behavior act on the MPOA. Implants of estradiol, prolactin, or oxytocin into the MPOA facilitate maternal responding. The MPOA and vBNST exert an influence on maternal behavior by interacting with the VTA and the NAcc. Activation of the MPOA stimulates the dopaminergic system originating from neurons in the VTA, resulting in dopamine release within the NAcc. Dopamine depresses NAcc activity which releases the ventral pallidum from NAcc inhibition. Disinhibition of the ventral pallidum would promote the processing of

pup-related stimuli by other brain regions so that appropriate maternal responsiveness occurs.

The MPOA/vBNST receive inputs from other parts of the brain, in particular from the olfactory and limbic systems which exert inhibitory influences on the functions of the MPOA/vBNST. Specifically, a major input to the MPOA comes from the medial amygdala (MeA) which, in turn, receives inputs from the OB and from the anterior and ventromedial nuclei of the hypothalamus (AHN/VMN). These two hypothalamic nuclei project massively to the PAG in the midbrain which regulates fear-related behavior. In virgin female rats, which do not show maternal responsiveness, pup odors would activate a MeA-AHN/VMN-PAG circuit and lead to escape responses. Since AHN, VMN, and PAG have neural projections to MPOA/vBST when these three areas are highly active, they also directly inhibit the MPOA/vBST. Around parturition, elements of this whole inhibitory circuit are depressed permitting hormonal events of late pregnancy and parturition to act on the MPOA/vBNST and to trigger the onset of maternal behavior.

In sheep, parts of the excitatory neural system have been characterized (Figure 2). Inactivation of the MPOA

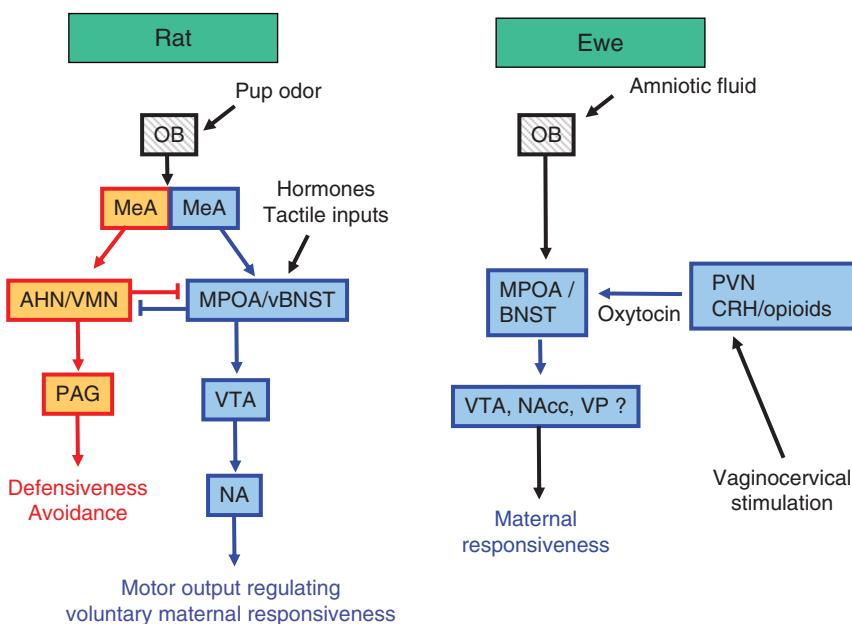


Figure 4 Composite neural model of the regulation of maternal behavior in the rat and in the ewe. Projecting lines ending in arrows signify excitatory connections and those ending in a vertical bar indicate inhibitory connections. In the rat, appropriate maternal responses occur when the medial preoptic area (MPOA)/ventral bed nucleus of the stria terminalis (vBNST) system is dominant over the anterior and the ventromedial nuclei of the hypothalamus (AHN/VMN). These latter nuclei project to the periaqueductal grey (PAG). The MeA/AHN/VMN/PAG system promotes fear responses and avoidance of pups (red circuit). Olfactory stimuli together with hormonal and tactile inputs activate the MPOA/vBST which consequently exerts a positive influence on maternal responsiveness (blue circuit) by interacting with the ventral tegmental area (VTA) and the nucleus accumbens (NA). In the ewe, the control of maternal responsiveness is mainly hypothalamic (paraventricular nucleus: PVN, MPOA, and BNST) and is triggered by vaginocervical stimulation. This stimulation activates mainly the oxytocinergic system of the PVN. Activation of the olfactory bulb by amniotic fluid is a requisite for the initial approach of the neonate in primipara and may act via the MPOA/vBST complex. Involvement of the VTA, NA, and VP is hypothetical.

at parturition impairs maternal motivation and little expression of maternal care is observed. In addition, inactivation of the BNST results in poor motivation to approach the young. It is hypothesized that at parturition, VCS activates oxytocin neurons in the PVN. This would coordinate the release of oxytocin in a number of brain regions and more specifically in the MPOA/BNST. From these two structures, brain regions that still remain to be identified may be activated to promote maternal acceptance behavior. Based upon findings in rats, the VTA, the NAcc, and its major efferent projections – the ventral pallidum – are good candidates for participation in a maternal neural network.

Onset of Recognition of the Young

Mothers of precocial species acquire the ability to recognize their own young soon after birth and refuse to nurse alien offspring (maternal selectivity). In sheep and goats, such discrimination is established within 2–4 h after parturition and relies on olfaction. The neural substrates controlling olfactory memory process have been extensively studied in sheep and differ from the brain regions regulating maternal responsiveness (Figure 5). Recognition of the young is mediated by the main OB in which coding of the familiar lamb odor is established. Norepinephrinergic inputs from the locus ceruleus to the OB are, in part, responsible for the formation of this

memory. Increase in norepinephrine release at parturition, with the help of oxytocin, causes the activation of OB cells permitting potentiation of the glutamate system by the retrograde messenger, nitric oxide. This results in an enhanced cellular activity in response to own-lamb odors. In this way, this output is decoded by subsequent olfactory processing regions. Among them, the cortical and medial nuclei of the amygdala play a preeminent role. Inactivation of either of these nuclei prevents mothers from learning to discriminate their own, from an alien, lamb. The basal forebrain cholinergic system is also activated at parturition and its lesion impairs the formation of olfactory lamb recognition.

Maternal Experience

Previous maternal experience is a requisite for optimal expression of maternal behavior at parturition in most mammals. Maternal responsiveness improves, and aggression declines, with increasing parity. Studies in rodents and sheep suggest that previous parity alters the underlying neural mechanisms making them more responsive to physiological factors at subsequent births. For instance, the number of estrogen receptors in olfactory cortices is higher in multiparous, than in primiparous, mice and ewes. In sheep, maternal experience enhances the expression of oxytocin receptor messenger RNA (mRNA) and the number of estrogen

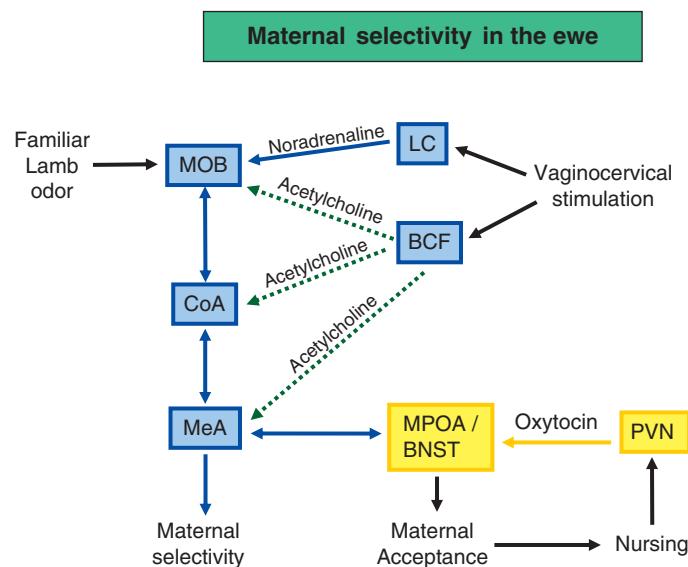


Figure 5 Neural model of the regulation of maternal selectivity at parturition in the ewe. Changes in the main olfactory bulb (MOB) reactivity toward lamb odor is triggered by vaginocervical stimulation via the noradrenergic system of the locus ceruleus (LC) and, presumably, the cholinergic system of the basal forebrain (BCF). Maternal selectivity is under the control of the cortical and medial nuclei of the amygdala (CoA, MeA, etc.) which receive input from the OB. The MeA interacts with the neural pathways involved in maternal acceptance – the medial preoptic area (MPOA), the bed nucleus of the stria terminalis (BNST) and the paraventricular nucleus of the hypothalamus (PVN). The hypothalamic oxytocinergic system is stimulated by nursing which, in turn, maintains maternal acceptance. Blue depicts the neural network involved in maternal selectivity; yellow represents the neural network involved in maternal acceptance.

receptors in the PVN. At parturition, the release of oxytocin, norepinephrine, acetylcholine, and glutamate in the OB is higher in multiparous, than in primiparous, ewes. In rats, experience-based changes are reported in glial function both in the hippocampus and the MPOA. Higher numbers of glial fibrillary acid protein-positive cells are observed in the MPOA following experience with pups in multiparous, than in primiparous, rats. Pregnancy and motherhood alter other forms of neural plasticity. Hippocampus dendritic morphology is also changed when experiencing parturition. Adult neurogenesis in the hippocampus is decreased at parturition in primiparous and multiparous mothers in comparison to nulliparous female rats. In contrast, increased olfactory neurogenesis is observed in dams during pregnancy and the early postpartum period. Other parts of the maternal neural circuit, such as the BNST and the NAcc, are also sites of increased cell proliferation induced by pup exposure. Thus, maternal experience induces changes in neurotransmitter release, receptors synthesis, neural morphology, and neurogenesis that could mediate the enhanced maternal responsiveness to hormones and neonate stimulation.

Conclusion

The study of parental care has provided one of the best opportunities to investigate how molecular and cellular changes in specific neural circuits influence behavior. The system is indeed complex, with multiple hormones and several neural systems involved, with marked differences between species in the type of maternal care, as well as in the neuroendocrine and neurobiological bases. Adding to this complexity, experiential factors influence maternal care not only through the dam's own previous experience as a mother, but also through her own experience of being mothered as an infant. For instance, infant rats who received more touch and licking stimulation from the mother in the nest show a higher level of pup-licking as adults with their own offspring, in comparison with less stimulated infants. Similar early maternal stimulation in the nest also produces a dampening of the offspring's emotional reactivity to novelty and stress when they become adult. In species in which mothers develop individual recognition of their offspring, as in sheep, similar mechanisms used to promote attachment of the young female to her mother seem to be reactivated when she gives birth and exhibits recognition and attachment to her offspring. This transfer of biological effects of early experience to later maternal responsiveness and behavior may be especially evident when considering species in which the parent is intimately involved in the socializing process of offspring, as in primates. In vervet monkeys, there is substantial evidence that the style of mothering

exhibited by adult daughters is similar to the style of mothering shown by their mothers. Vervet monkey mothers who engage in a high level of mother-ventral contact have daughters who also show high mother-infant contact. Abusive patterns of maternal care span generations as well and the incidence of abuse varies across matrilines in pigtail macaques, and studies have shown that the manner in which the infant was mothered did exert an effect on their adult mothering behavior. Among the human, it is commonly assumed that there are often intergenerational similarities in maternal behavior.

Based on our understanding of experiential effects on the brain in other animals, it is also possible that children who have been neglected or abused have experienced neurological changes which result in altered affective, perceptual, and cognitive function during development. Such changes are more than likely to affect how they perceive and respond maternally to their own offspring, how they approach maternal-care responsibilities initially, and how they are able to learn or modify maternal-care practices. One major theoretical issue, then, is to understand why this aspect of brain function which is critical for species survival is so malleable.

See also: Infant Bonding and Attachment; Maternal Deprivation; Social Bonding and Attachment; Social Learning and Behavior Transmission; Social Relationships and Social Knowledge.

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Mating Behavior

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Glossary

Courtship – It is a behavioral process whereby an individual attracts and courts partners of the same species to convince them to mate. Courtship is species specific and often stereotyped. Courtship can also refer to the period of time during which this behavior is displayed.

Display – It is a particular pattern of behavior used as a means of attracting attention and communicating sexual and/or social status.

Gamete – It is a cell containing only one set of chromosomes (haploid) which is capable of fusing with another gamete of the opposite sex to produce a fertilized egg that contains two sets of chromosomes (diploid) that will produce a new individual. Only two types of gametes exist: the small mobile spermatozoa made by the male and the large oocyte made by the female.

Sexual selection – It is the process that gives a reproductive advantage to some individuals over others of the same sex. Sexual selection is the result of competition between individuals of the same sex (intrasexual competition) and of mate choice (intersexual competition) and is a major force driving evolution of reproductive traits often to extremes, for example, the long tail of the peacock. The theory was first proposed by Charles Darwin in 1871 (*The Descent of Man and Selection in Relation to Sex*, John Murray, London).

Reproduction is a functional necessity for survival. It is also a central focus for natural selection and directly leads to the evolution of species. Most life forms reproduce sexually. In all cases, this is done through two specific cells, the gametes. One, the spermatozoa, is small, mobile, and produced in abundant numbers; the other, the oocyte, is large and produced in much smaller numbers. Generally, gametes are produced by different individuals: males produce spermatozoa and females produce oocytes. The male and female gametes, in most cases, do not come into contact by chance but because males and females meet when in appropriate physiological and behavioral conditions. Thus, the object of sexual behavior is to bring male and female gametes together via a mating ritual, so that fertilization can occur. This requires coordination between the maturation of both female and male gametes

and sexual behavior so that sexual partners meet and copulate at the appropriate time. However, in most species, the chance to reproduce and therefore to participate in the evolutionary process are not identical for all individuals. It also depends on other ecological and social factors that lead to specific choices of mating partners and to the evolution of a wide range of highly distinct mating systems. This article reviews how sexual behavior is organized and how a mate is chosen. It addresses the factors modulating the expression of this behavior: the sensorimotor and hormonal controls responsible for its coordination with the maturation of gametes and the mating systems that facilitate reproduction. In this article, sexual behavior and mating behavior are used synonymously.

Organization of Sexual Behavior and Courtship Displays

In all species, sexual behavior comprises a sequence of specific events: first, there is an attraction phase, then a precopulatory phase and finally mating itself. The first two phases of sexual behavior provide the opportunity to test partners for attractiveness and to select among potential partners.

Attraction Phase

To engage in sexual behavior, partners in the appropriate physiological state have to first find and then attract each other. This problem is obviously not the same for all species, for example, a solitary male elephant living long distances from a potential mate compared to a snake emerging out of the nest with lots of others. It also differs with the anatomy of each species and the environment in which they live. In birds, auditory and visual cues are predominant. Sexually active males often have plumage with bright colors or specific ornament ([Figure 1](#)) or they call loudly with specific songs, and some build elaborate and highly decorated bowers. Several amphibians use auditory cues to attract a mate. Sexual partners in many mammalian and reptilian species emit specific chemical compounds known as pheromones that indicate their reproductive states. Pheromones can be produced by special glands or as a by-product of metabolism and are present in urine, feces, vaginal fluid, and skin. The strong smell of the male elephant in musth, that is, during its



Figure 1 A male peacock displaying. Photo C. Eyquem, copyright Licence Art Libre.

period of sexual excitement for example, comes from pheromones secreted by the temporal glands. A few of these compounds have been identified, for example, dimethyl sulfide in hamsters and androstenol in pigs.

The Precopulatory Phase: Courtship Behavior

In the second stage, attracted partners engage in specific patterns of courtship behavior intended to lead the other partner to mate. These behaviors often have to overcome underlying aggressive or defensive patterns of survival behavior. This is done through complex and often stereotyped patterns of motor behavior that constitute the courtship display. The courtship display is species specific, but with similarities in closely related species. In bovids, males court females with an approach from the side accompanied either by movement of the head on the back of the partner or by a rapid pawing movement of a foreleg together with specific vocalizations ([Figure 2](#)). Courtship displays can last for several hours or even days and they may be quite spectacular as in many birds. They are organized as a precise sequence in which each step is the stimulus that induces the next. A well-known example is the courtship of the stickleback first described by Niko Tinbergen: the sight of a mature female elicits a zigzag dance from the male which in turn

elicits a presentation posture until the female releases her eggs and the male his sperm. Disturbing one step prevents expression of the following behavior.

Copulation

In most fish, fertilization is external so that copulation is not necessary and the last phase of sexual behavior simply consists in the emission of eggs and sperm in a specific location such as a nest. External fertilization is also the norm in most species of frogs and toads. But in these species, it involves the male holding the female in a specific pose called amplexus during which the male releases sperm over the female's eggs as they are laid. In other vertebrates, fertilization is internal, that is, it occurs inside the female body cavity. The male deposits sperm directly into the female either by simple contact with the genital opening as in birds, with a 'cloacal kiss' or via an intromitting organ. The release of sperm in some species occurs as soon as intromission is achieved; this is typical in ungulates whereas in other species, for example, the rat, prolonged stimulation through multiple intromissions is normal. Ejaculation is often accompanied by specific male postures and it is normally followed by a refractory period during which sexual activity is decreased.

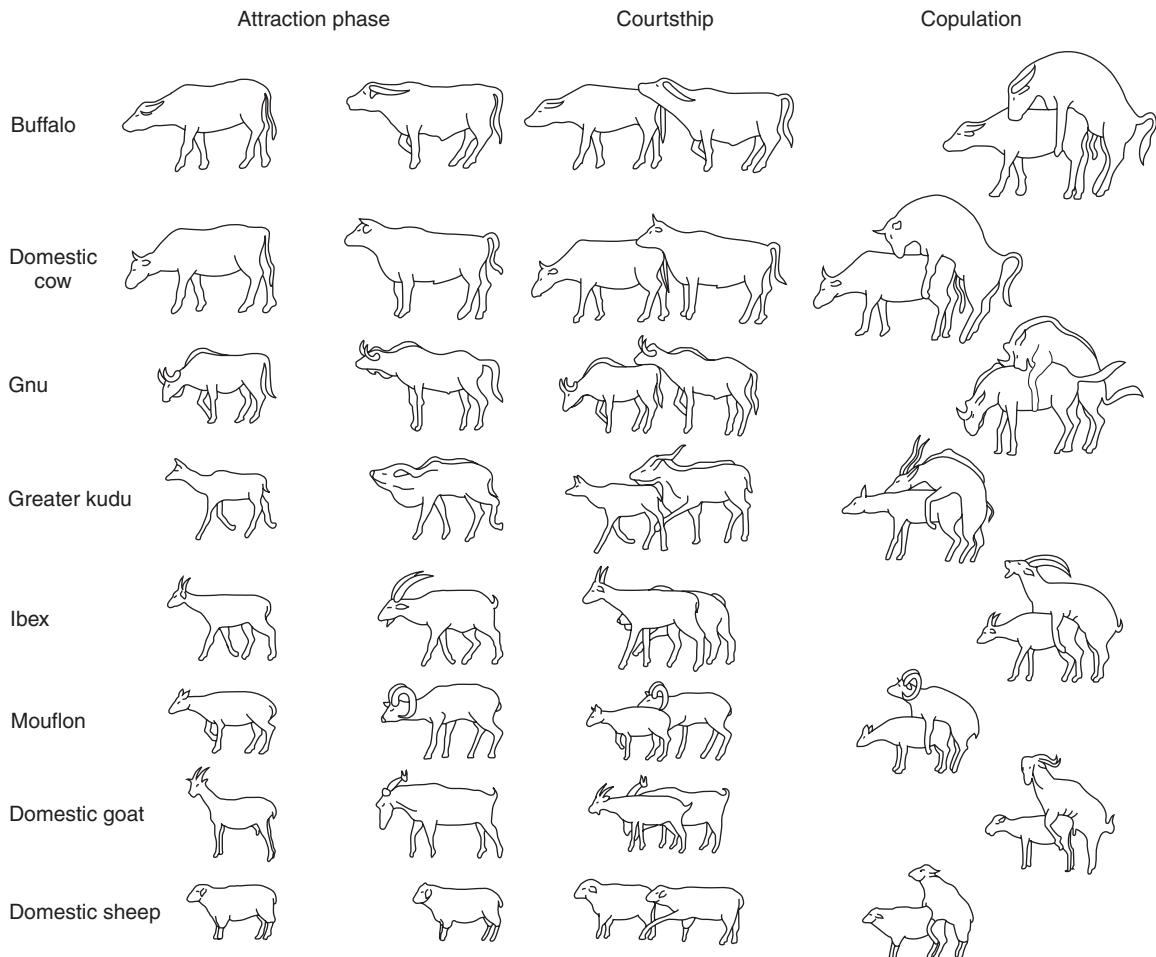


Figure 2 Patterns of mating behavior in different bovidae. Postures during mating are similar in all bovid species. When searching for females during the attraction phase, males hold their head horizontally with the neck extended. When courting, males approach the females from the rear and the side with either a placement of the head on the back of the female (e.g., buffalo, domestic cow, and gnu) or an outward rotation of the head at the side of the female accompanied by a rapid pawing of the foreleg (e.g., kudu, ibex, mouflon, sheep, and goat). Mating itself is accompanied by a downward movement of the male's head on the back of the female (e.g., buffalo, domestic cow, gnu, and kudu) or a jerking of the head backward (e.g., ibex, mouflon, sheep, and goat). Adapted from Rouger (1974) thèse de doctorat d'état, Université de Rennes.

Mate Choice

Reproduction requires a major investment of parental resources, especially in females who have to produce large eggs and nurture the progeny. Thus, although males generally have the most obvious role in sexual interactions, females have a critical role because they are mostly responsible for partner choice. Females choose their mates using multiple criteria that range from morphological traits, position in a dominance hierarchy, size of a territory, and quality of a gift to the nature of paternal behavior, to mention a few. In most vertebrates, females prefer the largest and strongest male controlling the best or largest territory. When as in the pipefish, the male has the greater parental investment, the situation is reversed. He is the choosy gender and will prefer the largest and most highly ornamented female that will compete to gain

access to him. In some species, such as the sage grouse, female choice is based on the intensity and quality of the courtship display. In others, females choose according to the form or intensity of a specific signal: the color of feathers in birds, the intensity of a call in frogs, or the size of antlers in red deer.

The characteristics of the mate that is chosen evolve because of the higher reproductive success of preferred males. It is considered a central process in sexual selection and leads directly to sexual dimorphism. Seeking the factors that drive this evolution has been the object of many studies and theories over the last 50 years. It has been proposed that these preferred characteristics are either directly or indirectly a sign of reproductive fitness that is that the male is strong, healthy, and is carrying genes that ensure the viability and reproductive success of the offspring. In some cases, the choice of a mate can be modified

by information acquired during a critical period in early life. This phenomenon was discovered by Konrad Lorenz in ducks and geese; he named it 'imprinting.' In adulthood, these birds will prefer the first moving object they saw in early life as a mating partner. Recently, imprinting has also been observed in goats and sheep reared from birth by a mother of the other species. At puberty, males and females preferred partners of the foster species to their own species. This preference reversed in females but was maintained in males during the 4 years of the experiment. This may explain why, in these species, males raised from birth in unisexual groups had an increased preference for partners of the same sex.

Hormonal Determinants of Sexual Behavior

Fertilization is only possible if maturation of the gametes and the expression of sexual behavior coincide with each other and between sexes. In most vertebrates, this coordination is achieved by the actions of reproductive hormones. Knowledge of a relationship between gonads and the expression of sexual behavior is ancient: castration has been practiced since antiquity. But the first confirmation of the role of the gonads in sexual behavior was in 1849 by Arnold Berthold. He discovered that roosters castrated as chicks did not grow a comb, never crowed in the mornings, and never mated. But they would do all these things if at the time of castration their own testis or that of another chick was reimplanted in the abdomen.

Males

In all species, ablation of testes is immediately followed by reduced sexual behavior that will eventually cease

altogether. The copulatory phase of sexual behavior is the first to stop, followed by the cessation of courtship behavior and then the cessation of the attraction phase of sexual behavior. The rate at which sexual behavior decreases is variable among species. In laboratory rodents, sexual behavior completely stopped by the end of 2 weeks after castration. In dogs, goats, and also humans some degree of sexual behavior can still be present several years after castration. The effect of castration on sexual behavior is also variable within species and several factors can slow the postcastration decline in sexual behavior. For instance, previous sexual experience, repeated exposure to sexual stimulation, and a highly stimulating sexual partner will all slow down the postcastration decrease in sexual behavior.

In most species, it is possible to restore male sexual behavior by treating castrated males for several weeks with testosterone, the major testicular hormone which is also responsible for the production of male gametes. This effect is dose dependent up to a plateau at which male sexual behavior reaches its precastration level (Figure 3). Larger quantities of testosterone will have no additional effect on male sexual behavior. The normal blood concentration of testosterone in a healthy male is always above this threshold. This explains why in most studies authors found no relationship between blood testosterone concentrations and sexual behavior. As demonstrated in laboratory rodents, genetic variation in the sensitivity to testosterone may explain part of the variability in the response. In some species, the role of testosterone is more subtle. For example, in the red-sided garter snake, the expression of sexual behavior is triggered by an increase in temperature but it depends on the presence of testosterone during the previous summer.

In the 1970s and the 1980s, scientists discovered that testosterone was not always directly active and that its

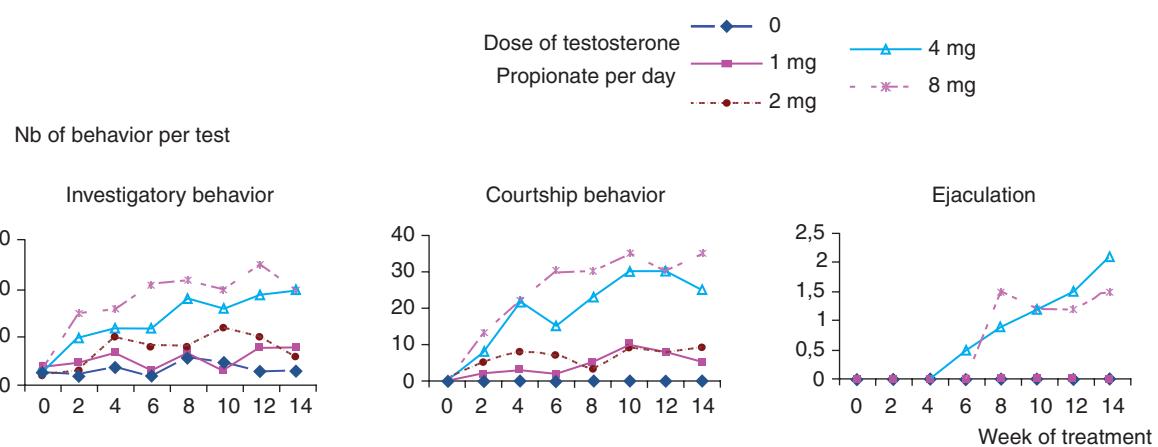


Figure 3 The effect of different doses of testosterone propionate on ram mating behaviors. The frequency of display of different behaviors increases with dose and duration of testosterone up to a plateau reached after 10 weeks at 4 and 8 mg day⁻¹. The testosterone threshold is lowest for investigatory behavior and highest for copulatory behavior. Adapted from D'Occhio MJ and Brooks DE (1982) Threshold of plasma testosterone required for normal mating activity in male sheep. *Hormones and Behavior* 16: 383–394.

action depended on prior conversion to biologically active metabolites in the target tissue. In general, 5α -dihydrotestosterone (DHT) is the active metabolite in the reproductive tract, whereas in the central nervous system (CNS) the active metabolite is 17β -estradiol. Thus, in the male rat and in the ram, 17β -estradiol is the active metabolite that induces male sexual behavior. However, in rat, some 5α -DHT is required to maintain penile sensitivity. In mice, 5α -DHT induces sexual behavior in some strains but not in others. In other species, the active metabolite depends on patterns of behavior. In the ring dove, courtship is composed of a bowing display, followed by chasing and nest soliciting. The first two behaviors are androgen dependent while nest-soliciting behavior is estrogen dependent.

In all cases, it is important to remember that hormones do not really trigger the onset of behavior but increase the likelihood that it will be expressed in presence of the appropriate sexual stimulus.

Females

As in males, ablation of gonads in females prevents the expression of sexual behavior. However whereas in the male, sexual activity can be displayed continuously during the breeding season, in most female mammals, the

expression of sexual behavior also called ‘estrous behavior,’ is restricted to a brief period around ovulation. In most species, ovulation is triggered by an increase in estradiol secretion by the ovary. This hormone is also important for the induction of estrous behavior. For a long time, apes and humans were thought to be exceptions because females may copulate at all times. Further studies have shown, however, that initiation of female sexual activity is more frequent just before ovulation in apes and humans.

In species like the cat, the pig, and the Japanese quail, exposure to estradiol alone is enough to induce estrous behavior. However, in many species, the behavior induced by estradiol alone is incomplete and castrated females need further exposure to progesterone, another ovarian hormone to present a behavior similar to that of an intact female. The exact time at which progesterone must be present differs among the species. In rodents that have short ovulatory cycles of 4 or 5 days, progesterone is required after a period of estradiol priming. In contrast, in sheep that have an estrous cycle of 17 days, estrous behavior is usually displayed only if the female has been exposed to progesterone for several days before the pre-ovulatory rise in estradiol (**Figure 4**). The quantity of estradiol needed differs between species ($0.5 \mu\text{g kg}^{-1}$ in sheep, $20 \mu\text{g kg}^{-1}$ in rat) but is always 100 times lower

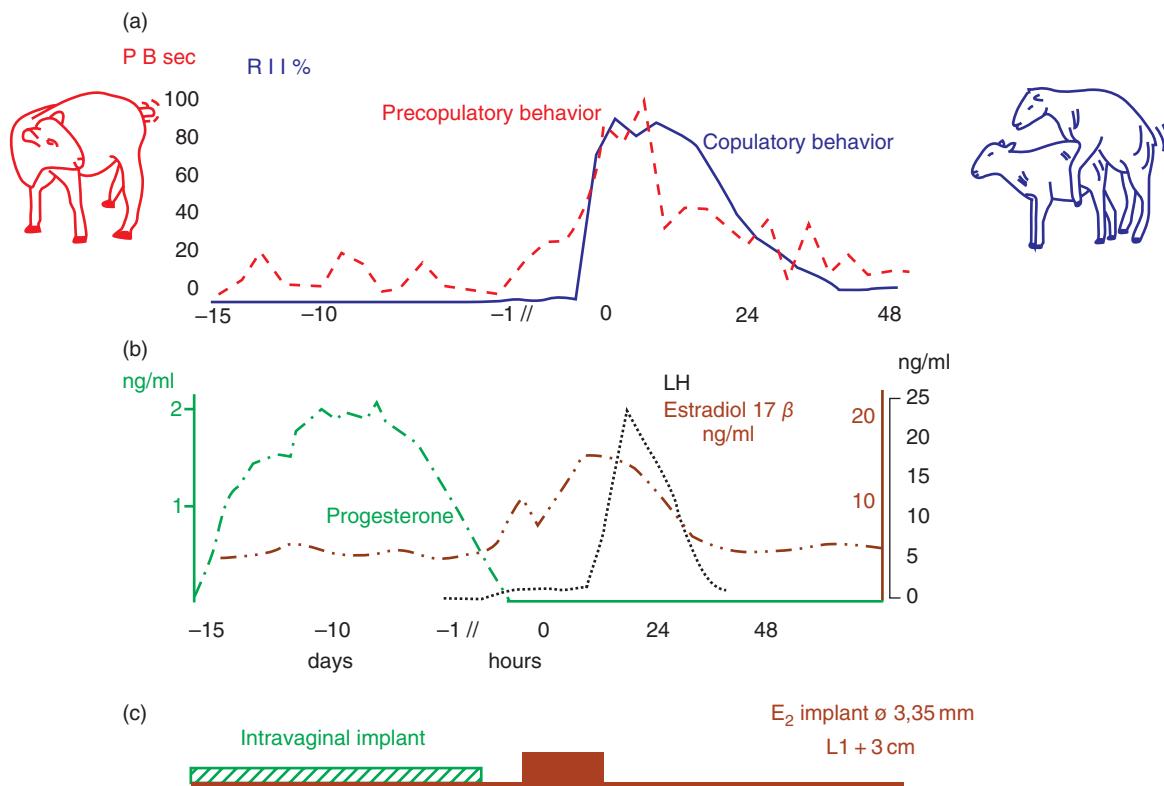


Figure 4 Behavioral (a) and endocrine (b) changes during estrus in intact ewes or ewes ovariectomized and treated with progesterone and estradiol (c). The intensity of precopulatory behavior (PB) estimated by the time ewes spent in contact with the ram in a choice test, is high for approximately 12 h. Copulatory behavior, measured as the percentage of male approaches that produce immobilization in the females (receptivity index (RI)), appears suddenly 8–10 h after estradiol increases and lasts for 24–26 h.

than the quantity of progesterone needed. Interestingly, the same type of estradiol action exists in the parthenogenetic desert-grassland whiptail lizard which contains only females that reproduce alone without males and, in which, female sexual behavior is expressed when the female is ready to ovulate but male sexual behavior is expressed at other times.

In most mammals, the progesterone produced after ovulation and during pregnancy also has an inhibitory action on sexual behavior.

In both males and females, the expression of sexual behavior can also be modulated by a number of peptides, the main ones being the gonadotropin-releasing hormone (GnRH) that also triggers ovulation and oxytocin, involved in parturition, lactation, and bonding with the sexual partner or the offspring. In amphibians, vasotocin has an important stimulatory role for sexual behavior.

Sexual Differentiation of Sexual Behavior

The type of sexual behavior expressed by males and females is generally quite different although in some species such as cattle or goats, male-pattern behavior can be part of normal estrous behavior. By treating females with androgen, it is possible in most species to induce the expression of part of the male repertoire of sexual behavior. But except in a few species, such as the ferret, it is very difficult to induce female patterns of sexual behavior in males.

Experiments in rodents have shown that differentiation in the ability to express male- or female-type sexual behavior is determined by the pattern and type of exposure to reproductive steroids in prenatal and neonatal life. If male rats are castrated at birth, as adults they can exhibit female sexual behavior following appropriate steroid treatment; they also have a reduced ability to express male sexual behavior. On the contrary, if testosterone is given to female rats between birth and day 10, the animals in adulthood will express male sexual behavior but not female sexual behavior. The testosterone, normally secreted by the testes of the male fetus or neonate, is said to have masculinized and defeminized the brain, both of which are distinct processes. An androgenized female rat will prefer an estrous female as a partner, whereas a male castrated during the critical period will prefer a male partner. This organizational action of the reproductive steroid hormones applies to most mammals although the time of the critical period and the degree of differentiation differs among species. For example, estradiol given in adulthood to male pigs, castrated at any time up to puberty, will retain the ability to express the immobile stance typical of female pigs in estrus. In this species, the critical period for brain sexualization extends to well after birth but the basic mechanism remains the same. The same phenomenon exists in birds but the deprivation

of hormones directs them to a male phenotype. In humans, sexual differentiation of the genital tract and the secondary sexual characteristics are well established but there is limited evidence showing that early exposure to reproductive steroid hormones has any effect on patterns of motor behavior during sexual activity or on the type of sexual partner that is preferred.

Sites and Mechanisms of Hormonal Action

Steroids can affect sexual behavior in acting directly on the CNS or indirectly by their action on peripheral target tissues.

Peripheral targets

Many of the visual, acoustic, and chemical signals involved in sexual behavior are steroid dependent. The bright color of sexually active male fish or birds or the colored sexual skin in monkeys are steroid dependent as also are the odor of the elephant in musth and the odor of the rat in estrus. In many cases, the sensitivity to the signals emitted by the partner is also steroid dependent. In the rat, ejaculation depends on the repeated stimulation of the penis which is covered by small spines. The density of spines on the penis is dependent on 5α -DHT and this explains why treatment of castrated male rats with only estradiol is not sufficient to restore complete sexual behavior. Androgens are also necessary for the correct development of the skeletal and smooth muscles specifically involved in sexual behavior, for example, the bulbocavernosus muscle in the penis.

Central targets

Steroids act on the CNS by first binding to specific receptors. Studies in mammals, birds, and reptiles have all found steroid-binding sites in the same areas: the preoptic-hypothalamic continuum, limbic structures such as the amygdala and the septum, and some mesencephalic structures. Experiments with localized lesion or the implantation of microquantities of crystalline steroids have shown that in all species studied, the medial preoptic area and the ventromedial hypothalamus are the most important targets of steroid action, respectively, for male and female sexual behavior. Steroids exert their action through the local modulation of the concentrations of neurotransmitters or neuromodulators by altering their concentration, turnover, or activity. Most neurotransmitters have been implicated in the central mechanisms that determine sexual behavior, but the precise action of sex steroids depends on the type of receptor, gender, endocrine conditions, and the particular type of behavior. Dopamine, for example, is important for the reward value of sexual behavior and is generally considered as stimulatory for sexual behavior, but in females its effect

may be either stimulatory or inhibitory depending on estradiol status and the phase of the mating sequence.

The CNS is also the site of the early organizational actions of gonadal steroids but the precise sites and mechanisms involved remain unclear. During this period, gonadal steroids have been shown to change the number and size of neurons, the density of synaptic connections, and the concentration of neurotransmitters in specific areas of the CNS, resulting, for example, in larger sexually differentiated nuclei in the hypothalamus of the male rat. The relationship between these anatomical changes and the patterns of sexual behavior induced by early steroid treatment still remain unclear despite some claims that structures implicated in homosexual behavior have been identified.

Mating Systems

In a species, the chances of an individual participating in reproduction are rarely identical. This is a direct result of competition among individuals of the same sex for access to partners and to the choice of partners. In most species, males compete and females choose. Together with the amount of parental care required and other social and ecological constraints, these phenomena lead to distinct mating systems across species, even among closely related ones. In addition, several types of mating systems are sometimes observed in the same species.

Monogamy

There are different definitions of monogamy. Here we consider an individual as monogamous if he/she mates with only one partner. This mating system is the rule in 90% of bird species but it is rare among mammals where its incidence is only 3%. It is especially common in species where all females are fertile at the same time or where the help of the male is needed to rear the offspring. It can be permanent – the partners forming a couple that lasts for life as in the snow bunting and in gibbons where males defend access to their territory and partner against other males. In other species, monogamy lasts for only one breeding period. Recent studies using DNA analysis have shown that many species considered as monogamous are not so exclusive; a significant proportion of the offspring results from extra-pair matings.

Polygamy

A system is said to be polygamous when an individual mates with more than one partner. ‘Polygyny’ is the term that describes a system in which the male has multiple partners, whereas ‘polyandry’ describes a system in which the female has multiple partners.

Polygyny

This is the most common system in mammals. Males monopolize permanently or temporarily several females grouped in a harem and prevent approaches by other males using aggressive behaviors. This is the case in bighorn sheep, gorillas, and bats. Sometimes, males do not defend the females themselves but the resources that females need to feed and rear their young. This is the case in cichlid fish, in the pipit, and the topi. In some species, the territory defended is a display area called a ‘lek’ from where females can choose their partner. This is observed in the black grouse, the hammer-headed bat, and the Uganda kob. In other species such as the Belding ground squirrel and the wood frog, receptive females are widely spread or they are all sexually receptive at the same time. Males compete to be the first one to mate; it is a mating system known as ‘scramble competition polygyny’.

In polygynous species, sexual dimorphism is very marked. The males are usually much bigger than females. Polygynous males also have larger testes than monogamous males, allowing greater sperm competition.

Polyandry

Polyandrous mating systems are very rare. One example is the Galapagos hawk where one female mates for several breeding seasons with up to eight males. Another example is the sand piper. In this species, the female produces a clutch of only four eggs and it has been suggested that polyandry allows the female to overcome this limitation.

Multimale multifemale groups

In this mating system, both males and females mate with multiple partners. This is reported in macaques, chimpanzees, domestic sheep, and some carnivores such as hyenas.

Alternative Breeding Tactics

Individuals excluded from reproduction by stronger competitors in the dominant mating system of the species may use alternative tactics to participate in reproduction. This is the case for several species of fishes. One way to achieve it is to look like and behave like the partner of the other sex. In the Mozambique tilapia, for example, the dominant male (also named the ‘bourgeois’ male) defends his territory and spawning pits. The subordinate male or ‘sneaker’ male is much smaller than the dominant male and behaves like a female enabling him to approach the nest and release his sperm. Another tactic is to stay close to the dominant male to take advantage of the fact that the dominant male cannot defend his territory and mate at the same time. This is the case in bighorn sheep, elephant seals, and green tree frogs. Subordinate males may also help the dominant male either to defend the territory or to take care of the eggs. In the rock blenny, the larger male, who is often the oldest, defends the nest with the help of smaller satellite males;

since the latter have access to the nest, they can occasionally fertilize eggs. These alternative tactics can be fixed for life, sequential or reversible depending on ecological conditions and the size of the population.

The sociosexual interactions in these mating systems can in turn affect the endocrinology of reproduction and, as a consequence, mating behavior. For example, in many species exposure to an active male stimulates ovarian cycles in the female hastening the onset of puberty or the breeding season. The influence can also be inhibitory as in an overcrowded group of mice or a marmoset family.

Conclusion

Mating is essential for the survival of species that reproduce sexually which is the case in most animal species. It is under tight hormonal control, which has been conserved through evolution. Furthermore, because the chance of participation is not identical among individuals, mating is also a major factor in species evolution. Through this uneven participation, the genotype of species can evolve. The fine-tuning of when, where, and with whom mating occurs is in turn modulated by the interactions between sexual partners themselves that modulate the endocrinology and behavior of the partners.

Acknowledgments

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See also: Animal Models of Sexual Function; Hormonal Contributions to Arousal and Motivation; Hormones and Female Sexual Behavior; Male Sexual Behavior; Sexual Motivation.

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Orientation and Navigation

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Glossary

Beacon – A conspicuous object that stands directly at the final goal location or at an itinerary reorientation point (transitory goal) along the route to the final goal.

Egocentric (or autocentric) – This applies to a place coding expressed in a frame of reference that is bound to the animal's current position, usually, but not necessarily, in terms of polar coordinates (i.e., direction and distance). The reference direction is then provided by the animal's body axis.

Exocentric (or allocentric) – This applies to a place coding expressed in an environment-bound frame of reference, usually as a content-addressable eidetic memory by which an animal can remember the location of a particular place it previously visited in the form of the image that can be perceived from this place, rather than in the form of Cartesian or polar coordinates.

Gradient – A vector indicating the direction in which a given parameter, continuously varying in space, varies with the maximal magnitude and the value of this maximal magnitude. The global area where this vector is constant or varies smoothly is referred to as a gradient field, and the local value of the parameter in question corresponds to the field potential.

Homing – This applies to a navigation process by which an animal returns to its home or some other specific goal (e.g., nest, burrow, or loft) as fast and straight as possible.

Landmark – A conspicuous object constituting an elementary component of an environment-bound frame of reference. Contrary to a beacon, it does not stand directly at a goal location.

Location-based information – A static, site-dependent information provided by the apparent configuration of the landmarks as perceived from the current place.

Route-based information – A dynamic, site-independent information provided by the kinesthetic systems and/or the optical flow about the movements performed.

orientation questions such as: In which direction should I move? Should I turn left or right? What is the shortest path to my home? As this type of questions certainly emerged early in life history, the ways animals nowadays orient themselves in their environment have evolved for an extremely long time in parallel in different taxa. *Sensu stricto*, orientation is just an angle measured with respect to a reference direction. Body orientation can be expressed relatively to the home or the previous movement direction, as well as to gravity, the geomagnetic field, or to some cardinal direction. The position of an animal in space is therefore fully defined by both its location (that is, the place it occupies) and the orientation of its body in the vertical and horizontal planes. For the sake of simplicity, we consider here only movements in a two-dimensional space, so that an animal's position is specified by two Cartesian coordinates and one single orientation value. Orientation behaviors then correspond simply to the behaviors that control the body orientation during movement so as to reach some specific goal.

There is a large diversity of orientation behaviors. The simplest ones involve elementary orientation mechanisms. They consist of getting-there procedures, which mainly specify the motor actions to perform in response to internal and/or external stimuli. Classical examples are provided by bacteria reaching high-nutrient-concentration places, first-year migratory birds reaching their wintering areas based on genetically coded vectorial information, or male moths moving upstream female-emitted pheromone plumes. Others, referred to as navigation behaviors, are more complex as they require some spatial representation. They are based on knowing-where processes, which enable animals to memorize the locations of their goals by using location-based or route-based information (see below). Hence, elementary orientation mechanisms make it possible to reach a new, unknown goal, whereas navigation processes are used to return to known, memorized goals. Although these two types of orientation behavior seem to be fundamentally different, it should be kept in mind that, as evolution is based on tinkering rather than on engineering, numerous animal species evolve original orientation solutions that in some way mix getting-there and knowing-where components.

Contrary to the satellite-based global positioning system (GPS), which now enables human beings to navigate worldwide with high accuracy levels, animal navigation systems are characterized by some working

Introduction

The behavior of most animals, at least during the early stages of their life, involves some movement of their body through the environment, and therefore results in

scale versus accuracy trade-off: those working at a large scale make it possible to only reach a more or less large area surrounding the goal location, whereas those that make it possible to pinpoint the goal can work only at a short scale, when the animal is relatively close to it. Consequently, animals that navigate over long distances have to successively rely on different, spatially nested navigation processes. For example, consider an albatross coming back to its nest after having foraged for a while (some several thousands of kilometers) at sea. It will have to rely on large-scale navigational processes through unfamiliar environment (the open ocean) until it can see its familiar breeding area (a specific island), then to shift toward a medium-scale, more accurate, navigational process to reach its nest area (a hillside or a cliff), and finally to shift toward a particularly accurate, small-scale navigation process to pinpoint the nest location. For numerous species that do not exhibit such very large movements, at least two pertinent scales – small and medium – have to be considered.

Orientation and navigation processes are fed by various sources of information. Environmental information is provided either continuously as stimuli smoothly varying in space (gradient fields) or discretely as punctual objects (beacons and landmarks). In contrast, other navigational systems, such as path integration, provide a vectorial coding of the goal location based on site-independent information. Once these various possibilities have been examined separately, it will be shown how they can be conceptually unified in a consistent theoretical framework.

Orientating in Physical or Chemical Gradient Fields

Numerous organisms and cells are able to orient themselves in a stimulation gradient field, that is, an environment presenting a physical or chemical factor to which they are sensitive and whose intensity varies more or less monotonically in space. Two types of elementary orientation mechanisms have been identified: taxis and differential-klino (DK) kinesis (other forms of kineses are space-use mechanisms not involved in spatial orientation). Taxis concerns species that can directly sense the gradient direction, for example, by comparing the values of the field potential (i.e., the stimulus intensity) measured by several accurate receptors distributed on the whole body. In contrast, DK kinesis concerns species that orientate indirectly with respect to the gradient direction (i.e., without sensing it) by controlling their local path sinuosity as a function of the variations of field potential perceived during

movement: a movement in the wrong direction, perceived as a decrease in the field potential (assuming an attractive stimulus), will lead the animal to locally lower the persistence in moving in that direction, and vice versa. This sinuosity regulation can take several forms. Bacteria orientating in a chemical gradient field rely on the most basic form: at each step, they choose a moving direction at random, and then move a distance whose magnitude is positively related with the rate of change in chemical concentration perceived. This simple trial-and-error mechanism enables bacteria to efficiently reach the areas presenting the highest concentration of some useful nutrient. It is usually misleadingly referred to as ‘chemotaxis.’

An interesting extension of this type of mechanism applies to animals, such as homing pigeons, procelariiform seabirds, and sea turtles, which are able to home through large-scale unfamiliar environments, presumably using two (or more) intersecting gradient fields. It might simply consist in locally decreasing or increasing the homing-path sinuosity depending on whether the current field potentials become closer to or further from the values that have been memorized at home. In this context, the memory of the goal location in terms of field potentials corresponds to the only knowing-where component in this otherwise getting-there-orientation process. It is usually assumed, however, that most species that have to home through large unfamiliar environments rely on a more sophisticated process involving additional knowing-where components. Indeed, they should, when moving around their home, memorize four key parameters enabling them to map the gradient fields (assumed to be more or less regular over large spatial scales beyond familiar areas): the two gradient directions with respect to a biological compass and the two gradient strengths. They may then compute a homing component for each gradient field, whose vectorial combination would enable them to infer the home direction with respect to their compass. Several biological compasses have been identified in birds (and other taxa such as hymenoptera): birds are able to learn the apparent movement of the sun azimuth (and associated changes in the skylight polarization pattern) with respect to an internal clock, as well as the rotation center of the star pattern, and so can use a sun compass by day and a celestial compass by night. They also rely on a compass based on the inclination of the geomagnetic field when the sky is overcast. In contrast, the nature of the gradient fields used to assess the current and home locations is still subject to debate. It may involve some geomagnetic parameters, such as the total intensity or the inclination, and the relative proportion of some atmospheric gas traces, which may be estimated by the olfactory system. Sea turtles may use a similar gradient map and compass navigation system to migrate between their foraging and breeding areas.

Place (or Eidetic) Navigation

At the other end of the scales, place navigation is a process that enables an animal to pinpoint a (hidden) goal location using the spatial layout of the nearby surrounding landmarks: the animal will return to this place by comparing the apparent landmark configuration memorized from the goal with the one perceived from its current location. The landmarks hence provide the basis for an exocentric, site-dependent representation of both the goal location and the animal's current location. Obviously, only landmarks that are sufficiently close to be submitted to a detectable apparent movement when the animal is moving are useful to build such representations. Indeed, if remote landmarks can provide directional cues, that is, serve as a kind of compass, they cannot provide reliable locational cues because their apparent configuration remains quite similar, whatever the animal's location. Consequently, close goal locations may be memorized, independently from each other, using a common set of landmarks, but distant goal locations have to be memorized each with respect to a specific set of nearby landmarks. It is also worth noting that only coarse-grain objects, which outline the geometric shape of the environment, should act as landmarks. In turn, objects generating high spatial frequencies should be filtered out because they provide unreliable spatial information as their locations are potentially fleeting. This filtering out is also likely to dramatically reduce the cognitive burden that bears on the exocentric localization system.

The place-navigation process used by mammals appears very flexible, notably thanks to some limbic neurons – hippocampal place cells and postsubiculum head-direction cells – that encode the animal's position (i.e., both location and orientation) with respect to the surrounding landmarks. After an exploratory phase, during which the animal learns how the apparent landmark configuration is locally altered when it moves around by jointly processing location-based information and route-based information, any place (including the goal) will be given an exocentric, content-addressable representation. Reaching the goal then involves a double change in frame of reference, from egocentric sensory inputs through exocentric localization and navigation computation to egocentric motor outputs. Birds appear to rely on a similar place-navigation system. Both mammals and birds have been shown to pay more attention to the global geometric shape of the environment, as provided by the whole landmark configuration, than to the individual characteristics of landmarks that can be used to identify them. A major difference, however, comes from that birds can use a sun- or magnetic-compass-based reference direction, whereas mammals have to extract a site-dependent reference direction by processing landmark-

based information. The ways other vertebrate taxa perform place navigation remain poorly known.

In turn, hymenoptera (bees, wasps, and ants) rely on a simpler but more rigid navigation process, which short-cuts the twofold change in frame of reference. They are indeed assumed to memorize the goal location by storing the bearings and angular sizes of landmarks in a (sun or magnetic) compass-oriented snapshot taken at the goal. Although it constitutes an exocentric, landmark-based representation of the goal location, such a snapshot is just a low-resolution, compass-referenced copy of the egocentric view (retinal image) perceived from the goal. Hymenoptera can then return to the goal by moving so as to progressively reduce the discrepancy, in terms of retinotopic coordinates, between the current retinal image and the memorized snapshot. As their image-matching process works egocentrically based on landmarks identified from their individual characteristics, hymenoptera have to head in the same compass-based direction as the one used when memorizing the goal location as a snapshot to be able to solve the navigational task, and the global geometric shape of the environment seems to play only a secondary role. Although much less studied, other insect species may process landmark-based information in a more or less similar way.

Path Integration

Although it has been studied mainly in mammals and insects, path integration is a site-independent process used by numerous other taxa such as birds and spiders. It enables an animal to build up an egocentric vectorial representation, in terms of direction and distance, of its starting point. (Human navigators rely on a similar system, called 'dead reckoning,' but the frame of reference is reversed: the boat location is expressed exocentrically with respect to the starting harbor and a compass-based reference direction.) When moving, the animal must therefore recurrently update the goal-pointing vector by processing route-based information, that is, dynamic information collected *en route* about its changes of direction (rotations) and changes of location (translations). The rules used by an animal to combine such rotational and translational information, in order to infer the relative movement of its hidden starting point by using information about its own movement, are formally similar to those specifying the apparent movement of a fixed conspicuous object (optical flow), but work in the reverse way. As a result, a simple rotation of the animal on the spot leads to simple counterrotation of the inferred goal direction, whereas the direction and distance to the goal vary interdependently during an animal's translation: the change in the goal direction (inferred motion parallax) is proportional to the sine of the goal direction and inversely

proportional to the distance, which itself decreases linearly with the cosine of the goal direction. Inferring the apparent movement of a goal when performing a naturally curvilinear movement, in which rotations and translations occur simultaneously, is therefore a very complex task. For this reason, it is usually admitted that only a single goal location – the starting point of the path – can be memorized using path integration, or possibly two goal locations in the simplest case of an animal shuttling back and forth between them (the inferred movement parallax is then null). Memorizing several goal locations simultaneously would require the concurrent use of several independent path integrators, but this possibility has not yet been demonstrated.

As it involves a recurrent updating of the vectorial coding of the goal location, path integration is a process prone to accumulate random measure errors, and so generates increasing inaccuracy in the memorized goal location during large-scale movements. It is therefore useful only to reach an area surrounding the goal. Afterward, the animal will have to shift to place navigation to pinpoint the exact goal location. However, it has been shown that path integration is relatively insensitive to errors occurring in the estimation of translations, which therefore need only to be roughly measured by kinesthetic systems or possibly by the visual system using the self-induced optic flow from the ground. In contrast, the way rotations are measured is of major importance, as it drives the speed with which measure errors accumulate. Hymenoptera, which are able to use a sun (or polarized skylight) and, possibly, a magnetic compass as a reliable external reference direction to estimate their rotations, can rely on path integration to return close to their home after a long sinuous outward foraging path. In contrast, mammals, which acquire this information only internally by means of their vestibular and other kinesthetic systems, can rely on path integration only for relatively short distances. In addition, a systematic error is generated in both mammals and hymenoptera when left and right turns are not balanced in the outward path. The occurrence of this error, which looks like an overestimation of the inferred motion parallax of the goal, indicates that neural computation of path integration is only able to approximate the correct mathematical rules. However, the neural structures involved are not yet well known. In mammals, the caudate nucleus seems to play some role.

Navigation through Large Familiar Areas

The landmark-based processes used by an animal to navigate through large familiar areas (home ranges) remain poorly understood, whereas they are of paramount importance, notably in mammals which often use several homes at the same time but whose path integrator

is not only restricted to a single goal location but also becomes quickly inaccurate when they move long and sinuous paths. It has been hypothesized that mammals and birds, and even bees, might be able to mentally build up a global exocentric frame of reference, based on the whole set of familiar landmarks. Such a global representation of the familiar area, usually referred to as a mosaic map, can be seen as a puzzle made by merging contiguous local exocentric frames of references, each one corresponding to the subset of landmarks that are within perceptual reach from a given location. It would be very useful to perform a generalized form of place navigation, from a current location surrounded by a specific subset of landmarks to a distant goal surrounded by another specific subset of landmarks (**Figure 1**, top). It has never been demonstrated in any species yet, excepted in human beings. This global-mapping ability may be linked to language – natural and mathematical. Indeed, using concepts such as Euclidean space and Cartesian coordinates makes the building of this kind of map relatively easy. Thus, early European navigators mapped the world in relying on dead reckoning (vectorial coding of the boat with respect to the starting harbor) to update their current location on a nautical chart, on which they indicated the locations of the new coasts they encountered as they went along. Because of the tinkering nature of evolutionary processes, it seems more likely that animals have evolved large-scale navigational solutions by mixing getting-there and knowing-where components that were already available rather than by designing fully rational cognitive maps as an engineer would do.

The simplest solution to reach a goal that is beyond perceptual reach involves the learning of purely motor sequences (praxis) or sensorimotor sequences (piloting), for example, walk a given distance toward a given beacon, or up to a given place, then turn left, and so on, or a chemical-trail-following mechanism, as used by numerous ant species. These getting-there procedures can be easily automatized, and so, are routinely used by numerous species (including human beings) when moving along usual itineraries through a familiar area. A simple set of independent and rigid itineraries is of limited use, however, as each one only enables an animal to reach a specific goal from a specific starting point. In turn, if a special status may be given to the choice points corresponding to the crossroads, the animal would progressively learn the global connectivity of a flexible topological network of itineraries, and learn at which neighboring crossroads the various possible roads lead when standing at any given crossroad (**Figure 1**, middle). For this purpose, the animal should, when arriving at a given crossroad, conjointly use an associative memory to link the current landmark configuration it experiences there with the backward direction leading to the crossroad it previously moved through, and a declarative

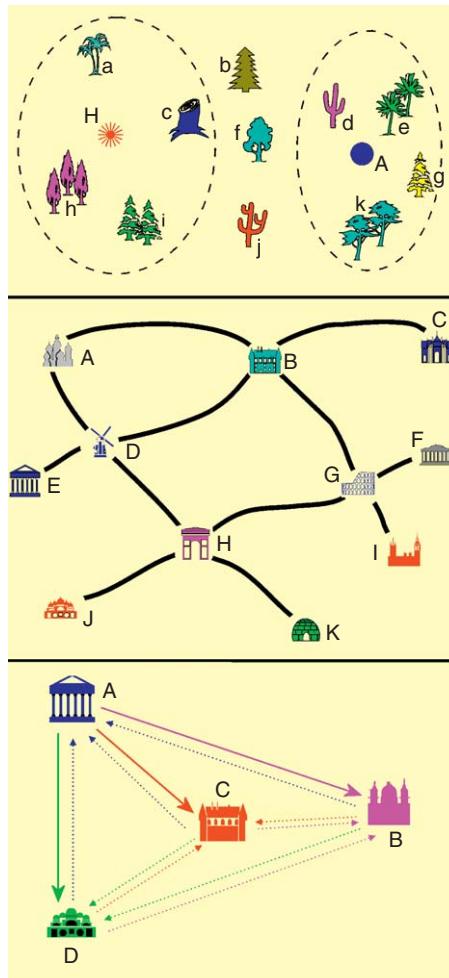


Figure 1 Schematic illustration of three models of navigation within a large familiar area. (Top) Mosaic map. The animal, at place A, knows where it is with respect to the subset of landmarks d, e, g, and k, which it can perceive from its current location, and also knows where its home H is with respect to the subset of landmarks a, c, h, and i, which it perceived from this place. The two dashed ellipses stand for the areas within perceptible reach from the two locations. Classical (i.e., short-scale) place navigation cannot be used to reach home because there is no common landmark shared by the two subsets. However, the animal might be able to determine its home location from a distant location if it has been able to map the whole set of landmarks (a-k) by mentally joining the various subsets perceived from other possible locations based on some common landmarks (e.g., subset of landmarks b, c, d, f, and j, which is assumed to be within perceptual reach at an intermediate location). (Middle) Topological network. Key places correspond to crossroads (and dead ends) occurring along familiar itineraries. For clarity, buildings stand for the subsets of landmarks perceived from these key places. The animal is assumed to learn as to how these various places are connected (e.g., place H is connected to places D, G, J, and K, place E is only connected to place D), and to recall which routes lead to which neighboring places when it perceives the subset of landmarks characterizing the current place. As movement is restricted on a topological network, the cognitive burden should be less heavy than the one involved by a mosaic map. (Bottom) Network of orientation tables. The animal is assumed to mnemonically associate the direction and distance (initially provided by path integration) of any key place with the subsets of landmarks perceived at the other key places (drawn as buildings). The animal is assumed not to know the spatial relationships between the various places (and their respective subsets of landmarks) independently of its own location, however. For instance, as illustrated here, the animal can recall where the places B, C, and D are (in terms of direction and distance) when it is at place A, but not where place B is with respect to place C at this time (this information is potentially present but will be recalled from memory only when the animal perceives the subset of landmarks corresponding to place C). In other terms, the animal is assumed to use a set of local, independent frames of references, each one being anchored to a key place, rather than a global cognitive map.

memory to assess that the current and previous crossroads are closely connected. In this way, it would progressively become able to quickly reach a remote crossroad, by relying on its declarative memory to sort the list of crossroads occurring along the best itinerary and on its

associative memory to recall the road to take at each intermediate crossroad to reach the next one.

Another parsimonious solution consists in associating the vectorial representation of the current starting point, as provided by path integration and assumed to

correspond to an important place, with the local landmark configuration experienced when arriving at another important place. After having explored its environment for a while, the animal may navigate thanks to a network of rudimentary orientation tables, the currently reached one indicating the direction and distance of the others (**Figure 1**, bottom). The animal is therefore not assumed to know the spatial relations between the important places of its home range independently of its current location (a useless possibility that is offered by a cartographic representation of the environment): it is only when the animal is standing in the vicinity of a given important place that the locations of other important places, relatively to the current one, may be recalled from memory. The ability to punctually associate a moving direction to a given landmark configuration has already been demonstrated in several taxa, but we do not yet know to which extent an animal may actually use it in a more global way to navigate through its home range.

Toward a Unified Approach of Orientation and Navigation Processes

Although they appear to be extraordinarily diverse in terms of information sources and mechanisms, all orientation behaviors can be conceptually unified by introducing the notion of generalized gradient field. In this framework, a goal is assumed to act as an attractor because it occupies the center of a radial gradient field, so that the goal direction systematically corresponds to the gradient direction, whatever the animal's location. The goal may by itself generate a radial gradient field by diffusing some light, heat, humidity, sound, or odor around it, but not necessarily. For example, hilltops can be said to attract uphill-moving animals (e.g., some butterfly species which aggregate there) in lying at the center of a radial altitudinal gradient field: the goal can be reached simply by heading in the direction in which the hill slope is maximum. The gradient field may even have no physical or chemical reality at all. For this purpose, we have to consider virtual stimuli, based on spatial memory, in addition to physical and chemical ones, giving birth to cognitive gradient fields corresponding to mental rather than actual buildings.

Consider first the role played by a visual beacon standing at the goal. If this beacon was luminous, the goal would be, by night, placed at the center of an illuminance gradient field which can be used by an animal to head toward it (e.g., an insect attracted by a lighting bulb). With an ordinary beacon in daylight, there is no more such a physical gradient field; however, if the animal has mnemonically associated the beacon

with the goal location, then the goal in some way occupies the center of a gradient field of beacon proximity, and orientating toward it corresponds to a form of taxis (similarly, the hot-and-cold game played by young people can be seen as a social form of DK kinesis). At least, the proximity gradient field generated by a beacon has some sensory, if not physical, reality: the field potential corresponds to the apparent size of the beacon, and the gradient direction can be directly inferred from retinotopic information. A further step is reached when considering an animal relying on place navigation or on path integration. Both provide a goal-pointing vector, and therefore in some way generate a radial proximity gradient field centered on this place, as would do a beacon set there, but which is a purely mental building: in path integration, it is generated by the recurrent updating of the goal location, thanks to route-based information, whereas in place navigation, it is generated by the discrepancy between the apparent landmark configuration currently experienced and the one that has been memorized at the goal location. A similar situation occurs in large-scale navigation based on two intersecting linear physical or chemical gradient fields. The home location has been memorized in the form of two coordinates corresponding to the potentials of these two gradient fields as experienced from this place, as hence can be said to stand at the center of a virtual radial gradient field whose potential corresponds to the discrepancy between the values of the current and memorized potentials of the two intersecting linear gradient fields.

With the generalized gradient field concept, taxes and DK kineses, which initially are elementary orientation mechanisms, can account for the operational working of much more complex navigation processes, possibly involving sophisticated spatial representations. This unified approach hence makes it possible to measure the orientational performance of an animal without having to make strong hypotheses about the actual level of its cognitive abilities, which can be assessed independently by analyzing the cognitive complexity of the task. This is a key point, because cognitive spatial abilities and orientational performances are not necessarily linked: despite having no cognitive abilities bacteria are quite efficient when orienting themselves in chemical gradient fields.

See also: Animal Models of Learning and Memory; Cognition: Learning and Memory: Spatial; Declarative Memory; Memory in The Honeybee; Neural Representations of Direction (Head Direction Cells); Place Cells.

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Parasite Infection and Host's Behavior

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Glossary

Behavioral fever – The raise in body temperature resulting from the active exposure of cold-blooded animals to heat sources.

Disease – An illness affecting humans, animals, or plants, often caused by infection. An infectious disease results from the presence and activity of a microbial agent.

Host compensation – The physiological or behavioral processes by which a diseased host improves on its condition, without getting rid of the parasite or pathogen.

Hypothetico-deductive approach – An approach that consists of proposing a hypothesis leading to predictions, the relevance of which can be tested empirically. Any contradiction between the observed facts and the prediction leads to the hypothesis being rejected.

Infection – The act or process of causing a disease, for a parasite, of getting diseased, for a host. Infection can also be the outcome of a parasite's encounter with and establishment in or on its host, a term sometimes restricted to bacteria and virus.

Parasitic manipulation – The ability, shared by several parasite groups, to modify their hosts' phenotype to their own advantage, often through increasing the probability of transmission from a first host to a second.

Pathogenicity – The ability of an organism (a pathogen) to cause disease in another organism.

Symptom – A phenotypic change that reveals that the individual is ill.

What makes a locust go bathing in the sun, maybe at the cost of increased conspicuousness to predators? Why would an ant climb to the top of vegetation, exposing itself to be swallowed by a sheep, or why would some suicidal cricket jump into the water? What makes a fox so aggressive or a rodent fearless toward a cat? Why would chimpanzees swallow clay or whole leaves of carefully selected plants?

These odd behaviors all have the same ecological and evolutionary framework: host–parasite antagonistic interactions. Reporting on the many cases where the behavior of infected hosts is altered, compared to that of uninfected conspecifics, raises the challenging problem of the

causality of observed phenotypical alterations. Is a more aggressive fox more likely to get infected or is it the infection that makes the fox more aggressive? Is the locust increasing its body temperature through bathing in the sun to stimulate its immune defenses, to slow down the pathogen's proliferation, or, on the contrary, is it the pathogen that manipulates its host to speed up its own proliferation? Is the biting rate of *Plasmodium*-infected mosquito increased as a side effect of energy depletion due to infection, or is it specifically enhanced by the parasite to increase its transmission rate?

These questions must be addressed following a hypothetico-deductive approach to establish who, if any, between the host and the parasite, is benefiting from the change in behavior. The consequences of altered behaviors on both host and parasite fitness, as the target of natural selection, constitute the ultimate causality of this phenomenon (**Table 1**).

A second level of causality must be addressed to understand how, from a mechanistic point of view, the intimate relationship between a parasite and its host modulates the host's behavior. Proximate causality of behavioral changes, therefore, implies unraveling of the molecular, neuronal, and endocrinological pathways used by a parasite to hijack its host's behavior.

Pathogenic Effects on Host Behavior and Host's Compensatory Behaviors

Pathogenic Effects

The pathological influence of parasites on the behavior of their hosts often results from neuroendocrine, nutritional, or organ dysfunction. Indeed, general modes of pathology, such as reduced nutritional levels or damaged sensory organs or muscles, have behavioral consequences such as lethargy or reduction in feeding, courtship, parental care, migration, territorial defense, and so on. Although the array of sickness behaviors experienced by infected animals ranges well beyond the scope of this article, we will start by reviewing a few common examples.

Reduced feeding or decreased activity is often reported in infected animals, from invertebrates to mammals. Reduction in feeding rate is interpreted as a stress-induced alteration in host behavior with no adaptive value either for the host or for the parasite. Decreased activity can result from depleted nutritional levels due to energy uptake by the parasite or reduced assimilation

Table 1 Categories of changes in the behavior of infected hosts (or of hosts exposed to disease risk) compared to uninfected ones, according to their direct consequences on parasite and host fitness

Host fitness	Parasite fitness	Behavioral changes
Decreased	Unchanged	Pathogenic effect
Decreased	Increased (transmission)	Parasite manipulation
Increased	Decreased	Host defense (avoidance or resistance)
Increased	Unchanged	Host compensation

Increase or decrease in parasite or host fitness is estimated relative to an infected host not producing the observed change in behavior.

efficiency (for instance, when the gut mucosa or the liver is damaged). Daily tasks can also be compromised if altered oxygen delivery (due, for instance, to blood parasites or parasites damaging lungs) reduces activity. Both may be linked if activity is decreased below a threshold required to forage for food.

Cognitive tasks may also be affected in parasitized hosts. Reduced learning ability has been reported in infected insects and mammals. Bumble bees naturally or experimentally infected with the trypanosome protozoa *Cryptosporidium bombi* are less able to learn the color of rewarding flowers compared to uninfected foragers. Similarly, the learning performance of bees whose immune system has been challenged nonpathogenically, is significantly impaired. The physiological basis of such trade-offs between immune defense and learning is supposed to lie in the proteins fuelling both functions. Mice infected with the coccidian protozoa *Eimeria vermiformis* display significantly poorer spatial acquisition and retention of the water-maze task. The modulation of opioid systems by parasites may be implicated in the alteration of these cognitive tasks. In humans, cerebral malaria is associated with both short-term and long-term cognitive impairments, in particular, for attention and working memory. Two years after the initial malaria episode, children with cerebral malaria have a 3.67-fold increased risk of cognitive deficit compared with other children. How can infection alter the central nervous system (CNS) to the point where learning, memory, and decision making are compromised, even in animals harboring parasites at sites physically distant from the brain? It has been proposed that the bidirectional connections between the immune system and the nervous system may allow such pathological effects. Not only are a range of behaviors affected by an immune challenge, but conversely, an individual performing an elaborate cognitive task (such as multiple-odor training in mice) or experiencing a stressful situation may be immunosuppressed. Such interactions among immune, endocrine, and neural systems are well established in vertebrates and are highly suspected in invertebrates.

Parasitized animals may also experience impaired antipredator or competitive behavior. This could come as a consequence of reduced stamina, decreased activity, and/or impaired cognitive capacities (such as chemosensation). For instance, birds with a high prevalence of protozoan blood parasites are more vulnerable to predation by hawks than individuals with a low prevalence.

Host Compensation

The depletion of energy and nutrient stores resulting from infection may be compensated for by increased food consumption (but at the expense of other costs associated with foraging activities such as predation) or by selective changes in dietary choice.

One of the best-studied examples of behavioral compensation for depleted endogenous reserves is that of the cestode flatworm *Schistocephalus solidus* in its intermediate host – the three-spined stickleback. Parasitized fish both increase the time spent feeding and change their choice of habitat, while simultaneously reducing their natural aversion to predatory cues. Change in dietary choice can be a more specific behavioral response to unbalanced nutrient depletion. Caterpillars of the lepidopteran *Spodoptera littoralis* infected with an entomopathogenic virus grow and survive better when reared on a protein-rich diet (**Figure 1(a)**). This confirms that resistance to parasite demands greater investment in proteins rather than energy (carbohydrates). Interestingly, infected caterpillars compensate for protein costs associated with immune defense by shifting nutrient selection toward a protein-rich diet (**Figure 1(b)**).

Reduced activity can also be interpreted as a compensatory behavioral response if it is employed by infected individuals to save energy and fuel immune responses. An extreme form of reduced activity is sleep. Both in the human and rodents, sleep is known to be altered in sick individuals. However, recent studies suggest that such behavior could be part of the host defense against infection rather than just a compensatory response to save energy (see below).

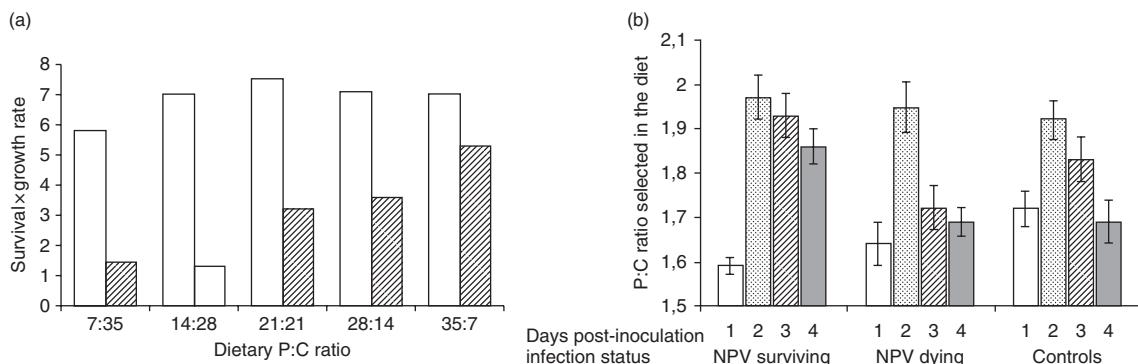


Figure 1 (a) Performance of *Spodoptera littoralis* caterpillars infected with entomopathogenic virus (NPV; dashed bars) or uninfected (control; empty bars), as a function of protein–carbohydrate ratio in their diet. Performance is estimated as a product of growth rate (body mass gain divided by development time) and survival. (b) Diet choice of *Spodoptera littoralis* caterpillars surviving infection with entomopathogenic virus (NPV), dying from infection or not infected (Controls), as a function of time after challenge. Diet choice is expressed as the protein to carbohydrate ratio selected from the two diets offered (P:C ratio: 35:7 and 21:21). A selected P:C ratio of 2:1 corresponds to a random choice.

Host Defense against Infection: Avoidance and Resistance

Several host behavioral traits affect disease risk, such as dietary choice, mating system, group living, and habitat use. Some are, therefore, expected to have evolved not only to optimize life-history strategy, but also to limit the risk or consequences of infection, sometimes under opposing selective forces. Although it may be difficult to demonstrate the adaptiveness of some behavior specifically in response to infection risk, the importance and taxonomic ubiquity of parasite-avoidance behavior should not be underestimated. Indeed, behavioral responses offer a number of advantages over the activation of physiological and cellular defenses. Avoiding infection saves the energy expenditure associated with mounting an immune response (and its side-costs, such as autoimmunity or immunopathology) and/or pathogen development. Some behaviors may also contribute to resistance of the infected host to parasite development or multiplication, prior to, or in combination with, physiological and immune responses. However, the exploration of the molecular and physiological basis of parasite avoidance and resistance behavior is still in its infancy, and only a few studies so far have addressed the molecular or neurophysiological pathways underlying the observed alterations in the behavior of infected hosts.

Foraging and Feeding

Host behavior associated with feeding is a route of transmission for many parasites (especially those of the digestive track), either directly through contaminated food or water or indirectly through infected preys. Consequently, a number of behaviors associated with

foraging for food and dietary choice have evolved to decrease infection risk (avoidance or prophylactic strategies) or to increase host resistance (therapeutic strategies).

For instance, the nematode *Caenorhabditis elegans* can modify its olfactory preference to avoid toxic bacteria. This roundworm lives free in microbe-rich environments in which it feeds on soil microorganisms. Although attracted from a distance by the bacteria *Serratia marcescens* which is part of its diet, the nematode can exhibit immediate physical evasion when it comes into contact with certain strains. The repellent compound is a surfactant secreted in large quantities by some *S. marcescens* strains as a necessary attribute for swarming motility. Physical pathogen avoidance depends on identified chemosensory neurons, and is controlled by unknown G-protein-coupled receptors and others such as Toll-like receptors. Escape is associated with feeding inhibition upon pathogen exposure, a combined behavioral defense involving an insulin-like signaling pathway. In addition, the nematode selectively avoids an odor experienced simultaneously with pathogenic infection, a criterion for associative learning. Aversive olfactory learning is rapid and is highly specific to the pathogenic species to which the worms are exposed. It is mediated by an enhanced serotonin signaling in the ADF sensory neurons. This combined behavioral strategy of defense against pathogenic bacteria in *C. elegans* seems to be part of a more general response to stress.

Infection can also alter the taste of a host and shift its food selection in order to increase the consumption of compounds that are toxic to the parasite it harbors. The ingestion of specific plant products by individuals exposed to infection risk or already parasitized is akin to self-medication. The use by chimpanzees of plants with antibiotic, antiviral, or worming properties (either

mechanically or biochemically) is interpreted as a form of self-medication. More generally, fighting parasites using plants, bark, or soil with pharmacological activity has been suspected in all major lineages of primates and several other species of nonhuman vertebrates for more than 30 years. However, experimental tests are still needed to show that these self-medication behaviors decrease infection rate or increase recovery; and interpreting the ingestion of stems, leaves, and roots with stimulating, antihelminthic, or antibiotic properties may still be controversial. Yet, its repeated evolution suggests that animals gained adaptive defense against parasitism through the evolution of innate dietary behaviors or by individual learning or cultural transmission in dietary choice. For instance, the absorption of small quantities of bitter substances is observed in several mammal species despite their aversion to it: it seems that innate 'repeat sampling' behavior of unpleasant plant substances has evolved in connection with their prophylactic properties relative to protozoan (plasmodium, amebae, etc.) and other parasites. When mice have a choice between drinking plain water or a chloroquine solution, they include 10–40% of the latter solution in their total fluid intake over 24 h. Mice infected by *Plasmodium berghei* (the specific agent of murine malaria) thereby develop less intense parasitemia and have a higher survival rate as long as they have access to chloroquine.

Sequestering phytochemicals from the diet is known as a strategy in phytophagous insects to repel predators. However, some phytochemicals also provide a biochemical defense against larval endoparasitoids. Caterpillars of the tiger moths *Grammia geneura* and *Estigmene acrea* parasitized by an endoparasitoid specifically increase gustatory responsiveness to pyrrolizidine alkaloids (PA) – a phytochemical toxic to the parasite. Specialist receptor cells (taste cells) of parasitized moths increase their firing rates in response to PA (but not to another deterrent compounds with no protective value against parasite). This specific signaling at phagostimulant cells stimulates feeding upon plants rich in PA in this otherwise generalist phytophagous lepidopteran.

Social Behavior, Mating

Resistance of infected hosts to infection most often relies on individuals changing their behavior as a consequence of their own health status (see sections titled 'Foraging and feeding' and 'Habitat use, hygienic behavior'). However, social behavior can also be implied in avoidance and resistance strategies. In that respect, discrimination of infected individuals is certainly the first mean of avoiding infection, if cues produced by infected conspecifics convey information on infection risk. In particular, recognition of a conspecific may often rely at least partly on chemical signals. Individual odors

that convey cues with regard to infection status can, therefore, provide valuable information mediating social relationships, such as territorial or foraging activities, group living, mate choice, and parental care. For example, rodents use social odors to distinguish between infected and uninfected individuals, displaying aversive responses to individuals infected with a wide range of parasites such as virus, protozoans, nematodes, or ectoparasites. In a choice test, estrous and nonestrous female mice avoid specific odors associated with either an infective or a preinfective male harboring the coccidian protozoan *E. vermiciformis*. The same discriminatory behavior is elicited by males infected with nematodes or virus. Analgesia is often an additional defensive response to the odors of an infected male since its anxiety or stress-associated behavior contributes to a reduced interest and avoidance of parasitized males by females. By contrast, such avoidance and stress-related responses in females are not elicited by castrated males (with low levels of testosterone) nor by physically stressed males. Male mice also face the threat of infection during social interactions involved in mating or during the establishment of social hierarchy. They discriminate against and selectively avoid the odors of other males (infected with the louse *Polyplax serrata* or with the nematode *Heligmosomoides polygyrus*) and of females infected with *H. polygyrus* or with the cestode flatworm *Taenia crassiceps*. Two highly polymorphic gene complexes, the major histocompatibility complex (MHC) and the major urinary proteins (MUPs) provide urinary odor cues that can be used for individual recognition. Changes in immune and endocrine functions induced by parasite seem to affect the production of MHC- and MUP-associated odor cues of parasitized male mice, hence signaling the infection to healthy individuals. Contrasting with studies on mate choice and infection risk in rodents and birds, there is so far no evidence that mate choice in primates acts to decrease the risk of disease transmission. Despite this, a large literature deals with the influence of parasites on mate choice. Females (being usually the choosy sex) may benefit in several ways from discriminating between infected and healthy males. A first direct benefit is, obviously, the avoidance of infection. However, when parasitic infection affects the body condition and stamina of males, females may benefit from discriminating between infected and uninfected males through securing various direct benefits such as larger nuptial gifts and/or increased assistance in parental care. Females may also benefit indirectly by increasing disease resistance in their offspring if resistance is heritable. It has, for instance, been suggested that the preference of female birds for ornamented males results from the fact that only males in good condition can maintain their bright colors or develop an extravagant plumage. More recently, it has been proposed that mating between genetically dissimilar individuals should result

in increased offspring heterozygosity, and, hence, better resistance to parasites and pathogens. Indeed, some studies suggest that genetic compatibility between partners, particularly at key loci such as the MHC, may influence mate choice in vertebrates.

Habitat Use, Hygienic Behavior

Hygienic behavior associated to the choice or maintenance of habitat ranges from simple cleaning to specific enrichment of a nest or burrow with plants having repellent or toxic properties. Rodents and birds bring to their home plants releasing volatiles naturally or after nibbling, thus reducing attraction, repelling, or killing ectoparasites or vectors. Leafcutter ants *Trachymyrmex* use bacteria with antibiotic properties to protect their fungal gardens from the pathogenic fungus, *Escovopsis*. Another famous example of hygienic behavior in social insects is the prospecting and cleaning behavior of *Apis mellifera* worker bees from chambers to remove dead pupae, thereby limiting the propagation of bacteria, virus, or mites.

Habitat choice as a defense against infection can be as simple as reaching the best place to boost the immune system or to limit proliferation of the parasite. In ectotherms, one behavioral response to infection is to choose an orientation in a thermal gradient or to sunlight, and/or a daytime activity, so as to increase body temperature. Such behavioral fever has been reported in several orthopteran species infected with entomopathogenic fungi for instance.

I Sleep Therefore I Fight

In humans, it has recently been suggested that the immune system would alter sleep architecture, when sick, to facilitate the generation of fever. Fever is an adaptive response to infection: it increases survival by potentiating immune response and by hardening the conditions for replication of the pathogen. Reciprocal interactions between immune signaling molecules such as cytokines and brain neurochemical systems have been evidenced in vertebrates. Thanks to these connections, neuromodulation of sleep (in particular, via the serotonergic system) may promote recovery by regulating three components of sleep architecture that allow the generation of fever: increased non-rapid eye movement (NREM) sleep (energy saving), shortened NREM-sleep bout length (reduced heat loss), and decreased REM-sleep amount (shivering permitted).

Parasite Manipulation

In the past 30 years, researchers have looked at the altered behavior shown by infected hosts from a perspective

different from pathological side effects or host compensation. Growing evidence indicates that in several host-parasite systems, the behavioral alterations observed in infected hosts can be viewed as an extension of the parasite's phenotype. Indeed, in several cases such behavioral alterations seem to benefit the parasite at the expense of the host's fitness. Therefore, the ability of parasites to manipulate the behavior of their hosts may have been favored by natural selection. In this line of reasoning, making a fox aggressive is like increasing a mosquito's biting rate, while having an ant firmly grasping the top of a grass exposing itself to be swallowed by a grazing sheep is similar to making an amphipod swimming toward its fish predator. In all cases, the parasite will increase its transmission chances to the next host, either by direct contact (of saliva on the wound), by a zealous vector (biting mosquito or tse-tse fly), or through the suicidal tendencies of its intermediate host. Any specifically altered behavior in the host that increases parasite survival at the expense of the fecundity and/or survival of the host will also qualify as parasitic manipulation.

As for avoidance and resistance behavior, studies exploring of the molecular and physiological basis of parasite manipulation are scarce, although the field is gaining much interest at present. A survey of the studies addressing the molecular or neurophysiological pathways used by parasites to hijack the behavior of their host already reveals some general patterns, such as the involvement of neuromodulators.

Manipulation by Parasites with Direct Transmission

Perhaps one of the most famous examples of behavioral alteration induced by a parasite is the increased aggressiveness of various mammals, such as foxes, skunks, bats, or dogs, infected with the rabies virus. The virus is inoculated via saliva when an infected individual bites a healthy one. During the period of cerebral infection and before the last stage of near paralysis, behavioral changes include signs of agitation and hyperexcitability. Abnormal aggressiveness develops and infected animals lose caution and fear of other animals including humans. The consecutive increased contact opportunities, mainly bites, thus facilitate the transmission of the virus by the saliva of rabid animals.

Among the diversity of ways a parasite can hijack the behavior of its host to facilitate its own transmission, an original strategy has been recently evidenced in a virus infecting the micro-hymenoptera *Leptopilina boulardi*, a parasitoid of *Drosophila* larvae. Infected females of this solitary wasp modify their host-acceptance behavior by increasing their tendency to superparasitize (i.e., to lay eggs in a host that is already parasitized). This behavioral alteration allows for the horizontal transmission of the

virus from infected to uninfected wasp lineage, within the superparasitized *Drosophila* larvae, extending transmission opportunities of the virus beyond vertical transmission.

Manipulated Vectors

Many studies have reported behavioral modifications in dipteran vectors infected with the mature transmissible stage of protozoa. The most frequently reported behavioral change induced by vector-borne parasites is the alteration of probing or biting behavior. The tse-tse fly *Glossina palpalis gambiensis* infected with the protozoa *Trypanosoma brucei brucei*, the sand fly *Lutzomyia longipalpis* infected with the protozoa *Leishmania*, and the mosquito *Anopheles gambiae* infected with the protozoa *Plasmodium falciparum* all increase probing or biting rate. These changes in feeding behavior include an increase in vector biting persistence (refeeding after interruption) and/or in feeding on multiple hosts; they specifically arise when the parasite has become infective to humans. In addition, human beings infected with the *Plasmodium*-stage infective to mosquitoes are more attractive for mosquitoes than those uninfected or individuals infected with asexual noninfective stages. Proteomic studies on mosquito-*Plasmodium* and tse-tse fly-trypanosome models have revealed a differential production in the head of infected vectors of proteins playing a crucial role in metabolism, neurotransmitter synthesis, cell-signaling pathways, and stress response (chaperones).

Manipulated Intermediate Host and Increased Trophic Transmission

Many parasites with complex life cycles bring about behavioral alterations in their intermediate hosts that appear to make them more vulnerable to predation by appropriate final hosts. For instance, crustacean amphipods infected with parasites that complete their life cycle in a fish, such as some trematodes or acanthocephalans, are typically attracted to light whereas uninfected individuals avoid lighted areas. Presumably, this reversed phototaxis makes infected amphipods more exposed to predation by fish. By contrast, amphipods infected with bird acanthocephalans do not show much alteration in their response to light, but show reverse movement in response to the stimulus of gravity. Swimming closer to the surface, they become more vulnerable to predation by waterbirds. Parasites may also directly affect the antipredator behavior of their hosts. For instance, it has been shown that *Gammarus pulex* amphipods infected with the fish thorny-headed worm *Pomphorhynchus tereticollis* become attracted to the odor of fish predators, whereas uninfected individuals are typically repulsed by the same stimulus. Similarly, rodents infected with the protozoa *Toxoplasma gondii* selectively lose their aversion to, and

fear of, cat pheromones. The consecutive increased vulnerability to predation by cat helps the parasite to reach its final host. This fatal attraction is not part of a parasite messing with its host neurophysiology, since infected rodents respond normally to other odors and no other behavior such as learned fear, anxiety-like behavior, or nonaversive learning is affected.

Manipulated Host and Increased Parasite Survival

In a few cases, parasite-induced alterations in host behavior do not contribute to transmission, but increase parasite survival. It is known that some parasites benefit from physically castrating their host, as the resources normally invested in reproduction by the host then remain available for the parasite to grow and mature. Parasites may also induce behavioral castration of their host. The infected hosts then reduce their courting activity or appear less inclined to engage in sexual activity. Such behavior can increase an intermediate host survival by decreasing predation risks associated with sexual activity, a pattern evidenced in the amphipod *G. pulex* infected with fish acanthocephalans. Similarly, *Plasmodium* oocytes (the nontransmissible developmental stage of the parasite) decrease the biting rate of its vector *A. gambiae* by decreasing the mosquito's motivation to bite. This behavior is exactly opposite of the manipulated feeding behavior seen above, when the parasite has reached the transmissible (sporozoite) stage. As biting is a major mortality risk for a mosquito, decreasing biting rate increases the probability of the vector-parasite pair to survive until transmission and manipulation can occur. Some parasites face evenmore risky interactions with their hosts. Parasitoid wasps must overcome the defensive behavior of their host upon stinging. One can imagine how risky approaching and stinging a host as large as a cockroach or a mantid can be for a parasitoid female at least 2 times smaller yet highly motivated to deposit an egg on it. Some fine-tuned strategies have evolved to control the host right from the first touch. The sphecid wasp *Ampulex compressa* first stings its cockroach target in the thorax, rendering the prothoracic legs transiently paralyzed. This leaves the wasp at ease to make a much more precise and time-consuming second sting through the neck into a specific part of the brain. The venom directly injected into the CNS induces a long-lasting lethargic state in the submissive but living cockroach, ready for consumption by the growing wasp larva in the burrow. A fairly astonishing case of behavioral manipulation has been described recently in the braconid wasp (*Glyptapanteles* sp.). This parasitoid wasp uses its caterpillar larvae host as a body-guard protecting the wasp pupae from predator attack. Several examples of parasitic manipulation investigated

so far suggest that the parasite interferes with host endocrine and neuromodulatory functions.

Conclusion

Behavior can be a major cause of the host's susceptibility toward or defense against infection, or of the parasite's transmission. However in most cases, there is an evolutionary trade-off between opposing selective forces upon the host reproductive success and the disease risk or consequences. Such compromise is expected to shape the host's behavior.

Besides, most of the time, it is a component of diversity of host traits that will determine encounters with and susceptibility to parasites, in conjunction with environmental factors and the parasite's transmission strategies. The intimate interaction that characterizes the partnership between host and parasite is multidimensional, in that the outcome of their molecular, cellular, and physiological cross-talks is expressed in several components of the host's life history, including behavior.

See also: Active Avoidance and Escape Learning; Analysis of Learning in Invertebrates; Animal Models of Learning and Memory; Cytokine Effects on Neuronal Processes and on Behavior; Neurobiology of Opioid Addiction.

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Primate Origins of Human Behavior

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Glossary

Culture – Information produced by innovation, acquired from others through social learning (e.g., by observation or imitation), and shared by a group of individuals.

Exogamy – Literally, out-marriage, or marrying out of one's group, however the group is defined: families, villages, clans, tribes, nations, or others.

Homologous traits – Variants of the same trait present in two or more species and derived from the species' common ancestor.

Homoplasious traits – Similarities that evolved independently in distinct species as responses to similar selective pressures.

Human nature – Humankind's psychic unity. The universal biological constraints affecting human thought and behavior and transcending cultural differences.

Philopatry, male – A primate residence/dispersal pattern in which males remain in their natal group for life, whereas females move to another group around puberty.

Polygyny – A mating arrangement in which a male forms stable breeding bonds with two or more females simultaneously.

Sexual promiscuity – A mating arrangement in which both sexes have short-term mating relations with several partners.

Symbolic capacity – The ability to use arbitrary signs (sounds, gestures, pictures, etc.) to represent any aspect of the reality (objects, actions, emotions, concepts, etc.).

cumulative integration of more elementary units over time. As was long remarked by Charles Darwin in his book *The Descent of Man*, "if no organic being excepting man had possessed any mental power, or if his powers had been of a wholly different nature from those of the lower animals, then we should never have been able to convince ourselves that our high faculties had been gradually developed." Comparative behavioral primatology is the single most informative means at our disposal for reconstructing the evolutionary history of human behavior. Other disciplines are certainly helpful for this purpose but they present serious limitations: the paleontological study of fossilized hominids is basically silent about the evolution of most aspects of social behavior and mental processes, while archaeology – the study of the artifactual remains of hominids – does not go back in time far enough.

Homologies and Homoplasies

One basic principle underlying the use of nonhuman primates to probe the origins of human behavior is as old as evolutionary theory itself: a trait may exist under various versions in species that have inherited it from a common ancestor. Variants similar through common descent are called homologies. The concept of homology has been used by generations of comparative anatomists to infer degrees of relatedness between species and, on this basis, to reconstruct their evolutionary tree. Presently, it is used by molecular biologists to reconstruct phylogenies based on deoxyribose nucleic acid (DNA) or amino acid sequences. However, it is also possible to reverse the reasoning and infer homologies from phylogeny. Knowing the phylogeny of a group of species, one may – for any given trait – identify similarities based on common descent and gain insights into past stages in the evolution of the trait. This is particularly useful for reconstructing the evolutionary history of social behavior, which leaves very few traces in fossils. Behavioral homologies were analyzed systematically by ethologists beginning in the 1940s. Ethologists were primarily concerned with the evolution of stereotyped behavioral sequences, such as courtship displays in insects and birds. However, any behavior-related phenomenon – provided it has a genetic basis – may exist in homologous forms in related species, including patterns of social

Human behavior is an all-inclusive and extremely heterogeneous category of phenomena. It includes relatively simple motor patterns such as reflexes, facial expressions, and modes of locomotion; however, it also comprises complex social phenomena like marriage patterns, political alliances, kinship systems, war, and rituals. It is with the evolutionary origins of such higher-order behavioral entities – and with the related methodological and epistemological issues – that we shall be concerned here. It is not immediately obvious that the study of nonhuman primates should have anything to contribute to our understanding of what appear to be uniquely human forms of social behavior. However, as with complexity in general, social complexity results from the

interactions, cognitive abilities, emotions, motivational systems, and developmental processes.

Another basic concept for assessing the origin of human behavior is that of homoplasies. These are inter-specific similarities based on common adaptive function and resulting from similar selective pressures. Functional similarities between the human and other animals which cannot be explained in terms of descent from a common ancestor may be homoplasious. Homoplasies provide information about the ultimate causes responsible for the very origin of a human trait. Indeed, if the adaptive function of a trait observed in a number of different species is well established, it is possible to infer the same initial function for the human form of the trait.

Beyond Homologies and Homoplasies

That said, reconstructing the origins of human behavior involves much more than the straightforward identification of homologous or homoplasious traits. This might be the case when one is concerned with well-circumscribed motor patterns like communicative signals and facial expressions. For example, Jan van Hooff described the separate phylogenetic origins of laughter and smiling from, respectively, the relaxed open-mouth display (or play face) and the silent-bared-teeth display of nonhuman primates. In such a situation, the homologous portion of the expression may be relatively easy to recognize across species – in the same manner, for that matter, that anatomical homologies are recognized.

But owing to the complexity and variability of human behavior, homologous and homoplasious components usually do not reveal themselves so easily. Human behavioral entities such as sexual competition for mates, kin favoritism, conditional reciprocity, intergroup hostility, monogamy, or the sexual division of labor are composite traits that express the interplay of a large number of mental and social processes. Accordingly, the comparison of any human behavioral phenomenon with its primate counterparts typically results in the phenomenon breaking down into a number of components – or building blocks – that have different evolutionary origins. First, some components may have homologous counterparts in other primates – either in our closest relatives exclusively, or in a larger taxonomic fraction of the primate order, and even beyond. Second, some aspects may have homoplasious counterparts in other primates, in which case the similarities are observed in certain species but not in all of those intervening between the human and the species exhibiting the trait. Third, a significant number of components may be neither homologies nor homoplasies, but ancillary consequences – or emerging properties – of other traits merging together, as will be illustrated later on. Fourth – and here lies the single most significant peculiarity of human behavioral evolution – many aspects

of the phenomenon are bound to be cultural innovations and, moreover, innovations that take various forms across groups. Indeed, human behavioral traits are the product of the intricate amalgamation of biological components with cultural innovations over evolutionary time – a process which accounts for the interminable and often acrimonious debates that have marked the evolution of the social sciences over the past centuries

Culture

Culture as a Biological Phenomenon

To better appraise the problem and its solution, it is useful to look at culture from an evolutionary perspective. A basic characteristic of behavior is that it is most often modifiable by learning – the exceptions forming the category of instinctive behaviors. In group-living species, individuals may learn from their own experience on the basis of various associative processes, but they may also learn by observing others, that is, through social learning processes such as observational conditioning, goal emulation, and imitation. The capacity for social learning is the most basic prerequisite for the possession of culture by a species. If a new behavioral sequence is invented by an individual, if it is adopted by other individuals through social learning, and if it generalizes to the whole group, it becomes part of that group's cultural repertoire. Species other than humans have the capacity to copy the innovations of others and hence have cultural repertoires. These are mostly composed of environment-related techniques – for example, termite fishing and nut cracking in chimpanzees – and amount to cultural traditions differing between groups. Social learning is thus basically adaptive: it allows animals to bypass trial-and-error learning and benefit from the inventive capacity, or chance discoveries, of others. Stated otherwise, culture itself is a biological phenomenon.

The Symbolic Step

If culture – as defined earlier – is relatively common in animals, symbolic culture is uniquely human. The evolution of the symbolic capacity in the course of human evolution deeply enriched the basic phenomenon by providing it with an extremely powerful medium. The use of arbitrary symbols – whether they were initially gestural, pictorial, or vocal – to refer to and communicate about objects, techniques, feelings, ideas, intentions, past events, and relations between things considerably widened the domain of what could be learned and transmitted through social learning. The evolution of language – a symbolic form of vocal communication – in the course of human evolution had at least four major consequences on cultural transmission. First, it amplified the repertoire of cultural

innovations to an extraordinary extent. In addition to physical techniques, innovations henceforth included, for example, verbalized ideas and stories. Second, language created a particularly effective medium for the long-term storage of information and its transmission across generations – a medium initially based on oral memory. Third, it greatly accelerated social evolution because symbolically-mediated ideas generate further innovations through associative processes and do so cumulatively, if not exponentially. Fourth, as described below, language considerably amplified cultural differences between groups, setting the stage for a ‘culturalistic’ – or abiological – conception of human behavior.

Culture and Human Nature

The impact of the symbolic capacity on culture is so important that to a majority of social scientists culture is a phenomenon more or less independent of the biological constraints set by human nature. The problem lies in the extent of cultural variation. Culture builds from the vagaries of individual innovations and evolves cumulatively. As a result, social practices are extremely variable both in their form and meaning cross-culturally, so much so that ‘social universals’ are practically absent in the human species – as has been amply documented by anthropology. The concept of marriage, for example, subsumes such a wide array of forms, practices, and beliefs across cultures that, after decades of ethnographic research and theoretical discussion, there is still no unanimity among anthropologists on how to define marriage. One example suffices to illustrate the issue. In some cultures, a sterile woman may marry another woman and have children with her through a male genitor. From then on the sterile woman is considered a man and called father by her children. The accumulation of innumerable such examples over the last century led a majority of social scientists to the view that the extent of cultural variation constituted solid evidence that the influence of human nature on social behavior was necessarily marginal and limited to a few basic drives and emotions. Accordingly, some authors went so far as to treat culture as a superorganic phenomenon that obeyed its own laws, independently of human nature.

The autonomy of culture in relation to human nature is a deceptive appearance. Cultural variation conceals human nature, and very effectively so, but it has not erased nor marginalized it. Human nature is buried beneath thick layers of cultural diversity, and here lies the relevance of the primate data for understanding our origins. Stepping out of human societies and looking at them from the perspective of other primate societies, differences between cultures give way to similarities between them, and our species looks remarkably

homogeneous. It is with the origins of the very content of this homogeneity that comparative primatology is concerned.

Primate Frameworks for the Origins of Human Behavior

The human reproductive system provides a useful example. All aspects of the phenomenon – from marriage type and the exact composition of domestic groups to the sexual division of labor – vary to a considerable extent cross-culturally. Moreover, all aspects are governed by cultural norms and institutionalized. To many social scientists, this implies that the phenomenon has little, if anything, to do with human nature. Primatology, however, belies such a view.

The Human Reproductive System

From a primatological perspective, the human mating system combines two major features: enduring breeding bonds and sexual promiscuity – short-term mating relations with several partners. Stable breeding bonds take the form of monogamous marital unions, or polygynous ones (a man paired simultaneously with two or more women). Polygyny’s mirror system, polyandry, is extremely rare in the human species. The second feature, sexual promiscuity, is practiced either premaritally – in which case it is most often accepted – or postmaritally in which case it is universally disapproved of. Stable breeding bonds and some levels of sexual promiscuity consistently co-occur in all human societies.

Stable breeding bonds appear to be parental partnerships based on a sexual division of labor between the father and the mother. Two major correlates of this view are verified. First, the costs of raising children are disproportionately high in our species owing to mothers feeding slow-maturing offspring well into adolescence. As a result, human mothers must feed more than one child at a time; for example, they may be suckling one child while provisioning two others. Nonhuman primate mothers never have to feed more than one offspring at a time. Second, ethnographical evidence indicate that the father (among other individuals) does alleviate the costs of maternity, principally through provisioning his family. Taken together, the two points are compatible with the view that marital bonds are, in effect, cooperative breeding units. Notwithstanding this, primate studies suggest that the human family did not initially evolve for that purpose. Stable breeding bonds in other primates appear to reflect mating strategies rather than parental strategies – a fact that suggests that the human family was a mating arrangement before it became a parental partnership.

Some theory is needed at this point. Owing to the fact that females do the bulk of parental care in mammals they are physiologically limited in the rates at which they may produce offspring. In contrast, the males' contribution to reproduction is most often limited to insemination, so that males who attempt to maximize the number of females they mate with do increase their reproductive success. For this reason male competition for females is much stronger than female competition for males, and polygyny is the basic principle governing mammalian mating systems, including primates. Notwithstanding this so-called polygyny pressure, males cannot simply do as they wish. Ecological factors such as the abundance and distribution of food impose strong constraints on the spatial distribution of females, hence on the capacity of males to monopolize them. Only in situations in which females forage alone or in small groups is a male in a position to monopolize either a single female – the outcome being some form of monogamy – or more than one female (polygyny). As a result, stable breeding bonds in mammals are best construed as ecologically constrained mating arrangements. Not surprisingly therefore, direct paternal care and parental collaboration are absent in many mammalian species that form stable breeding bonds. Applied to the human case, this reasoning suggests that stable breeding bonds evolved initially as mating arrangements, the latter operating as pre-adaptations for the evolution of paternal care – fathers provisioning their family – when the costs of maternity began to increase in the course of human evolution.

In multimale–multifemale primate groups, males compete aggressively for females within the framework of a dominance hierarchy. However, from the time fathers began to provision their family, the old primate heritage of male rivalry came to encompass new objectives – notably the control of resources valued by mothers. Such a situation was conducive to the formation of new types of social rankings in which a male's position was determined not so much by his strict physical prowess (dominance status) as by his capacity to control or produce valued resources (socioeconomic status). Female interests thus had a major impact on the nature and object of male competition. A closely related phenomenon is that nonhuman primates often compete through the use of coalitions. By allying with others they may overthrow higher-ranking individuals or maintain their position above lower-ranking challengers. The novel human mating system – with its emphasis on the control of resources – afforded males with new means and incentives for alliance formation. Sex and reproduction lie at the heart of human politics because they do so in other primates.

Primate frameworks such as the one adumbrated here are informative about the portion of human nature that we owe to our primate heritage. They shed light on human nature as it was before it got blurred

through the multitude of its cultural expressions. Human mating systems are certainly governed by cultural norms, but all such norms are derived from the same biological foundation, and constrained by it. When trying to make sense of these norms, one can only benefit from knowledge about their common biological basis.

The Incest Taboo

The incest taboo provides another instructive example. All human societies have rules proscribing sexual relations between close relatives. However, beyond the universal proscription of incest between parents and offspring, grandparents and grand-offspring, and between siblings, the types of unions considered incestuous vary extensively across cultures. To a majority of social scientists, the very diversity of incest rules would indicate that the incest taboo is a cultural construct that has nothing to do with human nature. On the other hand, all known primate species also avoid mating with their close kin, a fact that strongly suggests that the human and nonhuman phenomena have a common biological foundation.

An empirically based primatological framework of incest avoidance for human evolution includes the following principles. (1) Nonhuman primates recognize their kin, in the sense that they treat them preferentially. The domain of kin recognition includes the mother (and sometimes the father, depending on the mating system), offspring, siblings, grandparents, grand-offspring, whereas aunts, nieces, and nephews are often part of a grey zone. (2) Nonhuman primates do not recognize genetic relatedness *per se* but recognize its correlates such as high levels of developmental familiarity and, perhaps, physical similarities. (3) Inbreeding among close kin reduces an organism's reproductive success by lowering its fecundity and/or its offspring's chances of survival; incest avoidance is thus adaptive. (4) As a rule, individuals disperse from their birthplace or natal group around puberty – a pattern that considerably limits opportunities for incest. (5) Among sexually mature individuals that reside in the same group, mating is nonetheless avoided among kin that are part of the domain of kin recognition. When sexual interactions between kin do occur, they are performed mostly by immature individuals and in a behaviorally atypical manner. (6) Incest avoidance appears to be mediated mainly through the familiarity correlate of close kinship bonds. In 1891, the sociologist Edward Westermarck described this effect in the human species by noting that persons raised together from childhood developed 'a remarkable absence of erotic feelings' for one another. The so-called 'Westermarck effect' – an expression coined by Robin Fox – is at work in primates in general. (7) Male primates seem less motivated than females to avoid incest. This accords well with the fact that the costs of reproductive failure are higher for a female than for a male. (8) Sexual

avoidance between close kin was clearly documented among homosexual female macaques, indicating that developmental familiarity inhibits both heterosexual and homosexual sexual activity.

In all likelihood, these principles – and many others awaiting investigation – were part of humankind's primate heritage and constituted the universal substrate upon which hominids elaborated symbolically mediated rules about incest avoidance. Thus, the diversity of cultural rules about incest, rather than negating the universal biological foundation of the incest taboo, indicates that hominids built upon a basic feature of human nature and adjusted it to various subsistence economies, marriage practices, kinship systems, and so forth. Implied here, rules and morality – a trademark of human society – originated in the behavioral and social regularities of our primate forebears and later grew in complexity in the symbolic realm.

The Primate Origins of Human Society

Several articles in this encyclopedia describe the animal bases of human behavior in various areas – altruism, cooperation, competition, reproduction, parenting, social cognition, and so forth – and provide, implicitly, general frameworks for characterizing human nature. However, comparative primatology may also serve to enlighten the evolutionary history of human society as a whole. This is a topic that deeply intrigued early philosophers and social scientists and led to highly speculative schemes about the society of the 'primeval ape-man,' before it was abandoned early in the twentieth century following harsh criticisms from more empirically grounded anthropologists. The progress of behavioral primatology in the second-half of the twentieth century provides the needed empirical evidence and allows one to tackle this old question anew. What does characterize human society in relation to all other animal societies, and how did this specific configuration of traits evolve? Bernard Chapais recently proposed a comprehensive model addressing these issues, which is summarized below.

Human Society from a Primatological Perspective

The modal composition of human groups is the community of conjugal families, or multifamily community, a rare form in the animal world which combines two elements: a multimale–multifemale composition (several males and females living together on a long-term basis) and stable breeding bonds (families). Other primate species do form families, or multimale–multifemale groups, but most often the two features do not co-occur in the

same species. Another prominent feature of human social structure is its nested or multilevel dimension. Any multi-family community is an integral part of higher levels of social organization – for example, villages may belong to the same band, bands to the same tribe, and so on. In other primates, in contrast, groups are autonomous entities; chimpanzee communities, for example, are not part of any supracommunity social structure. The ethnographic record indicates that the most basic process binding human groups together is exogamy – literally, marrying out of one's birth group. As emphasized long ago by the anthropologist Claude Lévi-Strauss, marriage is universally a means of alliance formation. Marriage binds the spouses' respective families (in-laws) and creates new kinship bonds between the intermarrying groups – for example, parents have grandchildren living in the group in which their daughter transferred and married. In short, exogamy generates intergroup alliances based on kinship. As far as the evolution of human society is concerned, therefore, understanding the origin of exogamy and between-group alliances – hereafter the exogamy configuration – is the central issue.

Figure 1 portrays the phylogenetic relationships of the human species and of other anthropoid primates. This phylogeny is the likeliest one based on several sets of molecular data. It indicates that the two chimpanzee species (*Pan* genus) are more closely related to humans (*Homo*) than they are to gorillas. Interestingly, on structural grounds the exogamy configuration appears to be the product of the combination of two sets of elements – namely, the basic characteristics of chimpanzee society and a new mating system based on enduring breeding bonds. This view is compatible with the hypothesis that the society of early hominids, immediately after the *Pan-Homo* split, was chimpanzee-like in its broad outline – an assumption supported on phylogenetic, anatomical, and socioecological grounds. Chimpanzees and bonobos form multimale–multifemale groups in which mating is promiscuous, and so did early hominids, presumably. When stable breeding bonds did evolve in the hominid lineage, this produced the modal composition of human groups – the multifamily community – and set the stage for the evolution of a primitive version of the exogamy configuration as is now briefly explained.

Chimpanzees and bonobos are markedly territorial and exhibit male philopatry – males breed in their birth group whereas females emigrate around puberty and breed in other groups. In turn, male philopatry determines the group's genealogical structure: males live with their father, sons, brothers, uncles, and other paternally related kin, but not with their mother's kin. However, although patrilineal kin live together they do not appear to recognize each other on this basis. Mating being promiscuous, paternity is not recognized in any way comparable with maternity recognition, which is based

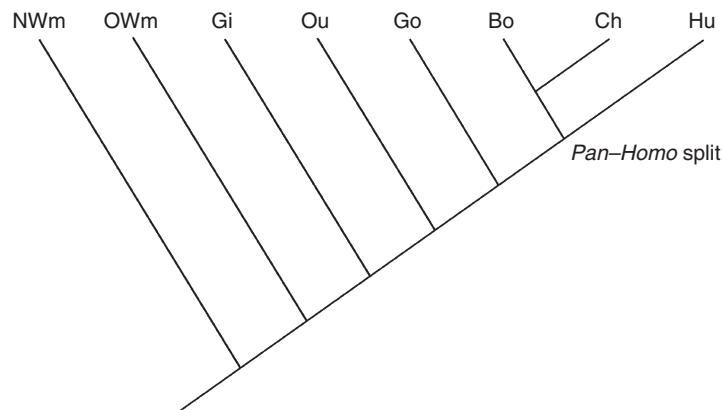


Figure 1 Phylogeny of anthropoid primates, one of the two suborders of primates. (Prosimians form the other suborder.) A phylogeny depicts the evolutionary relationships among species or larger taxonomic units such as genera or families. Intersections between two lines indicate speciation events – the divergence of two daughter species from a common ancestor. Hu: Humans. Ch: Chimpanzees. Bo: Bonobos. Go: Gorillas. Ou: Orangutans. Gi: Gibbons. OWm: Old World monkeys, which include cercopithecines (macaques, guenons, and other species) and colobines (guerezas, langurs, and others). NWm: New World monkeys (howlers, spider monkeys, and other species). The *Pan-Homo* split denotes the speciation that led to the separate evolution of the hominid (or hominin) lineage comprising the genera *Homo* and *Australopithecus* among others, and the chimpanzee lineage (*Pan*) comprising common chimpanzees and bonobos. The *Pan-Homo* split is dated between 6 and 7 million years.

on long-term, intimate bonds between mothers and offspring. If the father is not recognized, the father's kin cannot be either. Thus, chimpanzees and bonobos have a group-wide patrilineal kinship structure, but a socially silent one, and so would have early hominids. Notwithstanding this, chimpanzees and bonobos recognize their mother and their maternal siblings.

Stable Breeding Bonds as the Key to Human Society

Whatever their exact origins, stable breeding bonds had far-reaching consequences on human society. First, their integration to the pre-existent pattern of female transfer created a primitive form of exogamy. Indeed, from then on a female still moved into another group as before, but instead of mating promiscuously in it she formed a stable breeding bond. She was thus 'marrying-out' of her birth group and practicing a behavioral form of exogamy – one deprived of any exchange dimension or between-group agreement. According to this view, marital unions and the widespread practice of women-exchange by men would be cultural elaborations derived from this basic pattern. A further consequence of stable breeding bonds is that they revealed the hitherto silent kinship structure. Long-term associations between fathers and offspring enabled them to develop preferential bonds with each other – paternity recognition – while simultaneously making it possible for children to recognize their father's relatives, including their paternal grandfather and uncles. Stable breeding bonds generated what anthropologists long ago described as the most basic factor organizing social relationships in simple human societies: a group-wide kinship structure.

The extension of the domain of kin recognition, in turn, might have set the stage for the pacification of intergroup relations. Chimpanzees and bonobos are territorial. They avoid other groups and may even attack strangers. Again, presumably, so did early hominids. However, once kin recognition among paternally related kin had evolved, fathers, upon coming into contact with another group, could recognize their daughters and their newborn offspring – their grand-offspring – and refrain from attacking them. They could also recognize the preferential bond between their daughter and her 'husband' (their son-in-law) – a factor lowering the probability of conflicts between, this time, adult males. Similarly, grandfathers, brothers, and uncles could recognize their female kin and their in-laws living in other groups. In sum, a state of mutual tolerance based on kinship bonds would prevail between 'intermarrying' groups, a state that was possibly the phylogenetic antecedent of between-group alliances, or regional tribes.

Building upon this unique biological foundation and making use of their symbol-based capacity for culture, hominids have ever since been innovating in all areas of the social sphere, creating in the process a vast array of kinship systems and social structures in different physical and social environments. The upshot is that all such cultural variants, however peculiar, bear the stamp of humankind's stem social system. This reasoning illustrates the general principle underlying the solution to the old nature–culture opposition: culture certainly generates a tremendous amount of behavioral diversity, but it does so out of a universal set of biological constraints. Human nature is everywhere in cultural diversity, sometimes conspicuously but often

discreetly. If the neural and behavioral sciences are uniquely positioned to reveal the workings of human nature, only comparative primatology may shed light on its evolutionary history.

See also: Behavior Adaptation and Selection; Behavioral Development and Socialization; Brain Evolution in Vertebrates; Cooperation; Mating Behavior; Social Communication; Social Competition and Conflict Resolution; Social Cognition: From Behavior-Reading to Mind-Reading; Social Learning and Behavior Transmission; Social Relationships and Social Knowledge.

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Social Communication

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Glossary

- Coevolution** – The reciprocal selective pressures between species affect each others' evolution. Coevolution can thus be defined as covarying traits between different species in an environment. The evolution of prey–predator interactions constitutes an example of coevolution.
- Noise** – In the physical world, a noise is any time-varying or spatial-varying nonmeasurable quantity. It is an unpredictable event. In information theory, a noise is an undesired disturbance that may distort the information carried by a signal.
- Signal** – In the physical world, a signal is any time-varying or spatial-varying measurable quantity. It is a predictable event. In information theory, a signal is a codified message that carries information.

Communication is an important part of the behavioral repertoire of an animal. When the same individual both sends and receives any given signal, the process is called autocommunication. Echolocation (as used by bats and cetaceans) and electrolocation (as used by mormyrid and gymnotid fishes) correspond to this definition. Social communication involves at least two individuals. This process cannot occur in isolation and is consequently an inherently social behavior. It is the glue that holds animal society together. In general, sociality goes hand in hand with sophisticated communication. Sociality has not only several major advantages (e.g., numerous potential sexual partners, mutual aid for foraging activities, and better predator detection) but also some disadvantages. For example, it is well known that colonial breeding improves offspring care through communal protection. What is less known is the increased confusion in finding a young one or a mate. It is thus crucial for each individual to ensure an accurate identification. In animals, recognition between individuals is involved in the establishment of sexual and social relationships. In many cases, the ability to identify members of its own species and of different sex is essential to ensure the reliability of mating processes and reproduction. The discrimination between individuals of various social ranks or belonging to different social groups, between kin and nonkin, or between kin related by varying degrees can be of great interest in the context of sociality. Thus, the primary role of

communication signals is to ensure species, group, sex, or individual identification. Naturally and particularly within social groups, communication signals may also transmit information about prey detection, predator defense, spatial positioning, synchronization of activities, and hierarchy establishment within social groups. Finally, they can even reflect the motivational state of individuals.

Transmission of Information

According to the mathematical theory of information by Shannon and Weaver, the principle of animal communication could be summarized as follows: an emitter codes a message into a signal that propagates through the environment and that is transmitted to a receiver who decodes it to formalize a message (see **Figure 1**). This process corresponds to the sending of information. At the receipt of this information, the receiver reacts by modifying his behavior and consequently by sending back information. This exchange of information constitutes a communication process.

Several messages can be simultaneously encoded in the same signal. For example, numerous male birds use songs to delimit the boundary of their territory. A human translation of the message of the song should be as follows: "I am a territory owner and I am looking for a female." Besides this message, the bird provides numerous other information, principally about its species, sexual and individual identities, about its geographic location (the geographic variations of the songs are termed dialects, as for the human language), and about its motivational state.

When sending the signal, the emitter transmits a given volume of information and the receiver collects a part of this volume. The loss of volume between the information sent and received is due to the noise that impairs the system at different levels: at the level of the coding (bad motor control), propagation (bad transmission), and decoding (bad sensory reception). Thus, the semantic of the message sent and of the message received can differ, and the communication process will remain effective only if the parameters coding information are transmitted reliably between the emitter and the receiver. For example, for a sound, the volume of information is $V = FT\gamma$, with F representing the frequency bandwidth, T the duration, and $\gamma = \log_2(1 + S/N)$, S/N being the signal-to-noise ratio. If the sound signal propagates through a forest, the frequency bandwidth decreases due to selective filtering

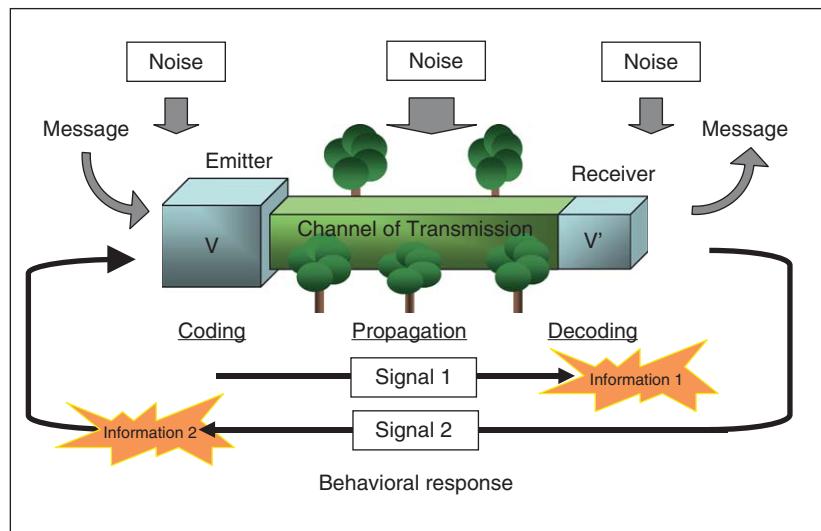


Figure 1 Principle of animal communication. A communication corresponds to an exchange of information (here signal 1 and signal 2) between an emitter and a receiver. In the process of being transmitted, different sources of noise and the properties of the channel of transmission are responsible for the decrease, which occurs between the volume of information sent (V) and the volume of information received (V'). This decrease affects the accuracy of the message finally received. Adapted from Shannon CE and Weaver W (1998) *The Mathematical Theory of Communication*. Chicago, IL: University of Illinois Press.

by obstacles (leaves, trunks) and the signal-to-noise ratio decreases due to the absorption and to the background noise of the environment. As a consequence, the volume of information collected by the receiver is weak. It can be so weak (e.g., in case of great emitter–receiver distance) that the message sent by the emitter will not be decoded by the receiver.

Sensory Channels

As seen above, communication implies exchange of signals between emitters and receivers. These signals can be light, sound (or vibration), chemistry, and electricity. The most widespread sensory channels used by animals to communicate are vision, hearing, and smell/taste. Other senses such as electrical, touch, or vibration perception are less common. Each signal modality has inherent properties, such as propagation speed, directionality, persistence, and resistance to obstacles. Thus, each sensory modality presents defaults and qualities that can be exploited by animals to establish efficient communication. Visual, acoustic, and even chemical signals operate at a long range contrary to tactile signals. The latter signals require a contact between two animals, and they are mostly used for reproduction, parental care, predation, and defense. Chemical signals have the particularity of being persistent, which can be advantageous (i.e., homing by tracks) or not (i.e., detection of the track by a predator). Chemical cues are used in a wide range of species, but mainly in insects and mammals. While chemical signals

are often emitted continuously during a given period, visual, acoustic, and tactile signals can be transmitted in temporally discrete sequences. Acoustic signals appear to be perfectly adapted for darkness or obstructed environments, and so they are intensely used by deep-sea fishes, nocturnal and forest birds, bats, and cetaceans. Contrary to chemical signals, visual and acoustic signals are rapidly transmitted, which may be an advantage with respect to localization for animals that move quickly in a three-dimensional space (e.g., flying insects, fishes, birds, and marine mammals). Thus, different sensory systems can participate in the communication processes, depending on the context and on the animal. Some communication processes require different senses that act in synergy. For example, identification of the mate or of the young requires olfactory and acoustic signals in shearwaters and seals (see [Video clip 1](#)) and identification of the species and of the sex requires tactile, visual, acoustic, and chemical signals in fruit flies *Drosophila melanogaster* ([Video clip 2](#)). In birds, selection by a female of a mate in arenas or leks associates the visual and acoustic signals displayed by males. Such communications are referred to as multimodal.

Physical and Biological Constraints

Animal species communicate within their own sensory worlds, and different species perceive their environment differently. The physical characteristics of the signals produced depend on the capabilities of their motor and

sense organs. For example, only big animals (e.g., elephants and whales) produce infrasound, and this ability is related to the sizes of their vocal and respiratory tracts. Among vertebrates, only some birds are able to simultaneously produce and modulate independently two frequencies. This process, called the two-voice phenomenon, is due to the low position of the vocal apparatus, the syrinx, on the two bronchi. Many insects, fishes, and birds are sensitive to ultraviolet light and to the polarization degree of light. This is not the case for mammals. Thus, physiological capacities of motor and sense organs define the physical and chemical properties of the signals used by a species.

As mentioned previously, the environment can be a source of noise (in the theory of information sense of the word) for communication. During propagation through environment, signals are subjected to attenuation and various other modifications, which decrease the reliability of information transfer. Physical or biological environmental noises can jam the signals and deeply modify their physical characteristics. In the acoustic domain, four different processes resulting in sound degradation can be identified: spherical spreading of sound energy, atmospheric absorption, scattering by heterogeneities of the propagation channel (e.g., physical obstacles, temperature gradients, and humidity), and boundary interference (interaction between direct and reflected sound waves). To these processes may be added the background noise due either to features of the habitat such as wind, ocean flow, and running water or to biological features such as the vocalizations of other animals. As a consequence, the signal arriving at the receiver is modified in the temporal, frequency, and intensity domains, and the information is degraded (see **Figure 2**). The

transmission of visual signals from emitter to receiver involves many of the same problems as sound transmission, such as attenuation, shape loss, and background noise. The main difference is perhaps the fact that light waves are more affected by obstacles since they cannot pass around an object as sound waves do. Indeed, only the sounds with a smaller wavelength than the obstacle's size are reflected. The environmental factors affecting the transmission at distance of chemical signals are mainly the properties of the current air or water flow of the habitat, and particularly its direction, speed, and type (laminar or turbulent). For deposited scent marks, other environmental factors, such as temperature and humidity, and physical and chemical characteristics of the substrate affect signal emissivity and longevity.

In some cases, the external constraints are very high and, by analogy with extreme arid or salty biotopes, the environment can then be considered as extreme for communication. For example, the absorbent environment of tropical forests or the noisy environment of bird colonies can be considered as extreme for acoustic communication.

Communication Strategies

Constraining environments constitute a strong selective pressure potentially driving strategies optimizing the reliability of communication processes. In this perspective, signal structure hypothesis predicts that the structure of animal signals will differ depending on the general features of the habitat. For example, again in the acoustic domain, bird songs optimized for long-range propagation in a forest should be as low in frequency as the sender can efficiently produce and slowly modulated in frequency. In

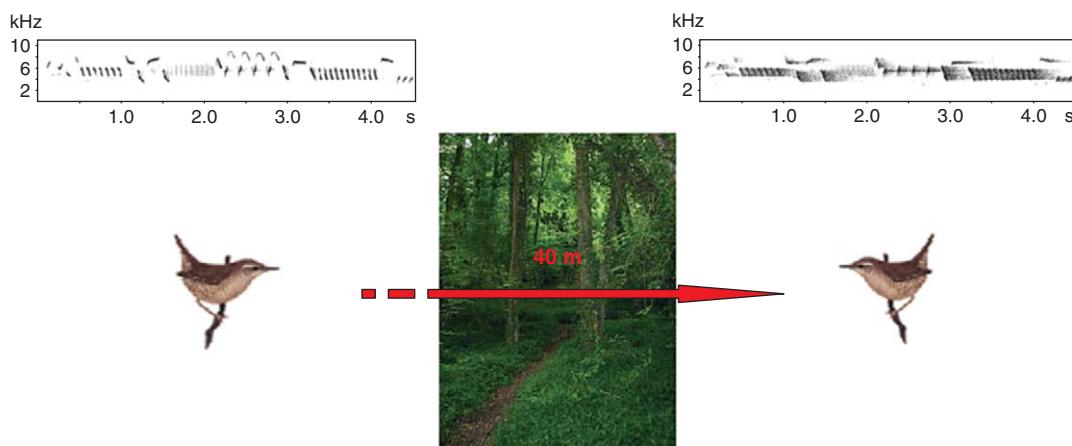


Figure 2 Effect of propagation through a forest habitat on the territorial song of the European wren *Trogolodytes troglodytes*. Spectrographic representations of songs recorded after a propagation of 1 m (left) and of 40 m (right). The effects of reverberations and high-frequency filtering are conspicuous in the 40 m propagated signal. Adapted from Mathevon N and Aubin T (1997) Reaction to conspecific degraded song by the wren *Trogolodytes troglodytes*: Territorial response and choice of song post. *Behavioural Processes* 39, 77–84.

addition, since the susceptibility of emitted signals to propagation-induced modifications depends on their acoustic characteristics, the emitter could enhance or, on the contrary, reduce the active space of its communication by coding information in more or less propagation-resistant parameters. This adjustment of the coding strategies according to the habitat has been experimentally demonstrated for the songs of some territorial forest birds. Due to the variable spacing of territorial individuals, some information may be coded to propagate only over a short distance and some might be coded such that it transmits over a long distance without much degradation. Thus, species-specific identity is encoded in propagation-resistant acoustic features, allowing individual to reach a wide audience. Conversely, individual identity is encoded by song features susceptible to propagation, this private information being reserved for close neighbors. Such processes have also been found in mammals. Pygmy marmosets *Cebuella pygmaea*, for example, emit a highly degradable call when individuals are close and a less propagation-sensitive signal when they are farther apart.

For visual signals, it is the contrast between the emitter and the background that determines the conspicuousness of the emitter. This contrast is based on four properties, which are brightness (or intensity), spectral composition (or color), spatial characteristics (e.g., size and shape features), and temporal variability (e.g., the pattern of movements). To maximize these parameters and thus to increase the signal-to-noise ratio, some animals choose particular light spots to communicate. This is the case of a jungle dancer: the Manakin bird (*Manacus* genus). It is a brightly colored bird of the forests of South and Central America. The courtship display of the male consists of rapid jumps and exhibition of its colored feathers on branches well exposed to sunlight.

In some social groups of animals, communication is particularly subjected to masking effects due to the numerous signals emitted by the conspecifics. In this case, the physical or chemical properties of the masker and those of the signaller are similar. The jamming effect is very important in these conditions of competing noise and increases the difficulty for a given individual to extract the information provided by the others. According to the information theory, an increased redundancy in signal improves the probability of receiving a message in a noisy channel. Experimental results corroborate this prediction. A nice illustration of this phenomenon can be found in penguin colonies. These birds use a particular call, the display call, to identify their mate, their chick, or their parent. Display calls of penguins appear to be highly redundant, consisting of more or less identical successive syllables with the same information, the individual identity, repeated many times. As penguins breed in dense colonies (up to one or two million pairs), calls emitted by other individuals generate

a huge background noise. The noise generated by birds in the colony is almost continuous, and periods of relative silence are short, infrequent, and unpredictable (listen to [Audio file 1](#)). Nevertheless, the redundancy of the vocal signature enhances the opportunity to find a quieter window in the almost continuous ambient noise. Experiments indicate that penguins detection is possible even when the intensity of the focus call is well below (-6 dB) that of the noise of simultaneous calls produced by other adults. This capacity to perceive and extract a given call from the ambient noise, and particularly from the calls of other individuals, is termed the ‘cocktail-party effect’ in speech intelligibility tests. Similarly, the information exchanged between individuals in insect and amphibian choruses is also redundant.

In addition to coding strategies, each animal tries to optimize its communication by managing where, how, and when to emit and how pertinent information can be gained from signals emitted by the others. In forests, the choice by birds of clear perching areas favors propagation of visual and acoustic signals. Numerous behaviors and movements enhance the transmission of chemical signals by spreading the odors or generating air or water current flows. Some insects thus disperse their cuticular pheromones by wing vibration. The choice of when to emit can be also an element allowing a sender to control the active space of its signal. For instance, some European bush-crickets mainly stridulate in the morning, avoiding problems of reduced transmission appearing in the afternoon due to negative temperature gradients. Similarly, a number of adaptations to environmental constraints allowing the decoding of information and the localization of the emitter can be found at the receiver level. Perching behavior of forest birds, besides improvement of the active space of emitted vocalizations, has a great influence on the potential received signals and, in reality, increases the active listening space more than the active space of the bird’s signals. Thus, during song reception in the Blackcap *Sylvia atricapilla*, a perching at 5 m high has the same consequence in terms of decrease in excess attenuation than a horizontal approach of 24 m to the emitter!

Signal Honesty

Communication systems are shaped by the extent to which emitters and receivers have mutual or conflicting interests. In behavioral ecology, traits that benefit the emitter are called signals, and those that benefit the receiver are called cues. For example, the roaring of stags constitutes a honest signal predictor of body condition. Nevertheless, roaring repeatedly may be costly in terms of detection by predators and in this case constitutes a cue. In the visual domain, sexual ornaments not only signal the quality of the male but also signal the emitter to

predators. It is the same for chemical tracks of ants, which allow homing not only for conspecifics but also for predators! So, the distinction between signals and cues is not clear. Similarly, the concept of signal honesty is controversial because it is difficult to determine the intent in animal communication. In fact, the term ‘honest signal’ must be used in a statistical sense. Signals can be considered honest if they carry information that will be on average useful to the receiver, particularly at the level of its fitness. Numerous honest signals have been described in the animal kingdom. For example, male and female blue-footed boobies have, as indicated by their name, colorful feet that are displayed ostentatiously during courtship. Feet coloration can change very quickly depending on food availability. Compared with males or females with duller blue feet, those with bright green-blue feet have better nutritional and health condition and are more attractive for the opposite sex. This has been experimentally demonstrated by modifying the male or female feet color with a make-up. Females decreased egg volume when the male feet color was manipulated to a duller blue. Similarly, experimental females with duller feet received less intra- and extrapair courtship (see **Figure 3**). In this species, feet color appears as a sexually selected trait.

The opposite of honesty is cheating (or deception). Cheating occurs when a signal has a positive informative value for the emitter but a negative one for the receiver. Senders are expected to try to manipulate receivers on their own, because the interests of senders and receivers conflict. Thus, animals are able to cheat when they communicate. A good example of cheating is the stag roaring, as described above. Formants (vocal tract resonances) provide a honest indication of body size in the rutting vocalizations of deer, and males use these formant frequencies in assessment during male–male contests. As adult humans, stags possess a descended larynx and this laryngeal descent serves to elongate the vocal tract and so to lower down the values of the resonant frequencies (see **Video clip 3**). This anatomical particularity allows some callers to exaggerate their perceived body size by decreasing vocal-tract resonant frequencies.

Through these different examples, we can see that the interests of emitters and receivers often conflict. As a consequence, the coevolutionary struggle between honest signal and cheating should lead to the extinction of honesty in animal communication. What prevents cheating from destabilizing signaling systems is the fact that receivers should ignore the signals if they are not useful to them.

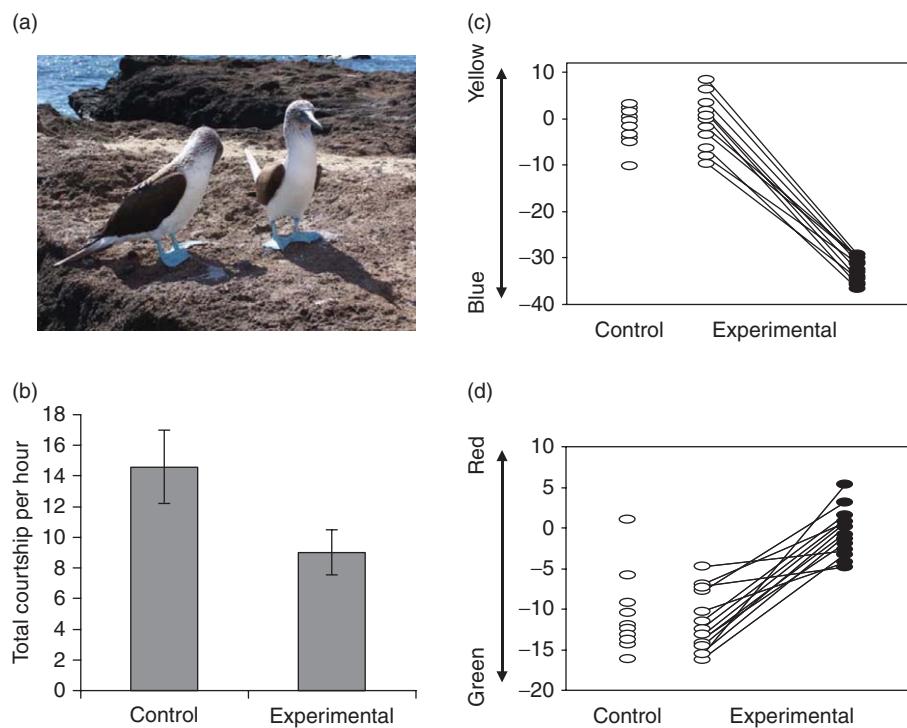


Figure 3 In the blue-footed booby *Sula nebouxii* (a) the foot color of paired females is a trait that influences male courting behavior (picture: T. Aubin). This was demonstrated by an experiment in which the foot color of females was modified with a make-up. (c, d) Chromaticity parameters of foot color (c: from blue to yellow; d: from green to red) of control ($n = 11$) and experimental ($n = 15$) females before and after the color manipulation. (b) Total courtship displays (e.g., parading and nest material presentation) received by the control and experimental females. Adapted from Torres R and Velando A (2005) Male preference for female foot colour in a seabird with a mixed mating strategy. *Animal Behaviour* 69: 59–65.

Communication Network, Eavesdropping, and Audience Effect

The simple relationship emitter–receiver mentioned previously gives a poor indication of the way communication occurs in animal groups or societies, because several individuals are often simultaneously involved in a communication process. In these communities of animals, communication occurs in a network rather than in a one emitter–one receiver dyad. Thus, studying communication in a network perspective appears as a necessity to decipher the behavior of complex groups such as social insects, birds, or mammals and to examine the dynamic nature of communication.

Social eavesdropping (interception of communication) is an illustration of communication network behavior. Eavesdropping occurs in a situation in which one or more observers (eavesdroppers) extract information from a signaling interaction between others. Eavesdroppers located near an emitter and receiver will collect information about their state, strength, and quality and will later use this information to adjust their own behaviors in following interactions with the original signalers. A classical example of visual eavesdropping is provided by experiments realized in laboratory conditions with Siamese fighting fishes *Betta splendens*. In these experiments, a subject sees two individuals in courtship interactions (male–female communication) or in aggressive interaction (male–male communication) and extracts relative information from these interactions. For example, a male assesses the competitive ability of two other males by watching their territorial contests. In future encounters, this male will avoid fighting the winner but will attack the loser. Eavesdropping can implicate different species. For instance, predators and parasites pay attention to signals of potential preys or host (e.g., bats preying upon calling frogs or insects, predator insects attracted by the sex pheromones of Lepidoptera or Coleoptera). This category of eavesdropping does not correspond to an exchange of information between preys and predators but has implications on the prey communication system. To avoid the attack of predators, the potential preys have then to keep their interactions secret. Reduction of signal range by the use of propagation-sensitive signals can be of a great interest to privatize communication. For example, numerous birds use quiet vocalizations that are less likely to be detected or localized when they want to limit eavesdropping. Similarly, in a species of cricket, *Teleogryllus oceanicus*, males of populations, in which a phonotactic parasitoid occurs, produce shorter and simpler sounds than males of parasite-free populations. Some acoustically orienting predators such as bats are so menacing for some species of crickets and katydids that they may have selected for the secondary loss of acoustic signaling. In these insects,

substrate vibrations partially replace vocalizations presumably because bats cannot eavesdrop on these signals.

Audience effect constitutes another illustration of communication network behavior. It corresponds to a change in signaling behavior of two interacting individuals in the presence of one or more external receivers (the audience). The audience is a nontarget receiver that nevertheless can act on the behavior of two individuals exchanging information. This process has been documented in numerous species and for different categories of signals. For example, it has been shown experimentally in a gregarious bird species, the zebra finch (*Taeniopygia guttata*), that the male pays attention to the mating status of conspecific pairs and uses this information to control its behavior toward its female partner. The experiment clearly demonstrates that the vocal response of males to their partner's calls and to the calls of familiar female changes depending on the composition of the audience – audience that was constituted either of males or of unmated pairs or of mated pairs (see [Figure 4](#)). This example reveals that signal production may depend on the social environment and the emitter's social status. A consequence of signaling in a network is that specialized behaviors or signals have evolved so as to direct a signal toward a particular part of the network rather than to the whole network. The capability of squids to produce visual signals on opposite sides of the body when surrounded by different individuals is an example of directing a signal to particular receivers and corresponds also to the privatization of information evoked above.

Referential Communication

When animals communicate, they typically convey information about their identity and their motivational state. In some cases, as mentioned previously, they can also indicate the discovery of food (resource-recruitment signals) or predators (alarm signals). The fact that an item that is present in an individual's proximal life space may be the topic of signal exchange constitutes what is termed a referential communication. The honeybee dance indicating a distant food source is an example of referential signaling in the visual domain. Sometimes, the information concerning the nature of the food or of the predator can be very precise. Thus, the chick-a-dee call of some tits (the chickadees) consists of four note types, and it is used in a wide variety of contexts, including not only contact between males but also alarm. Field scientists have observed that the number of repetition of the last note type (the dee note) was related to the size of the predator: the greater the repetition number, the smaller the predator size. Similarly, East African velvet monkeys categorize other species by giving acoustically distinct

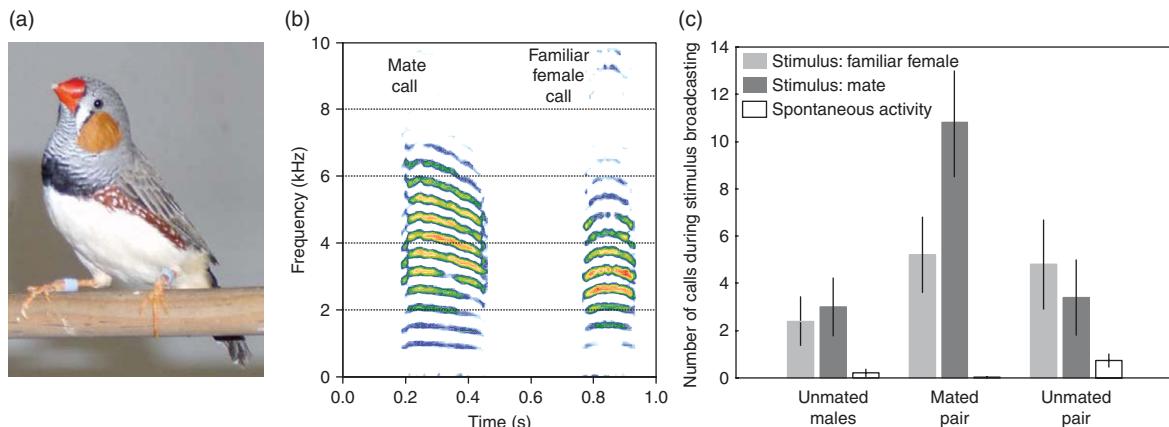


Figure 4 Importance of audience effect on the vocal response of male zebra finch to female voice. (a) Zebra finch *Taeniopygia guttata* (picture: courtesy of C. Vignal). (b) Male and female calls. (c) Response of males ($n = 15$) to the playback of female calls as a function of the mating status of the audience placed near the tested male in a companion cage: unmated male context: two single males; mated pair context: a normal male-female pair; unmated pair context: a male and a female not paired. During the experimental session, the male was presented with two sets of stimuli: a series of calls of its mate and a series of calls of a familiar female. Adapted from Vignal C, Mathevon N, and Mottin S (2004) Audience drives male songbird response to partner's voice. *Nature* 430: 448–450.

alarm calls to different predators. These calls allow the classification of three predator categories: two terrestrial predators, the leopard and the snake, and one aerial predator, the eagle. Associated with each alarm call type is a behaviorally appropriate response. The response to a leopard alarm is to run up into a tall tree. By contrast, the response to an eagle alarm is to run in a dense bush. Finally, the snake alarm elicits a mobbing behavior with the aim to move away from the intruder. Thus, in different animal species, there is evidence of referentiality, that is, of signals, which refers to significant targets in the environment such as food or predators. The ability to produce such signals is strongly related to the development of social life.

In animals, the communication systems play an important role in the realization of vital functions such as species, group, sex, individual identification, search of food, warning in relation to a danger, and contact between individuals. Some animals, principally social ones, are also able to use referential signals such as those related to the food or the predator nature. It is more particularly the development of the referential function, which is at the origin of the most sophisticated communication system: the human language.

See also: Animal Models of Sexual Function; Behavior Adaptation and Selection; Behavioral Development and Socialization; Behavioral Pathologies in Nonhuman Primates; Communication of Emotions in Animals;

Development and Language; Fear, Anxiety, and Defensive Behaviors in Animals; From Sensation to Perception; Mating Behavior; Orientation and Navigation; Parental Behavior; Social Bonding and Attachment; Social Communication; Social Competition and Conflict Resolution; Social Learning and Behavior Transmission; Taste Perception and Behavior in Rodents and Flies.

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Social Learning and Behavior Transmission

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Glossary

Asocial learning – Asocial learning describes individual learning that occurs in the absence of social interaction.

Cultural variants – Group behaviors, or traditions, are considered culturally variable when they are present in at least one area, but absent in other areas with similar, if not identical, ecological conditions.

Culture – Culture refers to group-specific variation in behavior that is a result of social influences. Some reserve this term for the human alone, due to the complex nature of human culture. Others place the main distinction between tradition and culture on the behavioral processes involved in the development and maintenance of cultural attributes (e.g., imitation, teaching, and language).

Cumulative culture – When gradual modifications to behavior are added over time, a cumulative effect may lead to more advanced and complex traditions. Human cultural complexity is often attributed to this phenomenon.

Imitation – In 1898, Thorndike broadly defined imitation as: “learning to do an act from seeing it done.” It is, perhaps, the most debated social learning mechanism, as it is seen by many to require more complex cognitive abilities and mental representations of others. Whether it is seen in animals or not is also highly debated, and it is considered a hallmark of human cultural development.

Social learning – Social learning refers to a range of learning mechanisms that are socially influenced either by the observation of others, or as a result of interactions with an individual and/or the byproducts of that individual’s actions. Certain social learning mechanisms are thought to play a critical role in the development and maintenance of group-specific behaviors.

Teaching – In observational learning, it is possible for one individual to gain information by watching another’s actions or behavior. Teaching is the term used to distinguish the condition in which the model of the behavior modifies his or her actions in such a way that allows the observer to acquire that information more rapidly. Teachers often do this at a cost to their own productivity, and may even encourage or discourage the observer.

Tradition – Group-specific behaviors that are exhibited by members of one group, but not another of the same

species, are considered local customs, or traditions.

Traditions are transmitted socially, rather than genetically, and are not merely the result of adapting to local ecological conditions.

Introduction

Learning, whether social or asocial, can provide an individual with the opportunity to gain experiences that will help it to better adapt to changes in its environment. An individual’s environment can change many times and in many ways over its lifetime. In some cases, it may be better for a naive individual to gain information from another individual via social learning, because the risks associated with individual learning may be costly (e.g., learning to avoid a poisonous food-source). Depending on the costs and benefits, an individual should employ different social learning strategies, changing between asocial and social learning.

Social Learning

Most reports of social learning in animals involve the acceptance or avoidance of novel foods, mate-choices, and predator-avoidance strategies; these behaviors may be learned by observing others, or from artifacts produced by others. In some cases, learning socially may or may not require reasoning about other individuals, or even reasoning about one’s own behavior. Less common examples of socially learned behaviors include specialized grooming, communicative signals, and tool-use techniques. These types of activities may require that an individual understand their own relationship to the demonstrator (e.g., for copying motor patterns), or understand the goal of the demonstrator’s actions – both of which involve relatively sophisticated cognitive abilities. Determining what cognitive abilities are involved in the learning process has been the primary objective of social learning researchers for over a century, with a great amount of attention focused on nonhuman primates.

Imitation was initially considered one of the few traits that animals had in common with the human, and many languages even have terms that involve the words ‘ape’ or

'monkey' to describe imitation (e.g., aping). However, great debate exists over whether or not monkeys, apes, or any other animals are able to truly imitate, with a particular emphasis being placed on what the definition of imitation should be. Sometimes, imitation can be confused with more subtle forms of social influence that lead to learning the same behaviors as others. For this reason, the literature on social learning distinguishes terms for the different ways in which learning can take place, such as social facilitation, contagion, stimulus enhancement, local enhancement, emulation, imitation, and teaching. While having many terms for learning contributes to confusion in the literature, it also highlights the various ways in which social influences affect animal learning and behavior transmission.

Social Facilitation and Contagion

The presence of another individual may enhance or inhibit existing behavior in an individual. This is commonly referred to as social facilitation, but is also referred to as social enhancement – defined as the motivational state of an individual being enhanced by another (be it an increase or decrease in motivation). The mere presence of another individual has the potential to lead to synchronization of behaviors over space and time, which is crucial in group-living species with regard to group cohesion, behavioral coordination, foraging efficiency, and predator avoidance. While social facilitation alone does not lead to complex behavior matching, it has the potential to increase the opportunities in which observational learning can occur. When coordination of behavior occurs among two or more individuals, it is referred to as social contagion, because the mere act of seeing another individual engaging in a species-typical behavior elicits that same response in others. For example, satiated brown capuchin monkeys will resume feeding when a familiar conspecific is seen eating nearby and will also consume more food in the presence of another monkey than if alone. This facilitated feeding behavior is seen in other monkey species as well as dogs, chicken, fish, and the human.

Stimulus and Local Enhancement

One of the most common forms of social influence occurs when an individual's attention is drawn to a stimulus by another individual. This is referred to as stimulus enhancement. Similarly, when an individual is drawn to a specific location because of the actions or byproducts of the actions of another individual, it is called local enhancement. It is not always possible to distinguish these two forms of enhancement, because an object or stimulus may also be associated with a specific location.

In Great Britain, blue tits were observed drinking from the top of milk bottles left outside homes by milkmen.

The birds were seen piercing the foil cap of the bottles to gain access to the milk within. When this was first observed, it was assumed to have spread via social learning, but the specific mechanism was unknown. Following the presentation of pierced-opened milk containers to captive chickadees, the birds were able to deduce on their own how to pierce unopened lids, suggesting that they did not need to observe the behavior performed by a conspecific, merely, being drawn to the location and seeing the resulting condition of the bottles was sufficient.

Some have argued that stimulus and local enhancement are not social learning mechanisms *per se*, rather they are social influences that lead to individual trial-and-error learning, or in some cases facilitate opportunities for observational learning to take place. Nevertheless, local and stimulus enhancement are generally recognized as important processes in social learning, as they may facilitate the acquisition of behaviors from one individual to another.

Emulation

Emulation refers to a form of learning about the affordances of a task, or changes in the environment, as a result of a conspecific's behavior. Within the literature, four distinct aspects of emulation learning, which all relate to learning about the affordances of an object, have been defined: (1) setting a goal, (2) learning the physical properties of objects, (3) learning relationships among objects, and (4) learning what can be done with an object. Furthermore, the changes taking place in the environment are the primary focus of the learner; therefore, the demonstrator's identity (or even presence) may be irrelevant in instances of emulation, so long as the movements are observed. Other terms for emulation include goal enhancement and affordance learning.

Although some describe emulation as a less complex form of learning than imitation, stating that it does not require the need for perspective taking, it may still be a more efficient method of acquiring new information. In certain instances, choosing to ignore irrelevant information or actions conducted by another, in favor of more efficient actions that result in the same endpoint, may be a more efficient strategy. For example, chimpanzees readily switch between emulation and imitation in an experimental foraging task when they are able to distinguish between relevant and irrelevant actions that are demonstrated for collecting food. In one condition, a foraging apparatus is painted so that the inside of the box is not visible. An experimenter demonstrates poking a stick into a hole in the top of the box, and then pokes the stick in another hole on the side of the apparatus. Food is then released from the box and the chimpanzee is given the opportunity to attempt to extract food. In the second condition, chimpanzees are shown the same actions but

the box is not painted and the inside is clearly visible. The chimpanzees now can see that poking the stick in the top hole only hits a shelf inside the box, whereas the second hole on the side actually releases food. In the first condition, chimpanzees copy all the actions that were demonstrated, but in the second condition, they copy only the actions that result in the end-state reward. When human children are presented with these same experimental conditions, they imitate the actions demonstrated irrespective of its relevance to food collection, suggesting that there may be some intrinsic value for the human to do as others do.

Imitation

Imitation is linked with high copying fidelity that not only includes the same end results as the original demonstrator, but also any relevant or even irrelevant behaviors and actions involved. However, over time, varying perspectives and approaches to the study of imitation in animals has led to the belief that imitation is a specific form of social learning – one that is considered to be more cognitively advanced as it may also require perspective taking, or theory of mind. This may very well be the case, as it seems those who are more aware of the social affairs of others are better social learners. Arguably, the best examples of imitation come from animals that are believed to have a theory of mind. For example, chimpanzees have been shown to imitate novel actions of a human experimenter. In the ‘Do as I do’ (DAID) experiment, a chimpanzee is trained to respond to the phrase, ‘Do this!’, which is followed by a range of behaviors, all of which are novel to the chimpanzee. The actions included touching areas of the body that were visible to the subject, such as touching an elbow, the stomach, or a shoulder, as well as nonvisible areas, such as the ear, nose, and back of the head. While the two chimpanzees imitated the human demonstrator, they had more difficulty matching behaviors that were not visible to themselves than areas that were visible.

Teaching

The study of social learning in animals has primarily addressed the perspective and abilities of the learner, since these are the main determinants of the learning process. A less common influence found in animal social learning is the active teaching of a behavior by a conspecific. An individual may perform a behavior in the presence of another group-member, but this does not necessarily imply that they are actively teaching for the benefit of the observer. Teaching differs from merely demonstrating, because the teacher modifies its behavior in some way due to the presence of the observer. This change in behavior does not benefit the teacher, and can

sometimes even be at a cost to the teacher. As a result of the teacher’s change in behavior, the observer learns at a faster or more efficient rate than it would alone.

Teaching by nonhuman animals is not limited to a specific taxonomic group, nor is it only seen in cognitively advanced species. The most convincing examples of teaching come from animals that cooperatively breed, such as ants, pied babblers, and meerkats. In meerkats, most group members are related to other members of their colony and lead a relatively cooperative life. Group members take turns monitoring for predators while others forage for food; and in some cases, a group member will present young offspring with prey that is partially maimed in order to provide them with direct hunting experience. By doing this, a meerkat may lose the prey if the offspring are not successful in killing it, thus it is a potentially costly act to be a teacher. However, if the young are successful, they gain the necessary experience for successfully hunting their own prey. For this reason, it has been suggested that the act of teaching is a cooperative behavior that facilitates learning in others.

Traditions and Cultural Behaviors

Differences in learning abilities across species may not only reflect specific abilities for social learning, but also the ways in which social behavior and group dynamics interact to support behavior transmission. As with the debate over imitation in animals, the question of whether or not animals have culture has been, for the most part, definitional with a focus on the learning mechanisms that support the transmission of cultural behavior. Under a small range of conditions, there is increasing evidence that animals are capable of imitation, though they rely on simpler forms of social learning more frequently. The study of animal learning has shown that even the most basic levels of social influence can lead to behavioral differences between populations, which are referred to as local traditions or culture. Distinctions are made between the terms culture and tradition because for many, culture is a term associated with complex cumulative culture seen only in the human.

Field Studies of Cultural Learning

One of the first reports of animal culture came from the island of Koshima, where a group of Japanese macaques washed sweet potatoes that were provisioned to them (**Figure 1**). This behavior originated with one female, Imo, and, within 10 years, most others in her group were also washing their potatoes. The spread of potato washing was initially slow, with only one or two individuals acquiring the behavior per year. It has been suggested that imitation is an unlikely explanation for the spread of



Figure 1 Potato washing Japanese macaques submerge potatoes on the shore at Koshima. Photo credit: Frans de Waal.

this behavior due to the slow rate of transmission. If Japanese macaques were imitating what they observed, then the transmission rate would be much higher; however, the strict hierarchical nature of macaques restricts the proximity between unrelated individuals and those with the greatest rank disparity, and therefore limits social opportunities for individuals to observe the behavior. Furthermore, it assumes that cultural behaviors can only spread by complex social learning mechanisms such as imitation. While it is now widely assumed that potato washing did not spread by imitation, it is accepted as a feeding tradition of the monkeys at Koshima, since the behavior not only spread to most members of the group, but also endured beyond the influence of the original innovator, to future generations.

Another tradition in Japanese macaques is the handling of stones as an object-play behavior. Stone handling in Japanese macaques may not have a specific functional purpose beyond social play, as the behavior is not rewarded and does not involve food or food processing. New reports are emerging for variants of stone handling between groups, including stone throwing, as well as a novel fish-eating method in Japanese macaques, adding further support for variability in the types of traditions monkeys have.

The best evidence for variability of traditions comes from the long-term study of white-faced capuchin monkeys in Costa Rica. Some 19 000 h of field observations from 13 social groups over 13 years were combined from four field-sites, Lombas Barbudal, Santa Rosa, Palo Verde, and Curu. Five social conventions were described: (1) hand-sniffing: when an individual takes the hands or feet of another individual and deeply inhales, (2) body-part sucking: when an individual engages in lengthy period of time sucking a body part of another individual (e.g., ear, finger, tail), (3) finger-in-mouth game: when an individual puts his or her fingers in the mouth of another individual that then clamps down firmly for a lengthy

period of time, (4) hair game: when two individuals take turns biting hair from each other, and (5) toy game: when two individuals repeatedly take turns pulling nonfood objects from each other's mouths. Additionally, different food processing methods were described for some of the 13 field-sites. For example, at Lomas Barbudal, capuchins hunt squirrels by biting them on the back of the neck, whereas this method is not seen at all in Santa Rosa, and in Lomas Barbudal and Palo Verde, groups within and between sites vary in their use of pounding and rubbing foods. These capuchins appear to share the same food-processing methods as individuals with which they associate, suggesting that these behaviors are socially acquired.

An influential breakthrough for studying cultural variation in primates came from a combination of longitudinal studies in wild populations of chimpanzee throughout Africa. By systematically comparing the cultural variants for each site taking account of ecological conditions, 39 distinct behavioral variants were identified among seven chimpanzee research sites. Chimpanzees in some areas of Africa use stones as tools to crack open nuts, while chimpanzees at other sites select and modify tools for ant dipping – a foraging technique for consuming ants by using sticks. Chimpanzees also engage in traditions that do not have direct survival benefits. One example of this is the handclasp grooming (**Figure 2**). The handclasp grooming involves holding and supporting the arm of a grooming partner by the wrist or hand over the grooming pairs' heads. This behavior is seen at specific field-sites, and entirely absent in others; for example, the handclasp grooming has been observed in the Mahale



Figure 2 Handclasp grooming in chimpanzees. Photo credit: Frans de Waal.

Mountains but not at Gombe, a chimpanzee research site only about 150 miles distant.

The same approach has been used to study cultural variants in wild orangutan populations in Southeast Asia. Orangutans are relatively solitary, although still social, animals whose interactions with conspecifics are mostly limited to mother–offspring pairs and mating partners. Opportunities for social learning are inherently limited by the number of social encounters between individuals, so the opportunities for studying cultural learning in orangutans have also been limited. Despite this confine, a comparison of six field-sites showed that orangutans also exhibit cultural variants, including six social signals (e.g., kiss-squeaks), and 10 specialized feeding techniques – two of which involved tool-use. Data collected from these different sites confirmed that the reported cultural variants could not be attributed to ecological differences across sites, and furthermore, found correlations between the opportunities for social learning and the size of the local repertoire.

Captive Studies on Cultural Learning

Longitudinal studies in the wild (such as those mentioned above) are costly, both in time and money spent by researchers, which has in turn limited the amount of information available. In order to assess if wild traditions are the result of social learning, it is also necessary to conduct captive experiments that allow us to control for all instances of social influence. Two of the most influential experimental paradigms for uncovering social learning in the spread of behaviors are the ‘two-action task’ and the ‘group-diffusion’ methods.

The two-action task paradigm has mainly been used to control for the effects of local and stimulus enhancement on social learning while examining the imitative abilities of subjects in observational learning experiments. The test involves two distinct methods for solving a task. One method for solving the task is demonstrated to an experimental subject before the subject is presented with the task. Although both methods are possible, the subject only observes one of the two tasks. Therefore, if a subject performs the same task as the demonstrator, it is most likely a result of imitative learning. The two-action task is considered one of the best methods for testing imitation in animals.

Animals differ in their physical abilities (e.g., primates use their hands, while birds use their beaks for most foraging behaviors), and so an advantage of the two-action test, is that it can be designed specifically for the physical abilities of a given species. Two-action task experiments have been used to study imitation in pigeons, budgerigars, Japanese quails, rats, hamsters, brown capuchin monkeys, gorillas, orangutans, and chimpanzees, among other species. These experiments have typically investigated test subjects in pairs, and have suggested imitative, or

imitation-like, learning in the context of the two-action task paradigm. However, it is apparent that each of these species is distinct from the next with regard to social structure and cognitive abilities, and it is, therefore, to be expected that we find differences in how socially acquired information spreads among group members, and how new behaviors develop into group-specific traditions.

While the two-action task paradigm provides insight into the kinds of learning mechanisms involved in transmitting behaviors, it does not necessarily determine what social influences maintain and spread a behavior to the point at which it becomes a group-wide tradition. One approach to studying traditions in animals is to investigate the diffusion of a behavior pattern throughout a social group. In the wild, it is virtually impossible to witness or recognize the innovation of a novel behavior. It is also difficult to study the transmission of that behavior in a controlled manner, with some exceptions – such as potato washing and stone handling in Japanese macaques. Even in the Japanese macaque studies, with years of available behavioral data, the underlying social learning processes involved still remain a point of issue and debate.

As described above, the two-action task test allows us to study the extent to which individuals learn to copy by observation, and in most experiments to date, this paradigm has been used in a dyadic context. The ‘diffusion experiment’ can provide us with further details about how a behavior spreads socially beyond the artificial dyadic context imposed upon subjects.

The lack of persistent and thorough imitative abilities may be one of the key features that distinguishes the development and scope of cultural behaviors in humans from nonhuman animals, but whether imitation is a necessary component of culture remains debatable. What is more noteworthy is the cumulative culture the human exhibits. This is likely a combination of human ability for imitation, teaching, and language. With language providing the means for building upon passed generations, imitation providing the fidelity to details necessary for producing cognitively complex behaviors, and teaching playing an important role in maintaining group social norms, as teaching allows for rewarding and punishing behaviors that fall outside the norm. Irrespective of how similar or dissimilar animal and human cultures are, the study of animal cultural learning is imperative to a biological understanding of culture.

See also: Brain Evolution in Vertebrates; Cooperation; Physical Cognition and Reasoning; Social Cognition: From Behavior-Reading to Mind-Reading; Social Communication.

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Relevant Websites

- <http://culture.st-andrews.ac.uk/solace/> – Centre for Social learning and cognitive evolution.
- <http://www.ip.usp.br/ebottini/EthoCebus/echome.html> – EthoCebus Homepage.
- <http://sociallearning.info> – Sociallearning.info.
- http://www.emory.edu/LIVING_LINKS/ – Website of the Living Links Center for the Advanced Study of Ape and Human Evolution.

Social Relationships and Social Knowledge

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Glossary

Attachment – Attachment is the social bond between young and their mothers. In most species, attachment manifests itself as proximity-seeking to an attachment figure, usually the mother, particularly in situations of perceived distress or alarm.

Friendship – Friendships are close, consistent relationships that do not involve mating between pairs of nonrelatives. They can occur between animals of any age or sex and have been described in many species. However, friendships have primarily been studied between adult male and adult female primates.

Inclusive fitness – Inclusive fitness is a measure of an individual's fitness that includes both 'direct fitness' (fitness of the individual) and 'indirect fitness' (fitness through shared genes with close relatives). Indirect fitness is calibrated by the degree of relatedness, or the relatedness coefficient. In many situations, animals perform altruistic acts for relatives because – despite reducing their direct fitness – the act results in a net increase in inclusive fitness.

Prolactin – Prolactin is a peptide hormone originally discovered in association with lactation. However, across a wide range of animals including nonmammals, prolactin is now known to play an important role in parent–offspring bond formation.

Reconciliation – Reconciliation is an affiliative interaction among a pair of animals that have recently had a conflict or aggressive interaction. Reconciliation may serve to repair a damaged relationship.

Operationally, reconciliation is a postconflict increase in the probability of affiliative interactions above the baseline levels of inter action.

Social cognition – Social cognition is one type of cognition (the acquisition, processing, and storage of information) dealing with members of the same species. Social cognition covers the cognitive processes involved with perceiving, understanding, and interacting with conspecifics.

involve stable patterns of association and affiliation that are hallmarks of social bonds. In many species, social bonds are critical for survival. Animals rely on their social companions to help them find and defend food resources, to avoid or defend against predators, and to engage in reproduction.

Most social interactions among animals are explained by one of two factors: kinship and mating. Animals socialize with their close kin largely because these behaviors can increase their own fitness through kin selection and inclusive fitness. For example, bonds between parents and offspring help the offspring survive, which increases the parent's fitness due to the large portion of the parent's genes carried in the offspring. Such inclusive fitness benefits extend to more distant relatives, and kin selection is a major influence on patterns of social behavior. Many affiliative social relationships in animals parallel kinship relations. For example, in groups of matrilocal Old World monkeys (groups where the females stay in their natal group for life), the strongest relationships are among closely related females (mothers, daughters, sisters, etc.). These bonds form as an extension of the mother–infant bond as the developing infant forms attachments with the mother and, by association, other animals with which the mother has social bonds. These bonds remain as long as both members are alive.

Kinship often explains many otherwise 'altruistic' behaviors through the action of kin selection. Kin selection occurs when one animal aids a close relative at a cost to themselves. If the benefits of shared genes (inclusive fitness) outweigh the cost of the behavior, such behaviors carry a fitness advantage. Kin selection explains why cooperation and altruism are more common among close kin than among unrelated individuals. The most widespread example of kin selection is the help parents provide to their offspring.

A critical component to kin selection is kin recognition, or the ability to differentiate kin from nonkin, since animals can only preferentially help kin if they are able to effectively recognize them. The ability to recognize kin is widespread across animals (and even occurs in some plants) and does not require sophisticated cognitive abilities. For example, it can be based on simple mechanisms such as chemical detection. Alternatively, however, kin recognition can occur via cognitively sophisticated mechanisms such as social knowledge using individual and attribute recognition (see below).

Introduction

Among the most salient and important aspects of animal behavior are the interactions animals have with members of their own species. These interactions frequently



Figure 1 A harem of geladas resting above a sleeping cliff in the Simien Mountains of Ethiopia. Harems are the basis of gelada society and consist of long-term relationships among an adult male (much larger than the females) and several adult females and their offspring. Within the harem, the male–female relationships are based largely on mating, while the relationships among the females are based on kinship.

Outside of kinship, the most common associations between animals occur in the context of mating. Such interactions can range from brief encounters that do not involve any social bonds, to lifetime monogamy – among the longest lasting social bonds in animals. For example, pairs of royal albatross have been known to stay together for over 20 years. Even many nonmonogamous species have social bonds in the mating context, as in the temporary consortships found in many primates. As such, kinship and mating are the major factors structuring animal social systems (Figure 1).

Types of Social Bonds

Attachment

Attachment is the social bond between young and their mothers. In most species, attachment manifests itself as proximity-seeking to an attachment figure (usually the mother), particularly in situations of perceived distress or alarm. Attachment is the fundamental bond in development. The bond with their mother is the first bond most animals experience, and other bonds later in life are influenced by the nature of this bond. For example, many birds attach to the mother (biological or foster) through imprinting and, later in life, male birds will seek mates that resemble their attachment figure (even a human). Furthermore, most social bonds involve similar behaviors and biological bases, and it is likely that many social bonds are rooted in attachment. As such, understanding attachment sheds light on all social bonds.

Human attachment has a long history of study, and many early theories, including Freud's, pointed to the food provided by the mother as the critical component of attachment formation. This view was rejected by John Bowlby in the 1960s. Bowlby's theory of attachment focused on the role of the social interaction and proximity between the mother and the infant. Bowlby's views were influenced by ethological studies of imprinting that suggested that there is a critical window for the bond to form and that the bond formed early in life with the mother can affect adult behavior. The importance of social contact over food provision was supported by Harry Harlow's studies of attachment in orphaned rhesus monkeys. When given a choice between a soft, cloth-covered dummy 'mother' that provided no food and a wire dummy that provided food, the infants attached to the soft mother. Thus, the 'cupboard versus comfort' debate has largely been resolved in favor of contact-comfort as the critical component driving the attachment of babies to their mothers. As such, attachment is now often studied from both directions – infants to their mothers, and mothers to their infants. Functional imaging studies in humans are now revealing the biological bases of attachment from the mother's perspective (see below).

Familiarity

Familiarity is the simplest and weakest form of relationship among members of the same species. While most social bonds involve high rates of proximity and affiliative interaction, familiarity only requires visual, olfactory, tactile, or acoustic exposure to another animal.

Therefore, even animals that have little or no social interactions can be familiar with other members of their species. Even in the absence of social interaction, numerous studies show that familiarity influences an animal's behavior. For example, guppies are more likely to find hidden foods in the presence of familiar animals, and male red-winged blackbirds are better able to attract females if they are familiar with their male neighbors. Many animals go beyond familiarity and individually recognize other animals, making familiarity also one of the simplest forms of social knowledge (see below). Sustained contact and social interaction are thought to be important in transitioning from familiarity to stronger bonds.

Friendship

Close, consistent relationships have been observed in many taxa, and these are sometimes referred to as 'friendships.' In many species, pairs of unrelated animals (female–female, female–male, or male–male) maintain proximity and interact affiliatively at high rates over long periods of time outside of a mating context. Thus, these relationships resemble human friendships. Friendships were first described in anubis baboons by Shirley Strum and Barbara Smuts, who noted consistent associations among adult males and females. Friendships can be among the strongest and most enduring male–female bonds in many species. Because little is known about the biological and emotional correlates of animal friendships, they are defined by measures of proximity and affiliation. To separate friends from other types of relationships, they are defined as close social bonds among nonrelatives outside of a mating context. Some definitions require that the relationship be symmetrical (with both sides being equally responsible for maintaining the relationship) to qualify as friendship, although this standard is not often applied in practice.

From the time friendships were first identified, they have also been described in macaques, and chimpanzees. However, similar relationships are more widespread than the use of the term friendship would suggest (as many researchers are hesitant to use the anthropomorphic term 'friendship' and refer instead to 'associations' or 'bonds'). Relationships that resemble friendships have been identified in many species, including many nonprimates (e.g., dolphins and wolves). Because friendships cannot be explained by kin selection or mating, there has been considerable debate about why animals might form friendships.

Why form friendships?

Parenting

Many friendships between males and females, including the first friendships observed in baboons, appear to be related to parenting. In some populations of baboons,

these friendships function as a form of paternal investment because male friends, who are often the father of their female friend's infant, protect the infant from harassment, predation, or infanticide. Thus, both the male and female benefit from the relationship through increased offspring survival. In chacma baboons, where infanticide is common, many lines of evidence gathered by Ryne Palombit and colleagues point to the role of friendships in protecting against infanticide. First, friendships usually involve a female with a dependent offspring and a male that mated with the female when she conceived that offspring. Second, males preferentially respond to distress calls of their female friends when paired with vocalizations of possibly infanticidal males. Third, baboon friendships often dissolve if the infant disappears or is weaned. Finally, during periods of infanticide risk, lactating females with male friends have lower levels of physiological stress (as determined by levels of 'stress hormones,' or glucocorticoids) than other lactating females. Friendships between males and females with dependent offspring in many species are likely to involve a parenting function.

Mating

While friendships are specifically defined to occur outside of mating contexts, some friendships may still have a mating function. For example, a friendship may increase the chances that the two friends will mate in the future. Thus, one of the participants (usually the male) can gain a fitness advantage through increased mating success. There is some evidence for this explanation in anubis baboons where friends often have not mated in the past yet subsequently mate. Nevertheless, in many species it is difficult to demonstrate that two friends are more likely to mate than they would be if the relationship did not exist, particularly after controlling for factors like dominance rank and previous mating history. Despite the lack of evidence, it remains possible that friendships serve both a parenting and a mating function, much as male–female relationships in monogamous species do.

Reciprocal altruism

Friendships may represent an extended form of reciprocal altruism in which an actor provides a service to another animal with a previous history of providing the actor with a similar service (such as grooming or alliance support). The classic example of reciprocal altruism comes from vampire bats that are more likely to share their food (by regurgitation) with hungry social companions if they have received food from them in the past. While theoretically appealing, examples of altruism in nature are rare and some researchers question if it even exists. However, recently, social interactions have been analyzed using a biological marketplace framework where animals exchange interactions as a type of commodity exchange. For

example, a low-ranking animal may groom a higher-ranking animal in exchange for support in an alliance. Such exchanges appear to represent a form of reciprocal altruism.

Given the lack of mating or parenting benefits, reciprocal altruism is most likely to occur in male–male or female–female friendships. Such friendships are less common but do occur, perhaps most famously in male chimpanzees. In chimpanzees, strong relationships among males can help in rank acquisition, mate acquisition, and group defense. Many of these relationships can be explained by kinship (male chimpanzees remain in their natal group and thus have the opportunity to interact with close relatives). However, recent genetic work suggests that many alliances among chimpanzee males are between nonrelatives and consequently resemble other primate friendships. Chimpanzees in captivity have been shown to monitor animals that help them and, in turn, use this information in deciding who to help. Thus, chimpanzees are capable of reciprocal altruism, and friendships among male chimpanzees may represent reciprocal altruistic relationships.

Stress reduction

Several lines of evidence indicate that social support and affiliative contact among individuals can reduce anxiety and stress. For example, across several primate species it has been shown that animals being groomed have lower heart rates. Additionally, following an aggressive interaction, reconciliation between opponents produces a rapid reduction in rates of self-directed behavior – a behavioral correlate of stress. In male baboons, basal cortisol levels are negatively correlated with social integration. Across many primate species, subordinates have higher baseline stress hormone levels in part because they lack social support. Similarly, in humans, social support protects against minor chronic stress. Together, these findings suggest that social affiliation may have the direct benefit of reducing stress. Thus, animals may engage in friendships for this benefit.

Alliances

An alliance is a social relationship in which two (or more) animals cooperate to compete with a third animal. Each competitive encounter is called a coalition, and repeated coalition formation among the same animals leads to an alliance. Coalitions and alliances are widespread across the Primate order, but have also been observed in species as diverse as hyenas, lions, canids, dolphins, and rooks. In primates, alliance formation often parallels kin relationships with close kin teaming up to threaten an unrelated animal. One of the most striking examples of primate alliances that are not necessarily kin-based are the boundary patrols of chimpanzees. During these boundary

patrols, males from the same community join together to walk the boundary of their territory and attack (and sometimes kill) any extra-community animals they find.

Alliance formation has important implications for social cognitive evolution, which may explain why alliances have been described only in species with sophisticated cognitive abilities. First, the formation of an alliance involves choosing an effective ally. For example, baboons routinely choose allies that are (1) higher ranking than their opponent and (2) unrelated to their opponent. Therefore, choosing an effective ally requires knowledge of third-party relationships such as relative dominance rank and family relationships. Depending on the size of the social group, it can be cognitively challenging to keep track of such third-party relationships. Furthermore, in cases where alliances are due to reciprocal altruism (i.e., animals preferentially ally with animals that have helped them in the past), animals must monitor and remember previous interactions. Consequently, the cognitive challenges associated with alliance formation may be one of the most important reasons why living in complex social groups is thought to drive cognitive evolution (see below).

Biological Bases of Social Bonds

The biological bases of social bonds are best understood in the realm of parenting and, to a lesser extent, mating behavior. It appears that all bonds share behavioral similarities and biological underpinnings with the mother–offspring bond or pair bonds and, thus, understanding these bonds sheds light on all social bonds. These bonds have been studied from both an endocrinological and a neurological perspective.

Endocrinology of Social Bonds

Prolactin is a peptide hormone primarily associated with lactation. However, across a wide range of animals, prolactin is also important in parent–offspring bond formation. Prolactin has been widely studied in birds, where it has been associated with both maternal and paternal care for the young. Among female birds, prolactin is responsible for ‘broodiness’ – a behavior where female birds seek contact-comfort that leads to protecting and covering eggs and young. Many seasonally breeding birds have high prolactin only while providing parental care. In many species, male birds demonstrate a trade-off between testosterone and prolactin when shifting from mating to parenting, with high testosterone and low prolactin in the mating season and low testosterone and high prolactin once chicks hatch.

A similar pattern has been observed in some mammals, with elevated prolactin and reduced testosterone being

associated with paternal care in California mice and common marmosets. Similarly, pituitary prolactin and placental lactogen play leading roles in maternal behavior. However, the biological basis of parental care is understood to be more complex in mammals with oxytocin playing an important role. Oxytocin, a neurohormone which is released during mating, has also been implicated in monogamous pair bonds among rodents. This finding is based on the distribution and density of oxytocin receptors in the brain. Similar data show that vasopressin is also important in rodent-pair bonds. However, antagonist studies indicate that the roles of oxytocin and vasopressin are complicated and, at least among prairie voles, there are sex differences. Oxytocin is important for pair-bonding in females, while vasopressin is important for males. Corticosterone, a steroid hormone, shows a similar sex difference, appearing important for bond formation in male, but not female, prairie voles. More generally, other hormones released around the time of birth or during mating, such as β -endorphin, dopamine, prostaglandins, relaxin, progesterone, and estrogens, also play a role in mammalian social bonds.

Less is known about the endocrine correlates of maternal behavior and pair-bonding in primates and humans. Experience plays a significant role in the expression of primate maternal behavior, suggesting that hormones may have a priming role in first-time mothers but are less important in experienced mothers. In solitary captive rhesus monkeys, nulliparous females avoid unfamiliar infants when they are placed in their cage, while experienced mothers express maternal behavior toward them, irrespective of their own endocrine status. Studies in marmosets show that estradiol can increase motivation relating to maternal behavior. In the human, hormone levels may affect maternal attraction toward infants and general arousal and responsiveness but they cannot explain the occurrence of basic parenting behaviors. Human paternal behavior appears to have more direct endocrine correlates with elevated prolactin and reduced testosterone found in expectant and recent fathers.

Neural Bases of Social Bonds

In birds, the preoptic area (POA) anterior to the hypothalamus is essential for the expression of parenting behavior, and prolactin receptor expression is high in this region throughout brooding. Cowbirds – a brood parasite that lays its eggs in the nests of other birds and does not provide parental care – have reduced prolactin binding in the POA. Lesion studies suggest that the ventromedial nuclei (VMN) and the paraventricular nuclei (PVN) are also involved in paternal care. In mammals, much of our understanding about the neural basis of parental care comes from studies of rats. In rats, estrogen promotes maternal behavior by enhancing offspring-stimulated

neural activity in the medial preoptic area (MPOA), the bed nucleus of the stria terminalis (BNST), and the dorsal and intermediate lateral septum. The shell of the nucleus accumbens appears to be important for pup-retrieval behavior. The vomeronasal organ and amygdala are involved in inhibiting maternal behavior in rats through inhibitory action on the MPOA. Hormones that favor maternal behavior (estrogens) apparently act by releasing the MPOA from this inhibition. One result of this release from inhibition may be the production of oxytocin receptors that, in turn, increase maternal care.

In humans, romantic and maternal love evoke similar pathways (involving the anterior cingulate cortex, ventral anterior cingulate cortex, medial insular cortex, caudate nucleus, and the striatum). These highly conserved neural systems underlying love have also been linked to addiction and obsessive compulsive disorders. Functional magnetic resonance imaging (fMRI) studies indicate that the anterior cingulate and right medial prefrontal cortex are involved in parental (and especially maternal) responsiveness. Amygdala activation also occurs in response to infant sounds. The orbitofrontal cortex is preferentially activated by images of one's own offspring compared to images of other infants. More recent fMRI studies have found that images of one's own infant smiling preferentially activate reward-processing regions (including the ventral tegmental area/substantia nigra regions, the striatum, and frontal lobe regions involved in emotion processing – medial prefrontal, anterior cingulate, and insular cortex). Thus, attachment may be maintained by the pleasure mothers and infants experience from being exposed to each other.

Social Knowledge: The Cognitive Aspects of Social Bonds

Animals keep track of many types of information about their social companions and in doing so, engage in social cognition. Social cognition covers the cognitive processes involved with perceiving, understanding, and interacting with conspecifics. While very little research has quantified the adaptive value of social cognition, it is likely beneficial because it allows animals to predict and influence the behavior of others in their group, to resolve conflicts efficiently, or to recruit allies successfully. Additionally, some argue that more complex types of social cognition (e.g., deception) are important in a Machiavellian way as animals try to outmaneuver each other in social competition. Indirect support for the value of social cognition comes from recent studies in baboons where, within a population, baboon mothers that are more social have greater reproductive success. What remains uncertain is how social-cognitive ability translates to social integration and status. However, there is considerable indirect evidence that social complexity and

cognitive ability evolve together, and it is likely that the cognitive demands of social life drive some cognitive evolution.

Types of Social Information

There are three types of social information that animals can ‘know’ about another animal. First, animals may recognize specific individuals or the groups that they belong to. Examples of this type of recognition include (in increasing specificity): species, sex, familiar versus unfamiliar, group membership, kin versus nonkin, and individual recognition. These recognition abilities are crucial for recognizing social partners and discriminating between appropriate and inappropriate ones. Second, animals may know various attributes of individuals. For example, an individual may attend to the dominance status, breeding state, and other attributes of its social partners. Third, some animals are able to track and understand third-party relationships, or how different individuals relate to each other. Understanding of third-party relationships includes cognitive abilities of transitive inference and hierarchical classification.

Individual recognition

This is the most specific type of recognition and has been demonstrated in many species from wasps to hyenas. Cues involved in recognition can be visual, olfactory, acoustic, or tactile. Recognition is most common between mothers and offspring. Mothers and offspring are most likely to recognize each other in species where mothers and offspring must find each other among many other members of their species (e.g., in a herd of ungulates). Many such species have specific mechanisms for improving mother–offspring recognition. For example, the calls of nestling sparrows are more variable (and thus, more identifiable) in colonial nesting species than in solitary nesting species. Recognition also varies adaptively across development. Dolphin mothers give their individually distinct whistles at much higher rates following birth, apparently to help their offspring learn to recognize the vocalization. Furthermore, in primates mother–offspring recognition is strongest at the age when infants first start to leave their mothers and move around independently.

In many species, recognition extends well beyond mother–infant pairs. In nearly all primates, individual recognition typically extends to all the members of the social group and, in some cases, to members of neighboring social groups. Individual recognition is necessary for the types of differentiated relationships observed in primate and other complex societies. Such species appear to have evolved specific mechanisms involved in recognizing others. For example, primates (including humans) and sheep have areas of the brain (in the temporal and medial prefrontal cortices) that are specialized for processing

faces. Additionally, variation across species of wasps suggests that species with dominance hierarchies have the ability to recognize other individuals based on derived facial markings that vary among individuals. Species without hierarchies lack such markings and recognition abilities.

Attribute recognition

Most animals are able to recognize at least some attributes of conspecifics. In some cases, attribute recognition is in addition to individual recognition. However, in other cases, attribute recognition can occur in the absence of individual recognition. For example, the ability for males to detect when females are fertile is widespread in the animal kingdom. Additionally, many species (including plants) show the ability to differentiate kin from nonkin. Individual recognition is not required for either of these tasks as many species differentiate fertile from nonfertile animals and kin from nonkin based on simple cues (such as odors). However, in many animals attribute-recognition involves differentiating aspects of known individuals. For example, many primates appear to both recognize an individual and categorize that individual as kin or nonkin. In species with stable dominance hierarchies, animals must differentiate the animals that rank above and below themselves, and it appears that they do so by recognizing and then categorizing individuals. Furthermore, in some species (e.g., vervets and baboons) animals not only know where they themselves fit into the dominance hierarchy, but they also know exactly where other individuals fit into the dominance hierarchy. For example, an individual (ranked number 15) knows that the third-ranking individual out-ranks the fourth-ranking individual, and so on. Because rank is a relative measure, knowledge of others’ ranks must involve an understanding of third-party relationships (see below).

Third-party relationships

Some animals are sensitive to relationships among others. Such knowledge of third-party relationships is among the most sophisticated forms of social cognition, and it is undoubtedly important for predicting the behavior of social companions. For example, an individual baboon may be less likely to attack a juvenile baboon if the juvenile’s mother is sitting nearby – presumably due to a higher cost (i.e., the mother may retaliate). Third-party information is also necessary for choosing allies in alliance formation. If you are seeking an ally to attack another member of your group, you want to pick an ally that outranks and is unrelated to your opponent.

Some of the earliest evidence for third-party knowledge in animals comes from the work of Dorothy Cheney and Robert Seyfarth on wild vervet monkeys. Responses to playback experiments suggest that vervets know the kin relations of others, even beyond their own family unit.

This ability has now been demonstrated in baboons and hyenas. Captive research on captive primates has additionally shown that long-tailed macaques can learn to match pictures of mothers with their offspring and can generalize this pairing to new mother–infant pairs, suggesting they understand something about the underlying relationship between the animals.

In addition to kinship relations, there is a large body of evidence indicating that primates keep track of the relative dominance ranks of others as well. This ability was first demonstrated in wild baboons and has also been found in several macaque species as well as in hyenas. Captive studies suggest that macaques are very good at list learning and transitive inference – two abilities that might be involved in learning dominance ranks of others in the wild. Recently, it was shown that social corvids (jays) with dominance hierarchies can use transitive inference to infer the ranks of other conspecifics. Other evidence of complex social cognition comes from experiments indicating that baboons simultaneously recognize others' kin and dominance relations and thus appear capable of classifying others hierarchically according to both individual attributes and kin-group membership. Such studies support the hypothesis that sociality is cognitively challenging and can drive cognitive evolution. It is perhaps not surprising that sociality can have such far-ranging consequences given the central role that social relationships play in the lives of animals.

See also: Animal Models of Sexual Function; Behavioral Development and Socialization; Brain Evolution in

Vertebrates; Brain Stimulation and Addiction; Cooperation; Hormones and Female Sexual Behavior; Infant Bonding and Attachment; Mammalian Parental Behavior and Neurohormonal Determinants; Mating Behavior; Measuring Stress; Neural Basis of Gender; Neural Basis of Working Memory; Pleasure; Primate Origins of Human Behavior; Protein Synthesis and Memory; Sex Hormones, Mood, and Cognition; Social Cognition: From Behavior-Reading to Mind-Reading; Social Communication; Social Learning and Behavior Transmission; Stress and Reward.

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History of Behavioral Neuroscience

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Glossary

Cognition – The process of knowing, perceiving, etc. (Webster).

Emotion – Strong feeling (Webster).

Brain – The central nervous system, where all the processes listed below take place.

Learning – The acquiring of knowledge or skill (Webster).

Memory – The power or act of remembering (Webster).

Mind – Memory, the seat of consciousness in which thinking, feeling, etc., take place (Webster).

Motivation – That which causes one to act (Webster).

Behavioral neuroscience is literally the study of the neural bases of behavior, which forms a major discipline within the field of neuroscience and also within the field of psychology. By the same token, behavioral neuroscience has two historical roots, one in psychology – physiological psychology – and one in the brain sciences.

The great questions of philosophy – the mind–body problem and the nature of knowledge – were also the questions that drove early developments in physiological psychology. Wilhelm Wundt, who founded experimental psychology, entitled his major work *Foundations of Physiological Psychology* (1874; see 1908). William James, the other major figure in the development of modern psychology, devoted a third of his influential text *Principles of Psychology* (1890) to the brain and nervous system. Both Wundt and James studied medicine and philosophy, and both considered themselves physiologists. Their goal was not to reduce psychology to physiology but rather to apply the scientific methods of physiology to the study of the mind. The other driving force in early behavioral neuroscience was the study of the brain and nervous system.

The major topics in modern behavioral neuroscience are the neural bases of learning and memory, motivation and emotion, and cognition. A number of other areas began in behavioral neuroscience and have spun off to become fields in their own right. Thus, ‘sensation and perception’ have become separate fields (e.g., vision, audition, olfaction, etc.). We first sketch very briefly the recent philosophical and physiological roots.

The Mind

The history of such issues as the mind–body problem and epistemology is properly the domain of philosophy. Our focus in this brief section is on the history of the scientific study of the mind, which really began in the nineteenth century.

Psychophysics

Perhaps the first experimental attacks on the nature of the mind were the observations of Weber as generalized by Fechner. Ernst Weber, a physiologist, was attempting in 1834 to determine whether the nerves that respond to the state of the muscles also contribute to judgments about weights. As the reader knows, he found that the “just noticeable difference” (jnd) in weight that could be reliably detected by the observer was not some absolute amount but rather a constant ratio of the weight being lifted. The same applied to the pitch of tones and the length of lines.

Gustav Fechner realized that Weber had discovered a way of measuring the properties of the mind. Indeed, in his *Psychophysics* (1860; see 1966) he felt he had solved the problem of mind and body. He generalized Weber’s observations to state that as the psychological measurement in jnd’s increased arithmetically, the intensity of the physical stimulus increased geometrically – the relationship is logarithmic. Fechner, trained as a physicist, developed the classical psychophysical methods and the concepts of the absolute and differential thresholds. The methods Fechner developed were of great help to the early experimental psychologists like Wundt and his student Tichner in their attempts to measure the attributes of sensation.

Tichner identified the elements of conscious experience as quality, intensity, extensity, protensity (duration), and attensity (clearness) (see Tichner, 1898). But for all their attempts at scientific observation, the basic approach of Wundt and Tichner was introspection, and other observers (e.g., Külpe at Bonn) had different introspections. Edwin Boring studied with Tichner and was for many years chair of the Department of Psychology at

Harvard. He attempted to recast Tichner's views in more modern terms by emphasizing that the dimensions related to discrimination of physical stimuli. His student S.S. Stevens showed that trained observers could reliably form judgments of sounds in terms of pitch, loudness, 'volume,' and 'density.'

Stevens, at Harvard, later introduced an important new method of psychophysics termed direct 'magnitude estimation.' The subject simply assigned a number to a stimulus, a higher one to a more intense stimulus, and a lower number to a less intense stimulus. Somewhat surprisingly, this method gave very reliable results. Using this method, Stevens found that the proper relationship between stimulus intensity and sensation is not logarithmic, as Fechner had argued, but rather a power function: the sensation (i.e., sensory magnitude) equaled the stimulus intensity raised to some power, the exponent ranging from below to above one. This formulation proved very useful in both psycho-physical and physiological studies of sensory processes.

The key point of all this work on psychophysics is of course that it is not necessary to be concerned at all about subjective experience or introspection. The observer simply pushes a button, or states a word or number, to describe his/her judgment of the stimulus. The more the observer practices, the more reliable the judgments become and the more different observers generate the same results. Psychophysics had become purely behavioral.

Signal Detection

The modern era of psychophysics can perhaps be dated to a seminal paper by John Swets in 1961: "Is there a sensory threshold?" His answer was 'no.' He and David Green developed the theory and methodology of signal detection. There is always noise present with signals. When one attempts to detect a signal in noise, the criteria used will determine the outcome. This approach has proved immensely useful in fields ranging from the telephone to psychophysical studies in animals to detection of structural failures in aircraft wings to detection of breast cancer. But where is the mind in decision theory? It has disappeared. The initial hope that psychophysics could measure the mind has been reduced to considerations of observer bias. A similar conclusion led to the downfall of introspection.

The Brain

In the nineteenth century, debate focused on how mental activities (or cognitive processes) are organized in the brain. An early idea, which became known as the localizationist view, proposed that specific mental functions were carried out by specific parts of the brain. An alternative idea, which became known as the equipotential view, held

that large parts of the brain were equally involved in all mental activity and that there was no specificity of function within a particular brain.

The 'Thinking Machine' to Phrenology

Our concepts of mind, soul, body, and brain relationships come from a long history, and for the Western world, from a long battle between church dogma and progress of science. During the Renaissance, physicians began to explore the body. They dissected the brain and described it similarly to the other organs, emphasizing an intestine-like, random appearance of the convolutions. A landmark conceptual advance was René Descartes, who conceived of the 'thinking machine' (*la machine pensante*) about a hypothetical machine in the brain. In his treatise *De Homine* (1632–33) he wrote: I would like you to consider that all the functions I have allocated to this machine all depend by nature from the arrangement of the organs – – no more no less as the movements of a clock do, or an automaton moved by its counterweights and its cog-wheels – ... so that we do not have to conceive in the inner world of the body a soul or any other principle of movement or of life... other than of its blood and its spirits moved by the heat of the fire that burns permanently in the blood and that is not of a different nature of what exists in the inanimate matters". And also "... I suppose that our body is nothing else than a statue or a machine made of organic substances..." To move this thinking machine, and to be in accordance with the church, Descartes argued that the soul was necessary, but he put it in the pineal gland, a convenient organ in the middle of the brain and on its surface... and close to the ventricles. At that time, the ventricles were where the 'functional regions' for all of the faculties were organized, and the part of that brain was considered the common site for the sensations, reason, and memory. The sensations were created in the first 'cell' of the ventricles; they were transformed in the second cell (reasoning); and what remained was placed in the third cell (memory). For the Catholic Church to integrate and incorporate the soul was too much, and Descartes had to escape to Amsterdam to save his life.

A very important transition was the work of Nicolas Stenon (1638–86), one of the greatest and most famous anatomists of his time. He stated in a meeting in Paris as part of a strong critique of the pineal gland hypothesis, "There are only two ways to succeed in the knowledge of the machine in the brain: either the Master who conceived it deliver to us His secret for this ingenious device, or we dismantle piece by piece all its springs and examine them separately, exactly as we do for the other machines, and then, in a second time we will consider what these pieces can do as they work together". From that point, an enormous effort began to understand and

describe the different pieces of the brain and attribute functions to those pieces. Thus, by the eighteenth century, the search for the role and function of the small machines in the brain were already underway, supported by materialist and monist (as opposed to dualist) points of view.

Perhaps the most influential idea about localization of brain function derived from Franz Joseph Gall during the early part of the nineteenth century. Gall's insight was that, despite its similarity in appearance, brain tissue was not equipotential but instead was actually made up of many discrete areas that had different and separate functions. Eventually, Gall was able to characterize 27 different regions, or organs, of the brain in a scheme that he called organology. (Later, the term 'phrenology' came to be associated with Gall's work.) However, this term was coined by Gall's colleague, Spurzheim, with whom he had a falling out, and Gall himself never used the term.

Gall's ideas about the localization of cognitive functions began to tear at the religious and social fabric of the nineteenth century. In particular, his notion that various mental faculties were represented in different places in the brain was seen by various governmental and religious authorities as being in conflict with moral and religious views of the unity of the soul and mind. (In some ways this is reminiscent of current attacks on evolution by fundamentalist religious groups.)

Gall's theory had a limited factual basis. The way in which Gall had determined the locus and extent of each of the 27 organs was questionable, to say the least. Another scientific issue raised by critics during the nineteenth century was the fact that Gall never specified the precise extent or the anatomical borders of any of the organs. This lack of rigor, it was argued, made it impossible to correlate a specific faculty with the size of an organ or cranial capacity. Related criticisms involved Gall's seeming failure to acknowledge that there were variations in the thickness of the skull (i.e., variations from one individual specimen to another and from one locus to another within the same skull).

In a sense, Gall seemed to be vindicated in 1861 with the publication of Broca's discovery of the anterior speech area, now termed Broca's area, in his study of the patient Monsieur Leborgne. (This patient subsequently was referred to by the name 'Tan,' the only utterance Broca ever heard Monsieur Leborgne make; [Broca 1861](#).) Broca's finding from his patient Tan has been regarded by some historians as the most important clinical discovery in the history of cortical localization. Moreover, within the decade, what some historians regard as the most important laboratory discovery pertaining to cortical localization was reported (i.e., Gustav Fritsch and Eduard Hitzig discovered the cortical motor area in the dog). This proved that cortical localization was not restricted to a single function. The discoveries of the

speech area by Broca and the motor area by Fritsch and Hitzig were seen as support for Gall's ideas and reestablished him as the father of localization.

Evolution of Phrenology

Thus, phrenology continued into the nineteenth century from the early arguments that: (1) the mental functional organs definitively migrate from the ventricle to the gray substance, the "cerebral lobes" and (2) "attention, memory, imagination . . . are not primary faculties of mind BUT solely forms of activities of any intellectual faculty." Phrenology has been at the origin of a 'vertical' and a modular organization of the brain, one with the definitive implementation of the mind into matter. Paul Broca inherited directly from these hypotheses. A second phrenological revolution came with Wernike when he claimed that aphasia resulted from (dis)connections between modules and then evolved the modern hypothesis of function resulting from connections and fibers.

Phrenology further matured into the pursuit of function during the twentieth century. Spearman (1937) argued that "the basic idea of the scientific enterprise consists in reducing the countless actual activities to a small number of underlying separable principles named faculties or capacities."

Herbert Simon argued in his parable of the two watch makers, Hora and Tempus, "The time required for the evolution of a complex form from simple elements depends critically on the number and potential intermediate stable forms." David Marr (1976) stated, "A large computation should be split as a connection of small subparts that are as clearly independent of one another as the overall task allows . . . if not, a small change in one place will have consequences in many other places."

Following the pioneering study by [Fritsch and Hitzig \(1870\)](#) on the localization and organization of the motor area of the cerebral cortex, localization of function quickly won the day, at least for sensory and motor systems. In the last three decades of the nineteenth century, the general locations of the visual and auditory areas of the cortex were identified. The field of physiology, in particular neurophysiology, for example, in the work of Sir Charles Sherrington, together with clinical neurology and neuroanatomy, were exciting new fields at the beginning of the twentieth century.

At this time, the only experimental tools for studying brain organization and functions were ablation and electrical stimulation. Neuroanatomy was in its descriptive phase; thanks in part to the Golgi method, the monumental work of Ramon y Cajal was completed over a period of several decades beginning near the end of the nineteenth century. Neurochemistry was in its descriptive phase, characterizing chemical substances in the brain.

Finally, the birth of neuropsychopharmacology, involving the discovery of the first antipsychotic and then of the biogenic amines in Sweden (1963–1964), has stimulated much research in the realm of ‘brain and behavior,’ leading to a sort of parallel ‘chemical phrenology.’ While it is now clear that the highly distributed monoamine systems have more specific actions related to the regions of the brain they innervate, residual roles for serotonin in depression, dopamine in incentive salience, and norepinephrine in arousal still carry significant weight today.

Neurophysiology

The first recording of a nerve action potential with a cathode ray tube was done by Gasser and Erlanger in 1922, but the method was not much used until the 1930s. The human EEG was rediscovered in 1929 by H. Berger, and the method applied to animal research and human clinical neurology, particularly epilepsy, in the 1930s, for example, by Alexander Forbes, Hallowell Davis, and Donald Lindsley.

The pioneering studies of Adrian in England (1940) and of Wade Marshall, Clinton Woolsey, and Philip Bard (1941) at Johns Hopkins were the first to record electrically evoked potentials from the somatic sensory cortex in response to tactile stimulation. Woolsey and his associates developed the detailed methodology for evoked potential mapping of the cerebral cortex. In an extraordinary series of studies, Woolsey and his colleagues determined the localization and organization of the somatic sensory areas, the visual areas, and the auditory areas of the cerebral cortex in a comparative series of mammals. They initially defined two projection areas (I and II) for each sensory field (i.e., two complete functional maps of the receptor surface were found for each sensory region of the cerebral cortex; e.g., two complete representations of the skin surface in the somatic–sensory cortex).

In the 1940s and 1950s, the evoked-potential method was used to analyze the organization of sensory systems at all levels, from the first-order neurons to the cerebral cortex. The principle that emerged was strikingly clear and simple: in every sensory system, the nervous system maintained receptorotopic maps or projections at all levels from receptors (skin surface, retina, and basilar membrane) to cerebral cortex. The receptor maps in the brain were not point-to-point; rather they reflected the functional organization of each system (fingers, lips, and tongue areas were much enlarged in primate somatic cortex; half the primary visual cortex represented the fovea, and so on).

The Microelectrode

The evoked potential method was very well suited to analysis of the overall organization of sensory systems in

the brain. However, it could reveal nothing about what the individual neurons were doing. This had to await development of the microelectrode (a very small electrode that records the activity of a single cell). Indeed, the microelectrode has been the key to analysis of the fine-grained organization and ‘feature detector’ properties (most neurons respond only to certain aspects or features of a stimulus) of sensory neurons. The first intracellular glass pipette microelectrode was actually invented by G. Ling and R.W. Gerard in 1949 – they developed it to record intracellularly from frog muscle.

Metal electrodes were generally found to be preferable for extracellular single-unit recording (i.e., recording the spike discharges of a single neuron where the tip of the microelectrode is outside the cell but close enough to record its activity clearly). Metal microelectrodes were improved in the early 1950s. R.W. Davies at Hopkins developed the platinum–iridium glass coated microelectrode, and D. Hubel and T. Wiesel at Harvard developed the tungsten microelectrode. The search for putative stimulus coding properties of neurons was on. The pioneering studies were those of V. Mountcastle and associates at Hopkins on the organization of the somatic–sensory system, those of [Hubel and Wiesel \(1959\)](#) at Harvard on the visual system, and J.E. Rose, J.E. Hind, C.N. Woolsey, and associates at Wisconsin on the auditory system.

Thanks to the microelectrode and the careful and painstaking studies of a number of investigators, we now know that each sensory ‘area’ of the cerebral cortex consists of a number of subfields, separate areas coding different aspects of the stimulus. There are now more than 30 functionally distinct areas within the visual cortex of monkey and human (Zola-Morgan, 1995). This is localization of function with a vengeance!

It was not until many years later that imaging methods were developed to study the organization and functions of the normal human brain (see below). Heroic studies had been done on human brain functioning much earlier in neurosurgical procedures (heroic both for the surgeon and the patient; e.g., Penfield and Rasmussen, 1950). However, these patients typically suffered from severe epilepsy. The development of positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and other modern techniques is largely responsible for the current explosion of information in cognitive neuroscience (see below).

Learning and Memory

Karl Lashley is the most important figure in the development of physiological psychology and the biology of memory in America. He obtained his PhD at Johns Hopkins University. At Hopkins, he studied with John

Watson and was heavily influenced by Watson's developing notions of behaviorism. While there he also worked with Sheherd Franz at a government hospital in Washington – they published a paper together in 1917 on the effects of cortical lesions on learning and retention in the rat. Lashley then held teaching and research positions at the University of Minnesota (1917–26), the University of Chicago (1929–35), and then at Harvard from 1935 until his death in 1958. During the Harvard years, he spent much of his time at the Yerkes Primate Laboratory in Orange Park, Florida.

Lashley devoted many years to an analysis of brain mechanisms of learning, using the lesion-behavior method, which he developed and elaborated from the work with Franz. During this period, Lashley's theoretical view of learning was heavily influenced by two congruent ideas: localization of function in neurology and behaviorism in psychology. Lashley describes the origins of his interest in brain substrates of memory and Watson's developing views of behaviorism in the following letter written to Ernest Hilgard in 1935:

In 1914, I think, Watson called attention of his seminar to the French edition of Bechtereve, and that winter the seminar was devoted to translation and discussion of the book. In the spring I served as a sort of unpaid assistant and we constructed apparatus and planned experiments together. We simply attempted to repeat Bechtereve's experiments. We worked with withdrawal reflexes, knee jerk, pupil. Watson took the initiative in all this, but he was also trying to photograph the vocal cord, so I did much of the actual experimental work. I devised drainage tubes for the parotid and submaxillary ducts and planned the salivary work which I published. As we worked with the method, I think our enthusiasm for it was somewhat dampened. Watson tried to establish conditioned auditory reflexes in the rat and failed. Our whole program was then disrupted by the move to the lab in Meyer's clinic. There were no adequate animal quarters there. Watson started work with the infants as the next best material available. I tagged along for awhile, but disliked the babies and found me a rat lab in another building. We accumulated a considerable amount of experimental material on the conditioned reflex which has never been published. Watson saw it as a basis for a systematic psychology and was not greatly concerned with the nature of the reaction itself. I got interested in the physiology of the reaction and the attempt to trace conditioned reflex paths through the nervous system started my program of cerebral work. (Letter of May 14, 1935, K.S. Lashley to E.R. Hilgard, reproduced with the kind permission of E.R. Hilgard).

It was in the previous year, 1913, that Watson published his initial salvo in an article entitled, "Psychology

as the behaviorist views it." He was elected President of the American Psychological Association in 1914.

As we noted earlier, localization of function in the cerebrum was the dominant view of brain organization at the beginning of the twentieth Century. In Watson's behaviorism, the learning of a particular response was held to be the formation of a particular set of connections, a series set. Consequently, Lashley argued, it should be possible to localize the place in the cerebral cortex where that learned change in brain organization was stored – the engram. (It was believed at the time that learning occurred in the cerebral cortex.) Thus, behaviorism and localization of function were beautifully consistent; they supported the then notion of an elaborate and complex switchboard where specific and localized changes occurred when specific habits were learned.

Lashley set about systematically to find these learning locations – the engrams – in a series of studies culminating in his 1929 monograph *Brain Mechanisms of Intelligence*. In this study, he used mazes differing in difficulty and made lesions of varying sizes in all different regions of the cerebral cortex of the rat. The results of this study profoundly altered Lashley's view of brain organization and had an extraordinary impact on the young field of physiological psychology. The locus of the lesions is unimportant; the size is critically important, particularly for the more difficult mazes. These findings led to Lashley's two theoretical notions of equipotentiality and mass action (i.e., all areas of the cerebral cortex are equally important, at least in maze learning; what is critical is the amount removed).

Lashley's interpretations stirred vigorous debate in the field. Walter Hunter, an important figure in physiological-experimental psychology at Brown University who developed the delayed response task in 1913, argued that in fact the rat was using a variety of sensory cues – as more of the sensory regions of cortex were destroyed, fewer and fewer cues became available. Lashley and his associates countered by showing that removing the eyes has much less effect on maze learning than removing the visual area of the cortex. Others argued that Lashley removed more than the visual cortex. Out of this came the long series of lesion-behavior studies analyzing behavioral 'functions' of the cerebral cortex. Beginning in the 1940s, several laboratories, including Lashley's and those of Harry Harlow at the University of Wisconsin and Karl Pribram at Yale, took up the search for the more complex functions of association cortex using monkeys and humans.

Lashley's pessimistic conclusions in his 1929 monograph subsequently put a real but temporary damper on the field concerned with brain substrates of memory:

This series of experiments has yielded a good bit of information about what and where the memory trace is

not. It has discovered nothing directly of the real nature of the memory trace. I sometimes feel, in the reviewing the evidence of the localization of the memory trace, that the necessary conclusion is that learning is just not possible. It is difficult to conceive of a mechanism that can satisfy the conditions set for it. Nevertheless, in spite of such evidence against it, learning sometimes does occur. (Lashley, 1950, pp. 477–478).

Milner and H.M.

Perhaps the most important single discovery in this field is Brenda Milner's studies with patient H.M. who, following bilateral temporal lobectomy (removing the hippocampus and other structures), lived forever in the present (see below). Work on higher brain functions in monkeys and humans is one of the key roots of modern cognitive neuroscience, to be discussed below. Since the discovery of H.M., the hippocampus has been of particular interest in biological psychology. Another facet of hippocampal study in the context of the biological psychology of memory is long-term potentiation (LTP), discovered by Bliss and Lomo (1973). Brief tetanic stimulation of monosynaptic inputs to the hippocampus causes a profound increase in synaptic excitability that can persist for hours or days. Many view it as a leading candidate for a mechanism of memory storage, although direct evidence is still lacking.

Yet another impetus to the study of the hippocampus is the remarkable discovery of 'place cells' by John O'Keefe (see O'Keefe, 1979). When recording from single neurons in the hippocampus of the behaving rat, a given neuron may respond only when the animal is in a particular place in the environment (e.g., in a box or maze), reliably and repeatedly. There is great interest now in the possibility that LTP may be the mechanism that forms place cells. A number of laboratories are making use of genetically altered mice to test this possibility.

Riesen and Hebb

Lashley's influence is felt strongly through the many eminent contemporary physiological psychologists who worked or had contact with him. We select two examples here: Austin Riesen and Donald O. Hebb. The basic problem of the development of perception fascinated Lashley and his students. How is it that we come to perceive the world as we do? Do we learn from experience, or is it told to us by the brain? Riesen did the pioneering studies of raising monkeys for periods of time in the dark and then testing their visual perception. They were clearly deficient.

This important work served as one of the stimuli for Donald Hebb to develop a new theory of brain

organization and function, *The Organization of Behavior*, published in 1949. This book had an immediate and profound impact on the field. Hebb effectively challenged many traditional notions of brain organization and attempted to pull together several discordant themes – mass action and equipotentiality, the effects of dark rearing on perception, the preorganization of sensory cortex, the lack of serious intellectual effects of removal of an entire hemisphere of the brain in a human child – into a coherent theory. Important influences of Gestalt notions can be seen in Hebb's theory. He is a connectionist but in a modern sense – connections must underlie brain organization, but there is no need for them to be in series.

One concept in Hebb's book has come to loom large (too large perhaps) in modern cognitive-computational neuroscience: The Hebb synapse:

When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased (Hebb, 1949, p. 62).

Pavlov and Classical Conditioning

But there were other major traditions developing. Perhaps the most important of these was the influence of Pavlov. His writings were not readily available to Western scientists, particularly Americans, until the publication of the English translation of his monumental work *Conditioned Reflexes* in 1927. It is probably fair to say this is the most important single book ever published in the field of behavioral neuroscience. Pavlov developed a vast and coherent body of empirical results characterizing the phenomena of conditioned responses, which he termed "psychic reflexes." He argued that the mind could be fully understood by analysis of the higher order learned reflexes and their brain substrates. As an example of his influence, Clark Hull, in his *Principles of Behavior* (1943) wrote as though he were a student of Pavlov.

W. Horsley Gantt, an American physician, worked with Pavlov for several years and then established a Pavlovian laboratory at Johns Hopkins. He trained several young psychologists (e.g. Roger Loucks and Wulf Brodgen) who became very influential in the field. Perhaps the most important modern behavioral analyses of Pavlovian conditioning are the works of Robert Rescorla and Allan Wagner (e.g., Rescorla and Wagner, 1972).

Although Pavlov worked with salivary secretion, most studies of classical conditioning in the West tended to utilize skeletal muscle response, a la Bechtereiv. Particularly productive have been Pavlovian conditioning of discrete skeletal reflexes (e.g., the eyeblink response)

characterized behaviorally by Isadore Gormezano and Allan Wagner and analyzed neuronally by Richard Thompson and his many students, showing localization of the basic memory trace to the cerebellum, specifically the cerebellar interpositus nucleus (Thompson, 1986; Christian and Thompson, 2003). Masao Ito and associates in Tokyo had discovered the phenomena of long-term depression (LTD) in the cerebellar cortex (see Ito, 1984). Repeated conjunctive stimulation of the two major inputs to the cerebellum, mossy-parallel fibers and climbing fibers, yields a long-lasting decrease in the excitability of parallel fibers – Purkinje neuron synapses. Ito developed considerable evidence that this cerebellar process underlies plasticity of the vestibular–ocular reflex. Thompson and associates developed evidence, particularly using genetically altered mice, that cerebellar cortical LTD is a key cortical mechanism modulating classical conditioning of eyeblink and other discrete responses.

Fear Conditioning and Consolidation

Fear conditioning was characterized behaviorally by Neal Miller and analyzed neuronally by several groups, particularly Michael Davis (1992), Joseph LeDoux (2000), and Michael Fanselow (1994) and their many students. They showed that at least for classical conditioning of fear, the essential structure is the amygdala, which may contain the basic memory trace for this form of learning. The process of LTP may serve to code the amygdalar fear memory.

Duncan's discovery of the effects of electroconvulsive shock on retention of simple habits in the rat, in 1949, began the modern field of memory consolidation. Hebb and Gerard were quick to point out the implication of two memory processes, one transient and fragile and the other more permanent and impervious. McGaugh and his associates have done the classic work on the psychobiology of memory consolidation (McGaugh, 1989). McGaugh and his colleagues introduced posttraining treatment and demonstrated memory facilitation with drugs and showed that these effects were not due to effects on performance or to possible reinforcement effects of the drugs (and similarly for impairment due to electroconvulsive shock (ECS) impairment).

The amygdala is critical for instrumental learning of fear. James McGaugh and his associates demonstrated that for both passive and active avoidance learning (animals must either not respond, or respond quickly, to avoid shock), amygdala lesions made immediately after training abolished the learned fear. Surprisingly, if these same lesions were made a week after training, learned fear was not abolished, consistent with a process of consolidation (see McGaugh, 2000). The apparent difference in the role of the amygdala in classical and instrumental learning of fear remains a puzzle to this day.

Chemical approaches to learning and memory are recent. The possibility that protein molecules and RNA might serve to code memory was suggested some years ago by such pioneers as Gerard and Halstead. The RNA hypothesis was taken up by Hyden and associates in Sweden and by several groups in America. An unfortunate by-product of this approach was the ‘transfer of memory’ by RNA. These experiments, done by investigators who shall remain nameless, in the end could not be replicated.

At the same time, several very productive lines of investigation of neurochemical and neuroanatomical substrates of learning were developing. In 1953, Krech and Rosenzweig began a collaborative study of relationships between brain chemistry and behavior. Krech did classic early work in animal learning (under his earlier name, Kreshevsky) and was a colleague and collaborator with Tolman. Soon after they began their joint work in 1953, they were joined by E.L. Bennett and later by M.C. Diamond. Their initial studies concerned brain levels of acetylcholinesterase in relation to the hypothesized behavior and included analysis of strain differences (see Krechel *et al.*, 1960). They discovered the striking differences in the brains of rats raised in ‘rich’ versus ‘poor’ environments. William Greenough (1984), at the University of Illinois, replicated and extended this work to demonstrate dramatic morphological changes in the structures of synapses and neurons as a result of experience.

Where does the field now stand relative to the key issue of the localization of function, in this case of memory traces, the neuronal substrates for memory? It is now clear that memory traces for more elementary forms of learning and memory do have specific loci in the brain. The memory traces for classical conditioning of eyeblink and other discrete responses are stored in localized regions of the cerebellum. The traces for fear conditioning appear to be stored in localized regions of the amygdala. But for more complex forms of learning, the answer is still out. Certain brain structures (e.g., hippocampus) are critical for initial learning of declarative memories (e.g., experiential and semantic memories; see below) but the memory traces are not stored permanently there, but rather hypothesized to be stored in the cerebral cortex. Hebb's notion of distributed sets of traces, each with some specificity, but overlapping among the neurons of the cerebral cortex, continues to have appeal. This is high on the agenda for the next generation of behavioral neuroscientists to solve.

Model Systems

The use of model biological systems has been an important tradition in the study of neural mechanisms of elementary forms of behavioral plasticity and learning.

This approach has been particularly successful in the analysis of habituation – itself a very simple form or model of learning (see article on habituation). Sherrington did important work on flexion reflex ‘fatigue’ in the spinal animal at the turn of the century. In 1936, Prosser and Hunter completed a pioneering study comparing habituation of startle response in intact rat and habituation of hindlimb flexion reflex in spinal rat. They established, for habituation, the basic approach of Sherrington, namely that spinal reflexes can serve as models of neural–behavioral processes in intact animals. Sharpless and Jasper (1956) established habituation as an important process in electroencephalographic (EEG) activity. Modern Russian influences have been important in this field – the key studies of Evgeny Sokolov (1963), first on habituation of the orienting response in humans and more recently on mechanisms of habituation of responses in the simplified nervous system of the snail.

The defining properties of habituation were clearly established by Thompson and Spencer in 1966, and the analysis of mechanisms began. Several laboratories using different preparations – *aplysia* withdrawal reflex (e.g., Kandel and his many associates; see Kandel, 1976), vertebrate spinal reflexes (e.g., Thompson, Spencer, and Farel), crayfish tail flip escape (e.g., Krasne and Kennedy; see Krasne, 1969) – all arrived at the same underlying synaptic mechanism for short-term, within-session habituation, a decrease in the probability of transmitter release from presynaptic terminals in the habituating pathway. Habituation, at least short-term habituation, is thus a very satisfying field. Agreement ranges from defining behavioral properties to synaptic mechanisms. However, the mechanisms underlying long-term, between-session habituation are not yet known (Ezzeddine and Glanzman, 2003).

History of Motivation and Emotion

Emotion can be defined as “a psychic and physical reaction (as anger or fear) subjectively experienced as strong feeling and physiologically involving changes that prepare the body for immediate vigorous action” (*Webster’s Ninth New Collegiate Dictionary*, 1984). Although the introspective emphasis on the ‘feeling’ aspect of emotions played a prominent role in the development of many theories of emotion, much progress in the neurobiology of emotion has been made with the experimental analysis of behavioral phenomena objectively defined. Indeed, Darwin argued as early as 1872 that both observable expressions of emotions as well as underlying brain processes (direct action of the excited nervous system on the body are not unique to humans) (Darwin, 1872). Key emotional expressions were considered innate, instinctive

recognition of expression but subject to the evolutionary process:

That the chief expressive actions, exhibited by man and by the lower animals, are now innate or inherited – that is, have not been learnt by the individual – is admitted by every one. So little has learning or imitation to do with several of them that they are from the earliest days and throughout life quite beyond our control; for instance, the relaxation of the arteries of the skin in blushing, and the increased action of the heart in anger (Darwin, 1872, chapter 14).

Emotions: The Search for Biological Bases

Historically, for emotional behavior, the measurement of bodily changes associated with emotional behavior focused on a peripheral response mechanism largely related to the autonomic and endocrine system. Peripheral measures of emotion ranged from galvanic skin responses to heart rate to salivary secretion to levels of autonomic hormones. Consistent with a key role of peripheral physiological variables was the famous William James theory of emotion in which he argued, “Bodily changes follow directly the *perception* of the exciting fact, and that our feeling of the same changes as they occur in *is* the emotion” (James, 1884, p. 189–190).

Nevertheless the brain was pulled into serious consideration as a key mediator of emotion by parallel advances in conceptual framework, neuroanatomy, and actual experimental studies, some of which were initiated early in the history of behavioral neuroscience. In the latter half of the nineteenth century, Ferrier (1875) showed that orbitofrontal ablations in monkeys had no major effect on an organism’s sensory abilities but produced a definite change in the disposition of the animal. Broca (1878) described the “grand lobe limbique” (limbic indicates that this lobe surrounds the brain stem) which included the olfactory tubercle, prepyriform cortex, diagonal band of Broca, septal region, hippocampus, and cingulate as a common circuit in all mammals. Brown and Shafer (1888) showed that certain brain areas, such as the temporal lobe, were involved in emotion in which lesions of the temporal lobe in rhesus monkeys again had little effect on sensory abilities but produced a loss of ‘intelligence.’ The demonstration of decorticate ‘sham rage’ in the 1920s led to the hypothesis that emotional expression involved specific subcortical structures. Lesion studies led the way, followed later by stimulation studies by Hess, Ranson, and Masserman, all of which pointed to subcortical structures, such as the hypothalamus, soon to be labeled ‘limbic’ structures in the neural circuitry of the expression of emotional responses.

Early on, Cannon argued against the James-Lange theory, largely on the basis of the observation that animals continued to express emotional behavior in the absence of information from the periphery. Later, he hypothesized that emotional experience and emotional behavior were a release from cortical inhibition of neural impulses originating in the thalamus (Cannon, 1927, p. 120). Bard removed the neocortex of cats, leaving the rhinencephalon intact, which produced placidity that could be changed to ferocity by removal of the amygdaloid complex (Bard, 1951). Bard's extensive work made modifying Cannon's theory possible so that it could better define the neurocircuitry of emotional behavior.

A key influential study in 1937 by Kluver and Bucy found dramatic emotional changes with extensive temporal lobe lesions in rhesus monkeys, in which normally intractable animals became tame and friendly, were compulsively mouthing anything, and were markedly hypersexual. Concurrently, the elaboration of the now famous Papez circuit was proposed in 1937 as a circuit for emotion and evolved into the terminology and conceptual framework of the limbic system which remains today. The Papez circuit, discovered by injecting rabies virus into the cat hippocampus and monitoring its progression, included the hippocampus, mammillary bodies, anterior thalamus, cingulate gyrus, and parahippocampal gyrus. It was one of the first organized attempts to delineate cortical mechanisms involved in emotion. Thus, the limbic system came to represent not only Broca's 1878 grand lobe limbique, but also most allocortical regions of the brain from the Papez circuit, and from the conceptualizations of MacLean (1949) the amygdala and the hypothalamus for emotional expression. Brain circuitry explorations of emotion via interventions (lesions) evolved into more modern approaches, such as neuropharmacological challenges and probes and imaging of the human brain.

Recent Perspectives of the Biological Bases of Emotion

Key conceptual advances that laid the foundation for the modern neuroscience of emotion was the suggestion by Schachter and Singer (1962) that cognitive factors may be major determinants of emotional states. More specifically, they showed that cognition arising from the immediate emotional experience, as interpreted by past experience, provides the framework for labeling one's feelings, and thus cognition determines whether a state of physiological arousal will be labeled as a given emotion. Schachter in fact predicted the direction of research on emotion:

We will be forced to deal with concepts about perception, about cognition, about learning, and about the social situation. We will be forced to examine a subject's perception of his bodily state and his interpretation of it in

terms of his immediate situation and his past experience (Schachter, 1975, p. 561).

A strong argument for a biological basis for emotion also came from the pioneering studies of Ekman and Friesen (1986), in which a universality of six emotions was proposed based on extensive cross-cultural work on facial expression: happiness, surprise, fear, sadness, anger, and disgust combined with contempt. Ekman hypothesized distinctive patterns of central nervous system activity. A watershed translational approach far ahead of the field was the pioneering work of Jaak Panksepp identifying similar emotional states in rodents, including distress, anger, social bonding, play, and laughter (Panksepp, 1998). Others, such as Russell (2003), have eschewed a specific categorization of emotion and argued that any emotionally charged event is a state experienced as simply feeling good or bad, energized or enervated – in other words, a free-floating mood or core affect that is subject to interpretation by the perception of affective quality. In this context, the modern somatic marker hypothesis of Damasio (1996) in a sense validates some aspects of the original James-Lange theory in which decision-making is a process that is influenced by marker signals that arise in bioregulatory processes, including those that express themselves in emotions and feelings.

Brain Imaging and Emotion

Modern brain imaging studies have not only confirmed earlier work from lesion studies but are extending the circuitry to domains of emotion previously inaccessible in animal studies. Morris *et al.* (1996) showed that the amygdala in humans responds differentially in subjects shown facial expressions of fear and happiness, with the neuronal response in the left amygdala significantly greater to fearful versus happy faces. Damasio (2002) in a series of studies has argued that the term 'emotion' should be defined as specific and consistent collections of physiological responses triggered by certain brain regions when the organism is presented with a certain situation. The substrates for the representation of emotions include homeostatic circuitry in the brainstem, hypothalamus, basal forebrain, amygdala, ventromedial prefrontal cortex, and cingulate cortex. By contrast, Damasio defined 'feelings' as the mental states that arise from the neural representation of the collection of responses that constitute an emotion, and as such should be reserved for the private, mental experience of an emotion. Key structures involved in feelings, he argued, include the brainstem, hypothalamus, thalamus, cingulate, somatosensory cortices of the insula, and somatosensory I and II, and to monitor cognitive processing, the prefrontal cortex is engaged. This approach has led to arguments in which specific brain systems,

including the posteromedial cortices (precuneus, posterior cingulated cortex, and retrosplenial region) and anterior insula, are recruited in addition to the basic homeostatic circuitry for specific types of emotions, such as social emotions (e.g., admiration and compassion):

Overall, the finding that homeostatic regulatory mechanisms are engaged in the experience of admiration and compassion supports the hypothesis that social emotions use some of the same basic devices involved in primary emotions (Moll *et al.*, 2005) and the salience system (Seeley *et al.*, 2007) (Immordino-Yang *et al.*, 2009, p. 8024).

Behavioral Analysis of Emotion: Bridge to Motivation

The role of emotion in motivation also profited from conceptual and experimental advances. The behavioral analysis of motivational and emotional interactions championed by Brady (1978) led to a response-inferred foundation for a unifying operational framework relating motivational and emotional function that fueled the dramatic advances forming the experimental analysis of behavior:

Even the ‘hedonic’ characteristics of motivational functions can be accommodated within the empirical framework of this conceptual analysis by appealing to the experimentally based distinctions between ‘positive’ and ‘negative’ reinforcement operations. The evident byproducts (e.g., ‘euphoria’) of ‘appetitive’ consequating relations which increase the likelihood of behavior, on the one hand, and the ‘dysphoric’ accompaniments of ‘aversive’ consequences which weaken behavior (or strengthen escape and avoidance performances), would seem to provide a fruitful point of departure for the experimental analysis of this eudaemonic dimension (Brady and Emurian, 1978, p. 83).

Here, the basic building blocks are the contingencies relating ‘occasions’ and their ‘consequences’ (three-term contingency of Skinner: occasion, behavior, and consequences), and thus a motivational function would be any operation that would affect the potency of the ‘consequences’ (e.g., food deprivation potentiates the consequence or increases the likelihood of consummatory behaviors). Under this formulation, the analysis of emotional function focuses on procedures that affect the efficacy of occasioning events or enhance the discriminability of occasioning events in the occasion component of the contingency (e.g., startle effects may disrupt the occasioning situation and decrease the likelihood of a consummatory behavior). Such emotional functions emphasize the prominent role of inner events (e.g., feelings), and as such, terms related to hedonic function can be accommodated in terms of positive reinforcement, negative reinforcement, and punishment. Thus, the hedonic dimension of Brady’s

“behavioral universe” refers primarily to the affective valence of the bridge between emotion and motivation. Indeed, emotions are often linked to motivation but not necessary for motivation.

History of Motivation

Consistent with this view, an early motivational theory involving a key emotional component was the Miller (1948) and Mowrer (1950) two-process fear theory in which fear consisted of “an innate (internal) response to certain stimuli, such as pain, and the fear response innately produces the fear stimulus, just as electric shock produces pain”; or, in other words, the conditioned stimulus serves as a danger signal and elicits a state which has been described as fear. For example, a conditioned stimulus that produces fear then elicits a fear-reducing response, and the fear emotion is an explicit part of the motivation needed for conditioned response learning. Finally, Olds and Milner (1954) discovered that electrical stimulation of the medial forebrain bundle was highly rewarding to animals, providing a key experimental bridge of the neurobiology of emotion with the neurobiology of motivation. Early on theorists such as J. Anthony Deutsch argued that brain stimulation reward activated two pathways: a pathway conveying reinforcement and a pathway conveying excitation (motivation), another early link between emotion (hedonic function) and motivation (Deutsch, 1960).

Motivation, similar to emotion, is a concept that has many definitions. An early definition reflected the mixed dualism of later theories. Motivation was defined as “an inner psychological process or function, a driving force to be found chiefly within the organism itself and a plan, purpose or ideal with the definite implication of an ideational element,” that may not be consciously and overtly recognized (Perrin, 1923). Hebb argued that motivation is “stimulation that arouses activity of a particular kind” (Hebb, 1949), and Richter argued that “spontaneous activity arises from certain underlying physiological origins and such ‘internal’ drives are reflected in the amount of general activity” (Richter, 1927). Bindra defined motivation as a “rough label for the relatively persisting states that make an animal initiate and maintain actions leading to particular outcomes or goals” (Bindra, 1976). A more behavioristic view is that motivation is “the property of energizing behavior that is proportional to the amount and quality of the reinforcer” (Kling and Riggs, 1971). Finally, a more neurobehavioral view is that motivation is a “set of neural processes that promote actions in relation to a particular class of environmental objects” (Bindra, 1976).

These definitions trace the history of motivation and point to certain common characteristics of our concept of motivation. It is a state that varies with arousal and guides

behavior in relationship to changes in the environment. The environment can be external (incentives) or internal (central motive states or drives), and such motivation or motivational states are not constants and vary over time.

Influential Theories of Motivation

An early and influential theory of motivation by Hull (1943), termed the ‘drive-reduction theory,’ was predicated on the hypothesis of homeostatic mechanisms of motivation in which behavior could be regarded as an outward expression of the organism’s pursuit of biological health. Here, all motivation was theorized to derive from biological imbalances or needs. A need in this formulation, such as hunger (here, the need is for more energy), was a biological requirement of the organism. Motivation, according to Hull, then aimed at making up for or erasing a deficiency or lack of something in the organism. The word ‘drive’ was used to describe the state of behavioral arousal resulting from a biological need and was the energy that powered behavior. The animal searched for food to reduce the hunger drive. Hull theorized that the animal would repeat any behavior that reduced a drive, whenever such a need arose again. Drive-reduction theory was not supported by most research because it became clear that many of its tenets were unsupported. In particular, much motivated behavior could be generated without any biological drives being manifest. However, the theory did engage reactions that moved the field forward.

A key component of the development of incentive motivation theory was the major assault on drive-reduction, largely led by Bolles in the period around 1972 (Bolles, 1975) and followed by the work of Bindra (1974) and Toates (1981). Bolles argued that many issues remained unexplained by drive reduction theory, but a major issue was that individuals were motivated by incentive expectancies or learned expectancies of reward (stimulus–stimulus [S–S*] associations or what in Pavlovian associations are conditioned and unconditioned stimuli [CS and US]). Bindra argued that the CS for a reward came to elicit the same motivational state as the reward itself, thus causing the individual to perceive the CS as reward, not just the expectancy of reward. Toates (1981) further refined the model by arguing that physiological states (drive states) could enhance the incentive value of the reward. Others, including Konorski, Young, Solomon, Rescorla and Dickinson, contributed significantly to the development of modern incentive-motivation theory which guides the neurobiology of motivation, and the reader is referred to their scholarly works.

Subsequent theories of motivation have linked motivation with hedonic, affective, or emotional states and have postulated changes in motivation over time and experience. The incentive–sensitization (or incentive salience) theory of Robinson and Berridge (1993) divides, but in a sense extends, the power of incentives and moves them to

a neuroadaptive trajectory. Here, incentives have been split into two components – ‘wanting’ and ‘liking’ – based on the hypothesis that different brain mechanisms mediate these separate components in which ‘liking,’ mediated by opioid systems in the nucleus accumbens and ventral pallidum, is defined as hedonic impact or the brain response to sensory reward or pleasure without motivational power. In contrast, ‘wanting,’ mediated by the mesolimbic dopamine system, is hypothesized to be the incentive salience or the motivational incentive value of the same reward.

The extrapolation of behavioral sensitization (increases in the locomotor response to psychostimulant drugs with repeated administration) to increases in incentive salience in the incentive–sensitization model has early roots in the facilitation of conditioned reinforcement and drug-seeking behavior described by Hill and Robbins. Here, drug-seeking behavior is controlled by a succession of drug-associated discriminative stimuli, which can also function as conditioned reinforcers when presented as a consequence of instrumental responses. In addiction, many have argued that by means of associative learning, the enhanced incentive salience state becomes oriented specifically toward drug-related stimuli, leading to escalating compulsion for seeking and taking drugs. Originally, Mogenson (1980) proposed that the process of motivation to action involved ventral striatal–ventral pallidal–thalamic circuits, and others emphasized the role of the mesolimbic dopamine system innervating the nucleus accumbens in incentive salience. Everitt and Robbins (2005) extended a key motivational role for dorsal striatal–pallidal–thalamic circuits in the plasticity of motivational processing. The recruitment of the dorsal striatal circuitry is hypothesized to mediate habits and compulsivity associated with aspects of drug addiction and other motivational pathology. The underlying activation of neural structures involved in maintaining the incentive–salience state persists, making addicts vulnerable in the long term to relapse. Thus, the incentive salience component of incentive sensitization theory has significant heuristic value as a common element of drug addiction in that it narrows the focus to drug-seeking at the expense of natural rewards, and drugs are hypothesized to usurp systems in the brain put in place to direct animals to stimuli with salience for preservation of the species. The clinical observation that individuals with substance use disorders have an unusual focus on drug-seeking to the exclusion of natural rewards fits the incentive salience view.

Emotion and Motivation in Opponent Process

An alternate, potentially complementary, motivational theory also incorporating a strong emotional component is opponent process theory. Solomon and Corbit (1974)

argued that hedonic, affective, or emotional states, once initiated, are automatically modulated by the central nervous system with mechanisms that reduce the intensity of hedonic feelings and provide an additional motivational state for directing behavior. Solomon hypothesized that there is affective or hedonic habituation (or tolerance) and affective or hedonic withdrawal (abstinence). More specifically, a-processes, which could be either positive or negative hedonic responses, were proposed to occur shortly after presentation of a stimulus, correlate closely with the stimulus intensity, quality, and duration of the reinforcer, and show tolerance. In contrast, the b-processes appear after the a-process has terminated, are sluggish in onset, slow to build up to an asymptote, slow to decay, and get larger with repeated exposure. As such, the affective dynamics of the opponent process produces new motivation (i.e., new opportunities for reinforcing and energizing behavior).

From a neurobehavioral perspective, the initial acute effect of an emotional stimulus or drug in brain motivational systems is hypothesized to be opposed or counteracted by homeostatic changes in brain systems. Certain systems in the brain were hypothesized to suppress or reduce all departures from hedonic neutrality. This affect control system was conceptualized as a single negative feedback or an opponent loop that opposes the stimulus-aroused affective state. For example, in the context of drug dependence, Solomon argued that the first few self-administrations of an opiate drug produce a pattern of motivational changes in which the onset of the drug effect produces a euphoria that is the a-process which is followed by a decline in intensity. After the acute drug effect wears off, the b-process state emerges as an aversive craving state. Thus, in opponent process theory, drug tolerance and dependence are inextricably linked, and affective states, pleasant or aversive, were hypothesized to be automatically opposed by centrally mediated mechanisms that reduce the intensity of these affective states:

We have been taught to think of aversion and trauma as the only affective sources of physiological stress. The opponent-process model implies that often repeated pleasures are just as fertile a source of physiological stress (Solomon, 1980, p. 709).

During this period, Cabanac (1971) hypothesized a process by which a given stimulus can induce a pleasant or unpleasant sensation depending on the subject's internal state. This state was termed 'alliesthesia' and was applied to both temperature regulation and appetite. Here, for example, pleasant responses to sweet solutions occur with low gastric load, but aversive responses to sweet solutions occur with high gastric load. The existence of alliesthesia implies the presence of internal

signals modifying the conscious sensations aroused from peripheral receptors and supports the hypothesis that both peripheral and central processes contribute to motivation.

More recently, opponent process theory has been expanded by Koob and Le Moal (2001, 2008) into the domains of the neurocircuitry and neurobiology of drug addiction from a neurobiological perspective. Both within-system (mesolimbic dopamine dysfunction) and between-system (recruitment of corticotropin-releasing factor) neuroadaptations are hypothesized to mediate the opponent process. An allostatic model of the brain motivational systems was proposed that explains some of the persistent changes in motivation that are associated with vulnerability to relapse in addiction, and this model may generalize to other psychopathologies associated with dysregulated motivational systems. In this framework, during the development of drug addiction, counteradaptive processes such as opponent-process which are part of the normal homeostatic limitation of reward function fail to return within the normal homeostatic range and are hypothesized to form an allostatic state. The allostatic state is hypothesized to be a chronic deviation of reward set point that is fueled not only by dysregulation of reward circuits *per se* but by recruitment of antireward systems (e.g., brain and hormonal stress responses).

Conclusion

Thus, emotion can be defined as passions or sensibilities of both physiological responses and mental states, and motivation can be defined as a persistent state leading to organized activity. Both are intervening variables, are intimately related, and have neural representations in modern neuroscience. Emotional responses have long been associated with classic 'limbic' system circuitry, and modern imaging studies involve a cognitive component, can dissociate emotional expression from feelings and empathy, and identify an important role for the physiological responses in how feelings are interpreted as originally outlined in the James-Lange theory of emotion. Motivation has long been hypothesized to involve an emotional intervening variable, whether it be fear or incentive states, and neural substrates have been identified that have key roles in the incentive, hedonic, and aversive aspects of motivation – notably the circuitry involving the nucleus accumbens and amygdala which recruit dorsal striatal circuits and stress circuits respectively – as motivation becomes pathological such as observed in impulsive and compulsive disorders. Emotional and motivational circuits also color key elements of brain cognitive function that have important roles in the decision-making processes that guide all behavior.

Cognition

The term ‘cognitive neuroscience’ is very recent, dating perhaps from the 1980s. The *Journal of Cognitive Neuroscience* was first published in 1989. Indeed, Posner and Shulman’s comprehensive chapter on the history of cognitive science (1979) does not even mention cognitive neuroscience (human imaging techniques were not yet much in use then). Here we note briefly the biological roots of cognitive neuroscience (see Gazzaniga, 1995).

Karl Lashley was again a key figure. One of the most important aspects of cognitive neuroscience dates from the early days at the Orange Park laboratory, where young scientists like Chow and Pribram began studies of the roles of the association areas of the monkey cerebral cortex in learning, memory, and cognition.

H.M. and Primate Models

The period of the 1950s was an especially rich time of discovery regarding how cognitive functions were organized in the brain. Pribram, Mortimer Mishkin, and Hal Rosvold at the National Institute of Mental Health, using lesion studies in monkeys, discovered that the temporal lobe was critical for aspects of visual perception and memory. Work with neurologic patients also played a critical role in uncovering the neural substrates of cognition. As noted earlier, one particular discovery became a landmark in the history of memory research. “In 1954 Scoville described a grave loss of recent memory which he had observed as a sequel to bilateral medial temporal resection in one psychotic patient and one patient with intractable seizures. In both cases...removals extended posteriorly along the medial surface of the temporal lobes...and probably destroyed the anterior two-thirds of the hippocampus and hippocampal gyrus bilaterally, as well as the uncus and amygdala. The unexpected and persistent memory deficit which resulted seemed to us to merit further investigation” ([Scoville and Milner, 1957](#)).

That passage comes from the first paragraph of Scoville and Milner’s 1957 report, “Loss of recent memory after bilateral hippocampal lesions.” This publication became a landmark in the history of memory research for two reasons. First, the severe memory impairment (or amnesia) could be linked directly to the brain tissue that had been removed, suggesting that the medial aspect of the temporal lobe was an important region for a particular aspect of cognition (i.e., memory function). Second, comprehensive testing of one of the patients (H.M.) indicated that memory impairment could occur on a background of otherwise normal cognition. This observation showed that memory is an isolatable function, separable from perception and other cognitive and intellectual functions.

More specifically, H.M. could not remember his own experiences and could not learn new factual information after his surgery. This aspect of memory is termed ‘declarative’ memory because normal individuals, when asked, can state or declare the memories, and they can consciously retrieve and express them. But there are other types of memory, often grouped under the heading ‘procedural’ or nondeclarative (see below) on which H.M. performs like normal people. These include motor skills, Pavlovian conditioning of eyeblink and other aspects of basic associative learning, and even complex ‘cognitive’ puzzles like the tower of Hanoi, forms of memory that can be performed without conscious awareness of the memories, only the procedural performance of them.

H.M., whom we now know as Henry Molaison, died of respiratory failure at the age of 82 on December 2, 2008. He lived for many years at an extended care facility in Windsor Locks, Connecticut, where he served as the subject in hundreds of memory experiments. Those who worked with him described him as a very generous man, soft spoken, polite, and with a good sense of humor. The extraordinary depth of his declarative memory deficit was described to one of the authors (R.F.T.) by a colleague, Karl Pribram, a very senior neurosurgeon/neuroscientist when the two were colleagues at Stanford. He interviewed H.M. a few months after the surgery. They talked for a while, and Karl was impressed at how very bright and normal H.M. seemed (he had an above-average IQ). Then Karl had to leave the room for a phone call. When he returned a few minutes later, H.M. said, “Who are you? I have never seen you before.”

H.M. once said that everything seemed clear at the moment, but he worried about what had just happened. He felt as though he were just awakening from a dream and could not remember. He provided his brain to science, and it will be prepared in serial sections (2600 of them) by Jacopo Annese and colleagues at the University of California, San Diego. Mr. Molaison has contributed more to our understanding of human memory than any other human, and we will always be profoundly grateful to him.

The findings from patient H.M. ([Scoville and Milner, 1957](#)) early on identified a region of the brain important for human declarative memory (i.e., the medial portion of the temporal lobe). The damage was originally reported to have included the amygdala, the periamygdaloid cortex (referred to as the uncus in [Scoville and Milner, 1957](#)), the hippocampal region (referred to as the hippocampus), and the perirhinal, entorhinal, and parahippocampal cortices (referred to as the hippocampal gyrus). Subsequent, magnetic resonance imaging of patient H.M. suggested that his medial temporal lobe damage did not extend as far posteriorly as originally believed and that damage to the parahippocampal cortex was minimal (the lesion appeared to extend caudally from the temporal pole approximately 5 cm, instead of 8 cm, as originally reported; Corkin *et al.*, 1997).

While these observations identified the medial temporal lobe as important for memory, the medial temporal lobe is a large region including many different structures. To determine which structures are important required that studies be undertaken in which the effects of damage to medial temporal lobe structures could be evaluated systematically. Accordingly, soon after the findings from H.M. were reported, efforts were made to develop an animal model of medial temporal lobe amnesia. During the next 20 years, however, findings from experimental animals with intended hippocampal lesions or larger lesions of the medial temporal lobe were inconsistent and difficult to interpret.

In 1978, Mishkin introduced a method for testing memory in monkeys that captured an important feature of tests sensitive to human memory impairment (Mishkin, 1978). This method allowed for the testing of memory for single events at some delay after the event occurred. The task itself is known as the trial-unique delayed nonmatching to sample task, and it measures object recognition memory. In Mishkin's study, three monkeys sustained large medial temporal lobe lesions that were intended to reproduce the damage in patient H.M. The operated monkeys and three unoperated monkeys were given the delayed nonmatching to sample task in order to assess their ability to remember, after delays ranging from 8 s to 2 min, which one of two objects they had recently seen. The monkeys with medial temporal lobe lesions were severely impaired on the nonmatching task, consistent with the severe impairment observed in patient H.M. on delay tasks. Thus, lesions that included the hippocampal region and amygdala, as well as adjacent perirhinal, entorhinal, and parahippocampal cortices, caused severe memory impairment. This work, together with work carried out in the succeeding few years, established a model of human amnesia in nonhuman primates (Mishkin *et al.*, 1982; Squire and Zola-Morgan, 1983). Although other tasks have been useful for measuring memory in monkeys (object discrimination learning, the visual paired-comparison task, see below), much of the information about the effects of damage to medial temporal lobe structures has come, until recently, from the delayed nonmatching to sample task.

Once the animal model was established, systematic and cumulative work eventually identified the structures in the medial temporal lobe that are important for declarative memory. The important structures are the hippocampal region and the adjacent perirhinal, entorhinal, and parahippocampal cortices (for early reviews describing the medial temporal lobe memory system, see Zola-Morgan and Squire, 1993; Mishkin and Murray, 1994; for recent reviews, see Squire *et al.*, 2007, Eichenbaum and Lipton, 2008, Suzuki, 2009).

More Than One Kind of Memory

The medial temporal lobe is necessary for establishing a kind of memory that is termed long-term declarative or explicit memory. Declarative memory refers to the capacity for conscious recollection of facts and events (Squire, 1992). It is specialized for rapid, even one-trial learning, and for forming conjunctions between arbitrarily different stimuli. It is typically assessed in humans by tests of recall, recognition, or cued recall, and it is typically assessed in monkeys by tests of recognition (e.g., the delayed nonmatching to sample task). The medial temporal lobe memory system appears to perform a critical function beginning at the time of learning in order that representations can be established in long-term memory in an enduring and usable form (see also Eichenbaum *et al.*, 1994).

Another important discovery that paralleled in time the work on the medial temporal lobe system involved the understanding that there is more than one kind of memory. Specifically, work with amnesic patients and with experimental animals who sustained lesions to specific brain regions showed that other kinds of abilities (including skills, habit learning, simple forms of conditioning, and the phenomenon of priming, which are collectively referred to as nondeclarative or procedural memory) lie outside the province of the medial temporal lobe memory system. Nondeclarative forms of memory are intact in amnesic patients and intact in monkeys with medial temporal lobe lesions. For example, as noted above, classical delay conditioning of skeletal musculature was found to depend on the cerebellum (Thompson and Krupa, 1994), conditioning of emotional responses depends on the amygdala (LeDoux, 2000; Davis, 1992), and habit learning (win-stay, lose-shift responding) depends on the neostriatum (Salmon and Butters, 1995; Packard *et al.*, 1989). Nondeclarative memory thus refers to a variety of ways in which experience can lead to altered dispositions, preferences, and judgments without providing any conscious memory content.

Further work with monkeys has demonstrated that the severity of memory impairment depends on the locus and extent of damage within the medial temporal lobe memory system. Damage limited to the hippocampal region causes significant memory impairment, but damage to the adjacent cortex increases the severity of memory impairment. It is important to note that the discovery that larger medial temporal lobe lesions produce more severe amnesia than smaller lesions is compatible with the idea that structures within the medial temporal lobe might make qualitatively different contributions to memory function. This is because anatomical projections carrying information from different parts of the neocortex enter the medial temporal lobe memory system at different points (Suzuki and Amaral, 1994, Squire *et al.*, 2007). This might be

viewed as a modern reformulation of Lashley's principle of "mass action."

Another important brain area for memory is the diencephalon. However, the critical regions in the diencephalon that when damaged produce amnesia have not at the time of this writing (mid-2009) been identified with certainty. The important structures appear to include the mediodorsal thalamic nucleus, the anterior nucleus, the internal medullary lamina, the mammillothalamic tract, and the mammillary nuclei. Because diencephalic amnesia resembles medial temporal lobe amnesia in many ways, these two regions together probably form an anatomically linked, functional system (Squire and Zola, 1997; Gold and Squire, 2006).

These findings in monkeys are fully consistent with the findings from human amnesia. Damage limited to the hippocampal region is associated with moderately severe amnesia and more extensive damage that includes the hippocampal region as well as adjacent cortical regions is associated with more severe memory impairment (Zola-Morgan *et al.*, 1986).

The same principle, that more extensive damage produces more severe impairment, has also been established for the hippocampus proper in the case of the rat (Moser *et al.*, 1993). The dorsal hippocampus of the rat is essential for spatial learning in the water maze, and progressively larger lesions of this region produce a correspondingly larger impairment. Thus, in all three species, it has turned out that the brain is organized such that memory is a distinct and separate cognitive function, which can be studied in isolation from perception and other intellectual abilities. Information is still accumulating about how memory is organized, what structures and connections are involved, and what functions they support. The disciplines of both psychology and neuroscience continue to contribute to this enterprise.

Roger Sperry was another key player in the origins of cognitive neuroscience. After receiving his PhD in zoology, he joined Lashley for a year at Harvard and moved with Lashley to the Yerkes Laboratory at Orange Park, where he stayed for several years. Sperry did his pioneering studies on the selective growth of brain connections during this time (see Sperry, 1951). Lashley was fascinated by the mind-brain issue – the brain substrates of consciousness (although he never wrote much about it) – and often discussed this problem with his younger colleagues at Orange Park (Sperry, personal communication). Sperry and his associates at the California Institute of Technology tackled the issue with a series of commissurotomy patients – the human 'split-brain' studies. This work proved to be extraordinary, perhaps the most important advance in the study of consciousness since the word itself was developed many thousands of years ago (Sperry, 1968).

Brenda Milner studied for her PhD at McGill under Hebb's supervision. Hebb arranged for her to work with Wilder Penfield's neurosurgical patients at the Montreal

Neurological Institute. Her work on temporal lobe removal in humans, including H.M., really began the modern study of the memorial functions of the hippocampus (see above). She also collaborated on studies with Roger Sperry and Karl Pribram.

Another very important influence in modern cognitive neuroscience comes from the Soviet scientist Alexander Luria, who died in 1977. Luria approached detection and evaluation of damage to higher regions of the human brain both as a clinician with extraordinary expertise in neurology and as a scientist interested in higher functions of the nervous system (e.g., his book *Language and Cognition*, 1981).

EEG and Evoked Potentials

Yet another origin of cognitive neuroscience is recording the activity of the human brain, initially using EEG. Donald Lindsley was a pioneer in this work. After his PhD, he took a 3-year postdoctoral stage at Harvard Medical School (1933–35). The neurophysiologist Alexander Forbes was at Harvard doing pioneering studies on brain evoked potentials and EEG in animals. The first human EEG recording laboratory was set up at Harvard, and Lindsley and other pioneering figures (e.g., Hallowell Davis) did the first EEG recording in America (Lindsley, 1936).

More recently, the method of averaging evoked potentials recorded from the human scalp made it possible to detect brain signals relevant to behavioral phenomena that could not be detected with individual trial recording. Donald Lindsley was a pioneer in this field as well, doing early studies on evoked potential correlates of attention.

Brain Imaging

But the techniques that have revolutionized the study of normal human brain organization and functions are of course the methods of imaging. The first such method was X-ray computed tomography, developed in the early 1970s. The major innovation beyond simple X-rays was complex mathematical and computer techniques to reconstruct the images.

Somewhat later, PET was developed. It is actually based on a long used method in animal neuroanatomy – autoradiography. In this technique, a radioactive substance that binds to a particular type of molecule or brain region is infused, and brain sections are prepared and exposed to X-ray film. For humans, PET involves injecting radioactive substances, for example radiolabeled oxygen (^{15}O) in water. Increased neuronal activity in particular regions of the brain causes a rapid increase in blood flow to the regions, determined years earlier in work by Seymour Kety and others. Consequently, the radioactive water in the blood becomes more concentrated in active brain areas, detectable by radioactivity detectors.

The most widely used method at present is magnetic resonance imaging (MRI). This is based on the fact that changes in blood flow cause changes in the blood magnetic properties, which can be detected as changes in a strong imposed magnetic field. This method was first used in 1990 (Ogawa *et al.*, 1990). The current procedure is termed fMRI, involving very fast acquisition of images. A landmark publication in human brain imaging is the elegant book by two pioneers in the field, Michael Posner and Marcus Raichle, *Images of Mind*, 1994. The fMRI procedures have several advantages, such as the fact that they are noninvasive – no radioactive substance is injected – and this technique provides better spatial resolution than does PET imaging. Functional magnetic resonance imaging exploits variations in magnetic susceptibility that arises from molecular binding of oxygen to hemoglobin, which can be used to detect blood flow changes associated with neuronal activity (blood oxygen level reduction, BOLD). At the present time, these neuronal activity-related signals can be derived from areas of the brain with a spatial resolution of 1–2 mm. Moreover, the temporal resolution of this functional imaging technique is compatible with the time course needed to carry out most perceptual and cognitive operations. An important and promising strategy for the use of fMRI is its use in conjunction with other kinds of neurobiological techniques, including neurophysiology and anatomical and behavioral analyses. Thus, fMRI provides an extraordinary new window through which one can probe the neural machinery of cognition (Schacter *et al.*, 1998; Albright, 2000; Persson *et al.*, 2006).

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Effects of Stress on Learning and Memory

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Glossary

Acquisition – Mnemonic processes engaged at the time of learning (i.e., during the training phase of a memory study).

Consolidation – Mnemonic processes engaged for a limited period after encountering the events for which memory is later assessed. These processes are necessary for memories to be stabilized and made long term or permanent.

Eyeblink conditioning – A form of Pavlovian conditioning in which an animal learns to blink in response to a conditioned stimulus that signals a blink-inducing paraorbital shock or puff of air to the eye.

Hypothalamic–pituitary–adrenal (HPA) axis – The neuroendocrine system that is activated by stress and results in the release into the blood stream of glucocorticoids from the adrenal cortex.

Inhibitory avoidance – A fear-conditioning paradigm in which an animal learns, often after only one trial, to avoid making a previously punished response, such as stepping down from a raised platform or entering the darkened compartment at the end of a runway.

Morris water maze (MWM) – A spatial learning paradigm in which an animal can escape an opaque pool by locating a platform submerged beneath the water's surface.

Pavlovian (or classical) conditioning – A form of associative learning in which a previously neutral ('conditioned') stimulus becomes capable of producing an anticipatory response after one or more pairings with an 'unconditioned' stimulus that naturally produces a response. The occurrence of the unconditioned stimulus is not contingent on the animal's behavior.

Pavlovian fear conditioning – A form of conditioning in which an animal learns to associate a conditioned stimulus with the occurrence of an aversive unconditioned stimulus, typically a shock. The conditioned stimulus can be an explicit (e.g., visual or auditory) cue or the context in which the shock occurs.

Learning is assessed by the expression of fear-related behaviors (e.g., freezing or potentiation of the acoustic startle reflex).

Retrieval – Mnemonic processes engaged when memories are reactivated some time after learning has taken place.

Sympathetic-adrenal medullary system – The neuroendocrine system activated by stressors that results in release of epinephrine and norepinephrine. This system is responsible for producing the 'fight or flight' response.

In 1894, the French psychologist Théodule Ribot described how traumatic experiences could produce vivid and abiding memories, such as one subject's repeated experiences of dread upon revisiting his boarding school as an adult, or the recollection by another of the sound of the dentist's drill: "When I think of it I feel a chill run down my back and a slight trembling of the arms." However, Ribot also noted that many individuals experience 'affective amnesia,' in which strongly emotional material seems to "glide off their minds as a thundershower does off the roofs." These early case studies illustrate what has become a recurring motif in research on the effects of stress on learning and memory, that memories are sometimes enhanced by stress and at other times diminished.

Ribot ascribed this dichotomy to individual differences. But a seminal study of avoidance learning by Robert M. Yerkes and John D. Dodson, published in 1908, suggested this not to be the case, at least in rodents. They reported that the rate at which rats learned to avoid footshock was related to task complexity and to shock intensity. When the behavioral demands required for escape were made relatively complex, the relationship between shock intensity and learning rate followed an inverted-U function, with learning occurring most

rapidly in response to an intermediate level of shock. These findings demonstrated not only the existence of a bidirectional relationship between stress and learning in the rat, but also its moderation by specific experimental parameters rather than by individual differences.

Research in the current era has begun to characterize biological mechanisms underlying both the promotive and disruptive influences of stress on memory. By and large, progress in the investigation of each has moved along separate paths that have shown little overlap. This is perhaps because the range of behavioral paradigms and stressors used in different laboratories makes it difficult to synthesize across multiple sets of findings. One approach toward a more unified conceptual framework is to first identify from within this varied landscape the critical dimensions that are likely to determine whether stress promotes or prevents the formation and retrieval of memory. Parsing the field in this way better positions us to ask, in biological terms, how these bidirectional effects are achieved.

Critical Dimensions of Studies of the Stress–Memory Relationship

This article discusses the effects of stress on learning and memory by focusing on a number of behavioral paradigms that can be categorized according to the dimensions discussed below. As will be seen, three dimensions emerge as critically determining the effects of stress on memory and the neural mechanisms mediating these effects: the duration of stress (acute vs. chronic), the nature of the learning task (cerebellum- vs. hippocampus-dependent), and the relationship of the stressor to the learning task (intrinsic vs. extrinsic).

Type of Learning Task

Different forms of memory – from Pavlovian stimulus–stimulus (S–S) and stimulus–response (S–R) conditioning to declarative memory – depend upon different neural systems. In rodents, the effects of stress have been investigated primarily within Pavlovian eyeblink conditioning, fear conditioning, and spatial learning tasks. Although there are some commonalities in the neural circuitry underlying these three types of learning, they differ in relying most critically on three different neuroanatomical structures: the cerebellum, the amygdala, and the septal pole (i.e., dorsal region in the rodent) of the hippocampus, respectively. Moreover, procedural variations within each of these classes of learning are also important in determining the dependence of learning on specific neural structures. For example, whereas both trace (i.e., when the conditioned stimulus (CS) and unconditioned stimulus (US) are separated by an interval) and contextual

Pavlovian fear conditioning are frequently dependent on the dorsal hippocampus, delay conditioning (i.e., when the CS and US overlap in time) is not. Thus, if hormonal responses to stress affect hippocampal and amygdalar function differently, one might expect trace and contextual fear conditioning to be more sensitive than delay fear conditioning to the effects of stress.

Nature of the Stressor

Relationship to learning task

The source of stress can be either intrinsic or extrinsic to the experimental task. In fear-learning tasks, the stressor is intrinsic because it is typically the emotive event about which the subject learns. In eyeblink conditioning and navigational learning experiments, the stressor is typically incidental to the task at hand. It should be noted that even in learning tasks where an extrinsic stressor is applied, an intrinsic source of stress may also be present (e.g., shock to the orbit or airpuff to the eye in eyeblink conditioning, or immersion in a water maze).

Relationship to behavior

The impact on memory of an organism's responses to intrinsic stress differs widely depending on whether the source of stress is avoidable or not. Stress responses have a substantial impact on memory retention in passive, or inhibitory, avoidance paradigms but more nuanced effects on Pavlovian fear conditioning. There are similarly stark differences in the effects of inescapable and escapable stress on subsequent active avoidance responding – where intense, inescapable shock produces a state of learned helplessness. This condition, however, reflects a change (either adaptive or maladaptive) in performance of an escape response rather than in the strength of the underlying fearful memory itself.

Severity

Stress can range from relatively mild (e.g., exposure of a rodent to a novel context) to severe (e.g., restraint combined with frequent tailshock). Its duration can also range from single and brief (i.e., acute) to intermittent or continuous over days or weeks (i.e., chronic). Rarely is the dose of extrinsic stress varied systematically within a study. This contrasts with fear-conditioning studies, where the intensity of the US is often varied in a systematic manner. In the latter studies, however, it is difficult to distinguish the effects of stress responses of varying magnitudes from the predictable relationship between the strength of the US and the rate and asymptotic level of conditioning.

Acute Stress

Intrinsic Stress: Memory for the Stressful Event

Inhibitory avoidance

In inhibitory avoidance tasks, the measure of learning is the increase in the latency to make a previously punished response, such as stepping down from a raised platform or entering the darkened compartment at the end of a runway. Learning occurs extremely rapidly; often only one training trial is used. This means that the period of memory consolidation – beginning as soon as the training trial is terminated – can be clearly distinguished from prior acquisition. In contrast, when multiple training trials are used, intracellular and systems-level consolidation processes are inevitably initiated before later training trials have occurred. Furthermore, consolidation of inhibitory avoidance learning has proven to be exquisitely sensitive to stress and to manipulations of the individual's neuroendocrine responses to stress. For these reasons, inhibitory avoidance has become a mainstay of research in rodents into the effect of intrinsic stress on memory.

The inhibitory avoidance literature points to specific components of the individual's response to a stressful challenge that enhance consolidation of memory (**Figure 1**). Stress causes rapid activation of the sympathetic-adrenal medullary system, and slower activation of the hypothalamic–pituitary–adrenal (HPA) axis. The rapid release of epinephrine from the adrenal medulla and slower release of steroid glucocorticoid hormones (corticosterone or cortisol, depending on the species) from the adrenal cortex in response to shock initiates a cascade of events that ultimately affects consolidation

processes in the amygdala, hippocampus, and extrahippocampal cortical areas. Epinephrine binds to β -adrenergic receptors on vagal afferents, leading to excitation of ascending adrenergic neurons in the nucleus of the solitary tract (NTS) and the release of norepinephrine (NE) in the brain. Glucocorticoids cross the blood–brain barrier and bind to mineralocorticoid receptors (MRs), mainly located in limbic structures, and glucocorticoid receptors (GRs), distributed throughout the brain. Since MRs have a higher affinity for glucocorticoids, they are predominantly bound at rest. Surges in corticosterone triggered by stressors such as footshock therefore primarily produce increased GR stimulation.

Binding of NE to β -adrenergic receptors in the basolateral complex of the amygdala (BLA) is the key event in the enhancement of consolidation. Other receptors in the BLA that have been implicated in consolidation include α -adrenergic receptors and corticotropin-releasing factor (CRF) receptors. Stimulation of each enhances memory for the inhibitory avoidance task through facilitation of the adenylyl cyclase and cyclic adenosine monophosphate (cAMP) pathway initiated by β -adrenergic receptor activation.

Although much evidence supports the role of NE in consolidation, dopamine β -hydroxylase knockout mice, which are unable to produce NE due to dopamine β -hydroxylase's crucial role in the NE-synthesis pathway, are not impaired in the inhibitory avoidance task. These results may be attributable to long-term neural adaptations in response to NE depletion through development. Alternatively, consolidation may be achieved through other neurotransmitters if NE is unavailable, even in the healthy animal. This is supported by the

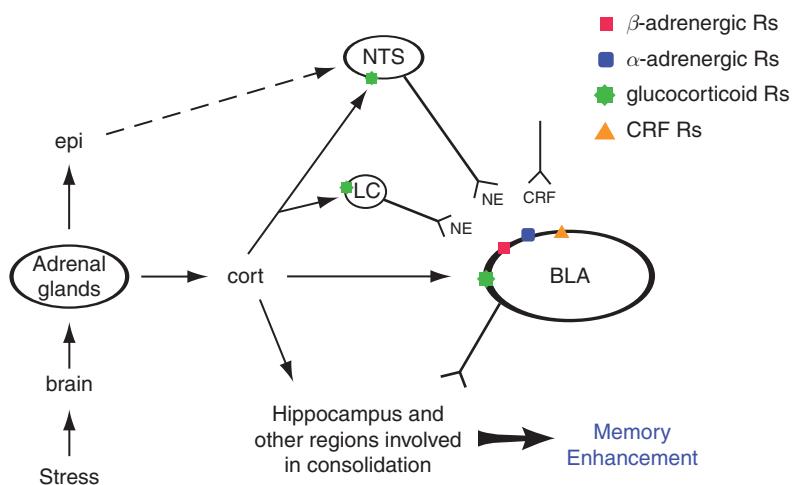


Figure 1 Neural mechanisms underlying inhibitory avoidance learning. A stressor such as footshock triggers the brain to send signals to the adrenal glands that cause release of corticosterone and epinephrine. Epinephrine binds to β -adrenergic receptors on the vagus nerve, sending an excitatory signal to the NTS and causing norepinephrine release in the BLA. Corticosterone binds to GRs in the BLA and facilitates norepinephrine release from the LC. The actions of NE, corticosterone, and CRF lead to changes in the BLA that ultimately cause memory enhancement. Abbreviations: basolateral nucleus of the amygdala (BLA), corticotropin-releasing factor (CRF), glucocorticoid receptor (GR), locus ceruleus (LC), norepinephrine (NE), nucleus of the solitary tract (NTS).

finding that learning occurs normally when β -adrenergic receptors are blocked pharmacologically before, as opposed to immediately following, training.

Beyond adenylate cyclase activity, the intracellular mechanisms through which β -adrenergic receptors in the BLA enhance consolidation processes remain to be determined. It is known that NE binding leads to facilitation of N-methyl-D-aspartic acid (NMDA) receptor-dependent plasticity, a necessary step in the consolidation of memories. BLA activation can also modulate long-term potentiation (LTP) in the hippocampus. Additionally, stimulation of β -adrenergic receptors in the BLA leads to expression in the hippocampus of *Arc*, an immediate-early gene (IEG) thought to play a role in synaptic plasticity.

Glucocorticoids enhance consolidation through two routes. First, they modulate NE release in the BLA via actions on noradrenergic cells in the NTS and locus ceruleus. Second, memory enhancement is achieved through binding to GRs in the BLA and hippocampus. In the BLA, GRs may interact with α -adrenergic and CRF receptors to enhance adenylate cyclase activity. Glucocorticoids may also act indirectly by sensitizing CRF systems in the amygdala. Importantly, GR binding in the hippocampus is ineffective if the BLA is lesioned. The BLA therefore seems to be critical in regulating the effects of stress on consolidation of memory in the hippocampus, and perhaps in other brain regions as well. This idea is supported by the finding that lesions of the stria terminalis, a major output pathway of the amygdala, also impair memory consolidation.

Water maze

In the spatial version of the Morris Water Maze (MWM), the animal learns to swim to a platform hidden in a pool of opaque water using distal spatial cues. Although the explicit focus of MWM studies is typically on the navigational learning component, this task actually represents a form of active avoidance learning; the act of swimming to the platform serves as a means of escape from the water. Findings in the limited number of studies that have focused on the role of the stress in MWM learning cohere with the inhibitory avoidance literature discussed above. Thus, corticosterone treatment after training increases retention, while intracerebroventricular and intra-BLA infusion of GR antagonists after training impairs memory, confirming that release of corticosterone from the adrenal glands and subsequent binding to GRs in the BLA plays a role in enhancing consolidation. A point mutation in the mouse's GR that prevents the receptor from binding to DNA also impairs spatial memory, suggesting that GR stimulation exerts genomic effects directly, rather than through interactions with other transcription factors. Studies involving posttraining infusions of specific noradrenergic drugs indicate the importance of stimulation of β -adrenergic receptors in the BLA in this form of learning as well.

Pavlovian fear conditioning

In Pavlovian fear conditioning, the organism learns to associate cues with the occurrence of an aversive US, typically a shock. Fear-conditioning paradigms differ from avoidance learning in that the incidence of the US is independent of the individual's behavior. This difference between S-S and S-R learning has important consequences in terms of the sensitivity of fear consolidation to modulation by stress system responses. Perhaps the most striking contrast between Pavlovian conditioning and avoidance learning is that post training, intra-BLA infusion of the γ -aminobutyric acid (GABA-A) receptor agonist muscimol, while profoundly impairing consolidation of inhibitory avoidance learning, leaves consolidation of auditory cue and contextual fear conditioning intact. Similarly, Pavlovian fear conditioning is not affected by systemic injection of a β -adrenergic receptor antagonist. These results suggest that NE release in the amygdala, critical for consolidation of inhibitory avoidance, is not necessary for the consolidation of Pavlovian fear memories. Despite being unnecessary for memory formation, NE nevertheless promotes it, since intra-BLA infusion enhances memory in contextual fear conditioning.

The role of HPA-axis activation appears to be more significant. Systemic administration of the GR antagonists RU38486 or RU40555 prior to conditioning disrupts acquisition of fear conditioning to contextual cues, but not to auditory cues. Because the dorsal hippocampus is a site of plasticity for contextual fear, it is likely that corticosterone ultimately has its modulatory effect in this region. Consistent with this idea, hippocampus-dependent trace fear conditioning, and not delay fear conditioning, is disrupted by the glucocorticoid-synthesis inhibitor metyrapone. However, changes in the dorsal hippocampus may be an indirect consequence of GR stimulation elsewhere, since contextual fear conditioning is disrupted by infusion of RU38486 directly into the BLA or ventral hippocampus but not by infusion into the dorsal hippocampus.

Interestingly, the critical window for stress-induced modulation of Pavlovian fear memory, like inhibitory avoidance, may be the phase of immediate posttraining consolidation. This is suggested by the discovery that injection of corticosterone itself potentiates contextual fear conditioning when administered immediately after, as well as before, training.

Extrinsic Stress: Memory for Events Incidental to the Stressor

Eyeblink conditioning

In Pavlovian eyeblink conditioning, the organism learns to blink its eye in response to a CS that signals a blink-inducing paraorbital shock or puff of air. Although these events are unpleasant and stressful, the measure of

interest is acquisition of a discrete motor response rather than the accompanying, coordinated set of diffuse, anticipatory fear responses. Eyeblink-conditioning paradigms also differ from fear-conditioning paradigms in that acquisition occurs over a much narrower range of temporal intervals between CS and US onset (usually <1 s) and over a much larger number of training trials. Finally, eyeblink and fear conditioning differ in the way in which the consequences of stress have been studied. To evaluate the effects of stress on eyeblink conditioning, the animal is exposed to an additional source of stress prior to the commencement of training. It may also be significant that the stressor is typically the same as that used to induce learned helplessness (one tailshock per minute over 60–90 min of restraint), and thus considerably stronger than the stress intrinsic to most fear-conditioning paradigms.

It is now well established that exposure to this form of stress facilitates acquisition of eyeblink-conditioned responses in male rats. This phenomenon is particularly intriguing because it occurs irrespective of whether training is initiated immediately after termination of stress or 24 h following it. *Per contra*, female rats show poorer eyeblink conditioning following stress exposure, apparently because stress disrupts the facilitative effects on learning otherwise exerted by estrogen. It is currently unknown whether as dramatic a sex difference characterizes other stress-related learning phenomena, since relatively little systematic research has been conducted on female rodents.

The neural mechanisms underlying the enhancement of eyeblink conditioning by stress in male rats have yet to be established, beyond the clear involvement – as with other stress-related phenomena described above and below – of the amygdala and activation of the HPA axis. Thus, both inactivation of the amygdala and adrenalectomy block the stress-induced enhancement of conditioned eyeblink responses.

A further potential region of interest is the cerebellum. Interestingly, the cerebellar cortex, where plasticity is necessary for normal acquisition of the conditioned response, is rich in corticosteroidal receptors. In contrast, the fact that stress enhances delay, as well as trace, eyeblink conditioning may make a role for the hippocampus less likely in this than in other stress-related memory phenomena. Consistent with this, the same stressful conditions that enhance eyeblink conditioning attenuate the cellular phenomenon of LTP in the hippocampus. Finally, the fact that eyeblink conditioning is enhanced immediately or 24 h after stress exposure suggests the influence of both nongenomic and genomic mechanisms.

Spatial learning

In contrast to Pavlovian eyeblink conditioning, exposure to stress prior to training has been found to impair male rats' long-term memory of the MWM. Retrieval or

expression of maze learning in rats is also disrupted by application of relatively mild acute stress prior to test. The time at which the stressor is given is critical: performance is affected when the stress is applied 30 min before test, but not 2 min or 4 h before test, thereby corresponding to the time course of corticosterone release. Consistent with this, exogenous corticosterone has the same effect. Local infusion of specific drugs indicates that this effect is mediated by the stimulation of GRs acting through a rapid, nongenomic mechanism to facilitate β -adrenergic transmission in the hippocampus.

The apparently divergent consequences of exposure to extrinsic stress prior to training, both across different paradigms and in relation to the well-established effects of intrinsic stress, require further exploration. The opposing effects of pretraining stress on eyeblink conditioning and spatial learning cannot be explained by differences in the intensity or timing of the stressor, since similar parameters were applied in both. Rather, the most likely, albeit as yet untested, explanation is that severe acute stress enhances the induction of cellular plasticity in the cerebellum while impairing the induction of plasticity in the hippocampus. Both phenomena are presumably mediated by activation of the amygdala, since its integrity is necessary for the impairment of spatial learning as well as for the potentiation of eyeblink conditioning.

It is perhaps more of a challenge to reconcile the deleterious effects of extrinsic stress on MWM learning with the facilitative effects of the stress of swimming that is inherent to the task. One possible explanation, reminiscent of the Yerkes–Dodson effect, is that this difference is simply related to the intensity of the stressor, with optimal learning occurring at intermediate stress levels. This notion is supported by the finding that learning in another spatial task (a Y-maze) is disrupted both when GRs are pharmacologically blocked and when they are highly saturated in adrenalectomized animals via application of corticosterone or a GR agonist.

A second possible explanation relates to the timing of stress. Extrinsic stress is applied before training and peak corticosterone release may, therefore, occur prior to the period of acquisition. Intrinsic stress, by definition, coincides with training and hence peak corticosterone release presumably coincides with the period of posttraining consolidation. As described earlier, the fact that almost all manipulations have been conducted immediately after training has shown that intrinsic stress specifically enhances consolidation of encoded memories. Interestingly, eyeblink conditioning is facilitated only when the stress is applied before, and not after, training. Whether and how exposure to extrinsic stress after MWM training would affect memory consolidation is not known, but it is intriguing to speculate that extrinsic stress, in general, may affect processes important for memory 'acquisition' and not those required for memory consolidation.

The neural mechanisms whereby extrinsic and intrinsic stressors exert different effects on memory are certainly worthy of further investigation. Whatever the specifics, the evolution of such distinctive mechanisms seems likely, since the idea is easily entertained that there is an adaptive advantage to having heightened memory for events directly associated with a traumatic episode, but not for events that are incidental to the trauma itself.

Chronic Stress

Where chronic stress, lasting at least several days, has been shown to have an effect on subsequent learning in rats, it has generally been to reduce the capacity for spatial learning. Three critical variables, all related to time, can be identified that influence whether spatial learning is impaired or spared by chronic stress: the age of the animals at the time of stress exposure, the duration of exposure to the stressor, and the interval between exposure to stress and the commencement of learning.

The broad picture is that only extensive periods of stress lead to impairments in memory and that these impairments may only be transient. A direct comparison of different periods of chronic stress revealed that spatial learning was impaired following 6 h/day restraint stress for 21 days but not following the same daily period of restraint for 7 days. Recovery of function is fairly rapid, even after the longer stress period, since no learning deficit is apparent when training commences 2 weeks later. In contrast, a deficit in spatial learning still remains 2 weeks after a much more extensive, 6-month, period of social isolation stress. This suggests that both the severity and persistence of memory impairments increase as a function of the duration of chronic stress exposure.

The third temporal variable, age, appears to be the most critical of all, for chronic stress-related manipulations beginning in infancy seem to have significant long-term consequences for mnemonic ability. Daily handling of rat pups during the first 2 weeks of life produces changes in reactivity of the HPA axis, and enhanced MWM memory is one of a complement of positive, long-term outcomes in the adult. As with other consequences of this intervention, the enhancement of memory is mediated by an increase in the organization of maternal behaviors on return of the pups to the nest. Indeed, even without handling, the pups of high-nurturing mothers show more rapid spatial learning as adults than the offspring of low-nurturing mothers. In addition, the offspring of mothers exhibiting low-nurturing maternal behavior show improved memory if reared by high-nurturing mothers. Hence, resilience is conferred through the quality of the mother's behavior during this critical period rather than through genetic factors alone.

Interestingly, rats may reenter a window of vulnerability later in life as well, because the 6 months of isolation stress referred to above affected spatial learning only when it began at 2 years, and not at 2 months of age, a time that corresponds to early adulthood.

The impairments to memory produced by chronic stress are likely mediated by alterations in regulation of the HPA axis. The effects of 21 days of restraint stress are mitigated by adrenalectomy and can be reproduced by 21 days of corticosterone supplementation, suggesting that chronic elevations in corticosterone are necessary and sufficient for memory impairments to ensue. A persistent increase in corticosterone secretion likely impacts memory via atrophy of apical dendrites in the CA3 region of the hippocampus; like the impairment of spatial memory, it is seen after 21 days stress exposure and subsides with the passage of time. As for mechanisms underlying the effect of early-life experiences on later measures of memory, expression levels of a wide variety of molecules in the brain of the adult are altered as a function of the repertoire of maternal behaviors experienced as a pup. Many, such as changes in GRs and CRF receptors, are closely associated with HPA-axis regulation, whereas others, such as NMDA-receptor-subunit composition, are associated with variations in plasticity and memory. It still not clear which of these variables interact with one another to specifically affect cognitive function later in life.

Although the extent to which other forms of memory are sensitive to chronic stress has not been established, it is noteworthy that prior exposure to the 21-day chronic stress protocol does not blunt either tone or contextual fear conditioning. Indeed, the expression of both forms of fear is enhanced by this treatment, suggesting a residue of anxiety following daily, repeated periods of stress. Thus, as in the case of acute stress, some forms of learning may be more sensitive than others to the ravages of chronic stress.

Conclusions

As first observed in studies of the human and animals over 100 years ago, stress can either enhance or diminish the efficacy of learning and the vigor of resultant memories. Through an overview of contemporary research in rodents, one can begin to discern the circumstances that determine which of these opposing effects are seen and how the HPA axis and sympathetic responses play their part in them. Three significant factors emerging from this analysis are the duration of the stressor, its relevance to the learning experience, and the nature of the learning task employed (see **Table 1**).

The most thoroughly studied phenomenon in this field is the strengthening of consolidation of memory for the stressor itself, measured as the animal's tendency to avoid future encounters with it. In contrast, consolidation of

Table 1 Summary of the effects of stress on memory in a variety of rodent models. Several factors determine whether stress enhances or impairs memory in a given paradigm, including the type of learning task, the timing of the stressor, its severity, and its relationship (i.e., intrinsic or extrinsic) to the learning task. These factors and the known neural mechanisms through which they exert their effects are summarized for each of the paradigms discussed in the text

	<i>Paradigm</i>	<i>Stressor</i>	<i>Memory processes affected</i>	<i>Effect on memory</i>	<i>Mechanisms</i>
Acute					
Intrinsic	Inhibitory avoidance	Shock	Consolidation	↑	β-adrenergic receptor stimulation in the BLA, GR stimulation in the BLA and hippocampus
	MWM	Water	Consolidation	↑	β-adrenergic receptor stimulation in the BLA, GR stimulation in the BLA and, possibly, hippocampus
	Pavlovian fear: Context	Shock	Consolidation	↑	GR stimulation in the BLA and ventral hippocampus
	Pavlovian fear: Auditory cue	Shock			
Extrinsic	Pavlovian eyeblink	Restraint and shock	Acquisition	↑	Amygdala and HPA-axis activation
	Spatial	Restraint and shock	Acquisition or consolidation	↓	Amygdala activation
		Shock	Retrieval	↓	GR stimulation in hippocampus acting rapidly to facilitate β-adrenergic transmission
Chronic					
	Early life	Reduction in maternal behaviors	?	↓	May relate to changes in regulation of HPA axis or proteins associated with plasticity
	Adulthood	21-days restraint	?	↓	Dendritic atrophy in the CA3 of hippocampus

BLA, basolateral amygdala; GR, glucocorticoid receptor; HPA, hypothalamic–pituitary–adrenal; MWM, Morris Water Maze.

simple Pavlovian (S-S) associations between environmental stimuli and the stress-inducing event are generally less affected by endogenous responses to the stressor. Stress can also affect learning of incidental information, but likely through an influence on its acquisition rather than on its subsequent consolidation. The nature of the learning task is important here as well, with diametrically opposed effects on Pavlovian motor learning and spatial learning. Finally, chronic stress, like acute stress, may also impact memory formation, but its timing is critical. Thus, stress of sufficient duration impairs the ability to form new memories, but only for a limited window of time after its cessation.

The diversity of approaches to the study of the relationships between stress and memory makes it difficult to identify unifying neurobiological mechanisms. Nevertheless, several commonalities emerge. Most significantly, both the enhancing and deleterious effects of acute stress appear to be mediated by activation of the amygdala that in turn influences plasticity in other regions of the brain. Second, HPA-axis activation has also been implicated in effects in both directions (and in both cases, through GR activation), as well as in the memory impairments caused by chronic stress. NE release plays a critical role in strengthening consolidation of stress-related information, but its involvement in the other interactions between stress and memory is still unknown.

Given that the same physiological responses to stress can have such discrepant effects on memory depending on the nature of the learning paradigm and the nature of the stressor, the interactions between stress and memory must be moderated by the specific neural changes associated with individual situations. Understanding the variables following trauma that cause some events to slip from the mind like ‘a thundershower does off the roofs’ and others to endure, sometimes to an unwelcome or even to a pathological degree, will bring us closer to a fuller understanding of the relationship between stress and memory.

See also: Animal Models of Learning and Memory; Cognition: Learning and Memory: Pavlovian; Emotion–Cognition Interactions; Eyelid Classical

Conditioning; Fear, Anxiety, and Defensive Behaviors in Animals; Fear Conditioning; Fear: Potentiation and Startle; Hormones and Memory; Measuring Stress; Memory Consolidation; Neurogenesis and Memory; Neural Substrates of Conditioned Fear and Anxiety; Neural Substrates of Unconditioned Fear, Defense, and Anxiety; Stress and Brain Morphology.

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H

Habituation

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Glossary

Dishabituation – An increase in the size of the habituated response to the habituating stimulus following presentation of a (usually) strong other stimulus.

Habituation – A decrease in the size of a response to repeated presentations of the same stimulus.

Sensitization – An increase in the size of a response to a given stimulus following presentation of a (usually) strong other stimulus.

Stimulus frequency – Here the rate at which the habituating stimulus is given.

Stimulus intensity – Here, the strength of the habituating, or sensitizing or dishabituating, stimulus.

some nine basic parametric properties or characteristics exhibited by behavioral habituation.

1. *Given that a particular stimulus elicits a response, repeated applications of the stimulus result in decreased response (habituation). The decrease is usually a negative exponential function of the number of stimulus presentations.* Examples of response habituation can probably be found in essentially all behavioral studies where a stimulus is regularly presented. In earlier experiments devoted to habituation, *per se*, parametric characteristics were studied for a variety of responses ranging from postrotatory nystagmus to startle and the galvanic skin response. With the exception of the knee jerk reflex, habituation was a consistent finding, usually exhibiting an exponential course.

2. *If the stimulus is withheld, the response tends to recover over time (spontaneous recovery).* Spontaneous recovery has come to be the most common method of demonstrating that a given response decrement is an example of habituation. The time course of spontaneous recovery is markedly influenced by many variables and is not necessarily characteristic of a given response. Thus, the habituated startle response to sound in the intact rat may recover in 10 min or fail to recover in 24 h depending upon details of testing. Consequently, any categorization of the types of habituation based solely on recovery time is likely to be somewhat artificial.

3. *If repeated series of habituation training and spontaneous recovery are given, habituation becomes successively more rapid (this might be called potentiation of habituation).* Konorski describes this effect for the orientating response, and it has been described in many studies where repeated habituation series were given.

4. *Other things being equal, the more rapid the frequency of stimulation, the more rapid and/or more pronounced habituation is.* Numerous examples of this were noted in the earlier reflex studies as well as in the work by Welker on curiosity. The effect occurs in terms of real time course and within certain limits in terms of the number of trials as well.

Habituation is defined simply as a decrease in response to repeated stimulation and considered a basic form of nonassociative learning. Response decrements due to injury, drugs, or other abnormalities are excluded. The responses studied in habituation vary from reflexes to autonomic measures to electroencephalogram (EEG) arousal and a wide range of behavioral phenomena such as activity, exploration, and response to novelty.

Following classical early studies by Humphrey, Prosser and Hunter, Harris, and Sharpless and Jasper, habituation is considered a central phenomenon, at least in animals with nervous systems; receptor adaptation and neuromuscular fatigue are excluded.

Sensitization is simply an increase in response to a stimulus due to the action of another (often strong) stimulus. Dishabituation is typically defined as the disruption of a habituated response, also due to the action of another (often strong) stimulus.

Some of the basic properties of habituation were described in the classic works noted above. In 1966, Thompson and Spencer surveyed the by-then very extensive behavioral literature on habituation and identified

2 Habituation

5. *The weaker the stimulus, the more rapid and/or more pronounced habituation is. Strong stimuli may yield no significant habituation.* This relationship is characteristic of most types of responses ranging from simple reflexes to complex exploratory behavior.

6. *The effects of habituation training may proceed beyond the zero or asymptotic response level.* Additional habituation training given after the response has disappeared or reached a stable habituated level will result in slower recovery. Although relatively few experiments have studied below-zero habituation as such, the observations may be viewed as an extension of the relationship between the number of stimulus presentations and degree of habituation. Zero response level is of course to some degree dependent upon the particular response measures used.

7. *The habituation of response to a given stimulus exhibits stimulus generalization to other stimuli.* This has been observed in many studies using auditory, visual, or tactile stimuli.

8. *Presentation of another (usually strong) stimulus results in recovery of the habituated response (dishabituation).* This phenomenon appears to be as ubiquitous as habituation itself and is commonly used to demonstrate that habituation has occurred. Pavlov was perhaps the first to describe this process (i.e., disinhibition) in relation to an extinguished conditioned response (CR), and also applied it to the habituated orienting response. Humphrey studied dishabituation extensively in lower vertebrates. Essentially, all responses of mammals that can be habituated can also be dishabituated. It is not always necessary for the dishabituating stimulus to be strong. In fact, Sokolov reported that a decrease in the intensity of an auditory stimulus results in dishabituation of the habituated orienting response in humans. Dishabituation, viewed as neutralization of the process of habituation, has perhaps been the most important method of distinguishing between habituation and fatigue.

9. *Upon repeated application of the dishabituating stimulus, the amount of dishabituation produced habituates (this might be called habituation of dishabituation).* Most studies of dishabituation (see above) have noted its habituation.

Thompson and Spencer argued that these nine common characteristics may consequently serve as the detailed operational definition of habituation, replacing the more general definition given above. The extent to which any other response decrements satisfy these characteristics will thus determine whether they can be called habituation.

However, Davis and Wagner challenged the parameter concerning stimulus intensity. They used the acoustic startle response in the rat and found that habituation to an intense stimulus caused a greater degree of absolute response decrement when tested with a weak stimulus than habituation to the weak stimulus. However, they also noted that if habituation and test stimuli were of identical

intensities, then the relative degree of habituation increases as stimulus intensity is decreased, in accordance with Thompson and Spencer's characteristic number 5. Therefore, the key is absolute versus relative measures of habituation. As Groves and Thompson stressed, characteristic number 5 must refer to relative rather than absolute measures of response strength.

Using the spinal flexion reflex, Spencer and Thompson were able to rule out changes in skin receptors, cutaneous afferent nerve terminals, and in motor neurons as loci of the decremental process underlying habituation, in accordance with Sherrington's earlier speculations. The decremental process must occur in interneurons. Perhaps their most important discovery was the fact that dishabituation was not a disruption of habituation but rather an independent superimposed process of sensitization. The decremental process underlying habituation was not disrupted at all by dishabituation. Indeed, in the flexion reflex, dishabituation (sensitization) always produces an increase in excitability of motor neurons. To the extent tested in mammalian systems, dishabituation is in fact a separate process of sensitization, but an exception has been noted in *Aplysia* by Rankin and Carew. These observations led Groves and Thompson to develop the dual-process theory (see below).

A number of theories, or at least hypotheses, concerning the process of habituation have been proposed over the years. In fact, many of these theories were more generally concerned with processes of learning and not developed specifically to deal with habituation. Three theories specific to habituation have been relatively successful and are still prominent today: Eugene Sokolov's stimulus-model comparator theory; Allan Wagner's revision of Konorski's gnostic hypothesis; and Groves and Thompson's dual-process theory. Each of these is briefly treated here.

Stimulus-Model Comparator Theory

Evgeny Sokolov developed a most influential stimulus-model comparator theory of habituation (see [Figure 1](#)). It was based primarily on his observation of the orienting response, often measured as arousal in EEG activity. The basic notion is that as a result of repeated stimulation, a stimulus model is formed in the brain, specifically in the cerebral cortex. In addition, there is an amplifying system that normally subverses behavioral output. A novel stimulus will result in a large orienting response, mediated by the amplifying system, identified with the ascending reticular activating system in lower brain regions. As the same stimulus is repeated, the stimulus model develops and as it develops, it exerts increasing inhibition on the amplifying system via descending corticofugal influences, thus yielding habituation. If a new

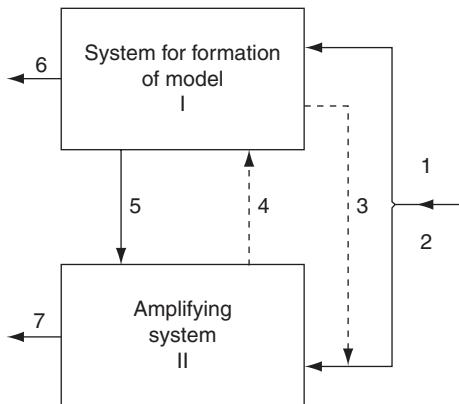


Figure 1 Sokolov's model of habituation. Sensory input through lines 1 and 2 projects to both the model formation and the amplifying systems. With repeated stimulation, the model develops and inhibits the amplifying system. See text for details. From Sokolov EN (1960) Neuronal models and the orienting influence. In: Brazier MA (ed.) *The Central Nervous System and Behavior: III*, pp. 187–275. New York: Macy Foundation.

or altered stimulus occurs which does not match the model, then inhibition is released and response strength recovers accordingly.

Wagner-Konorski Gnostic Unit Theory

Konorski briefly developed a theory of habituation that is, in many ways, analogous to Sokolov's theory. Allan Wagner elaborated Konorski's notion with greater emphasis on the roles of short-term memory and the existing associative network. This model is shown in [Figure 2](#). A stimulus

(SO = stimulus object) is processed via afferent fields to project to a memory system, the gnostic assembly, and to the arousal system. As the stimulus is repeated, a gnostic unit is formed, an increasingly accurate neuronal model or memory of the stimulus. As this model develops, it increasingly activates an inhibitory system that inhibits the arousal system, resulting in habituation. Wagner added two processes to Konorski's model, a reverberating circuit of transient memory (short-term memory – the two solid circles in the gnostic assembly) and the influence of the preexisting associative network. Indeed, Wagner's reformation of Konorski's model is in the mainstream of the information-processing theory, for example, Anderson and Bower. A key notion introduced by Wagner is that contextual cues may act via the associative network to excite stimulus representations in memory, that is, in the gnostic assembly. An important implication of this view is that some response systems should show context-specific long-term habituation.

Groves and Thompson's Dual-Process Theory

The basic assumption is that any effective stimulus will result in two independent processes in the central nervous system, one decremental (habituation) and one incremental (sensitization), that interact (see [Figure 3](#)). It is further assumed that habituation develops in the stimulus-response (S-R) pathway for whatever stimulus-evoked response is being habituated and that sensitization develops in a separate state system which then acts on the S-R pathway to yield the final behavioral outcome. Strong

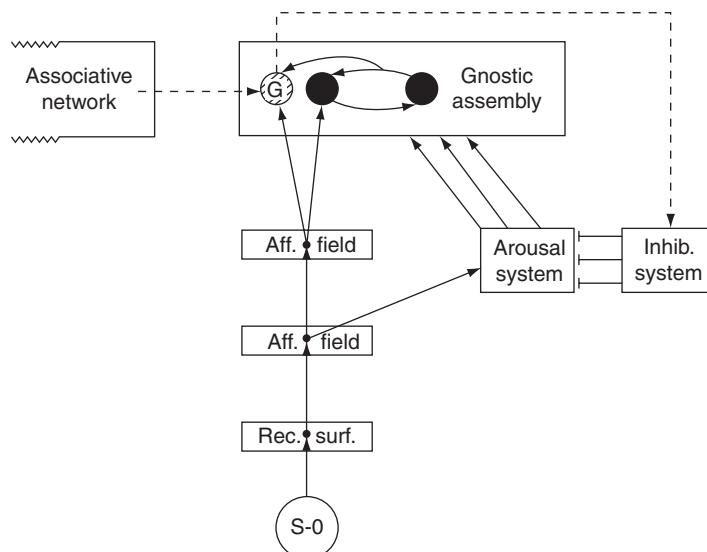


Figure 2 Wagner-Konorski's model of habituation. Sensory input forms a memory model in the gnostic assembly. As the model develops, it inhibits the arousal system. See text for details. From Wagner AR (1979) Habituation and memory. In: Dickinson A and Boakes RA (eds.) *Mechanisms of Learning and Motivation: A Memorial Volume for Jerry Konorski*, pp. 53–82. Hillsdale, NJ: Lawrence Erlbaum Associates.

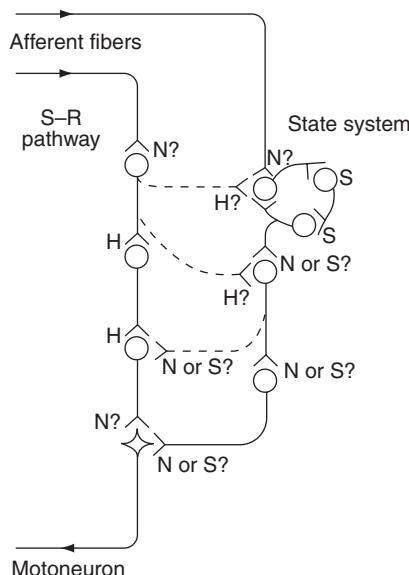


Figure 3 Neuronal model of the dual-process theory. The S–R pathway develops habituation and the state pathway may develop sensitization. They interact to yield behavior. See text for details. From Groves PM and Thompson RF (1970) Habituation: A dual-process theory. *Psychological Review* 77: 419–450.

supporting evidence for this theory came from studies of the activity of interneurons in the spinal cord.

All three theories have much in common. First, they all propose that a model of the stimulus develops as a result of repetition. This model is viewed essentially as a memory trace in the Sokolov and Wagner–Konorski models and simply as a decrement in synaptic transmission in the dual-process model. Such a synaptic decrement can of course qualify as a memory. Second, all three theories have an arousal system (amplifying in Sokolov; arousal in Wagner–Konorski; and state in the dual-process model).

In terms of differences, both Sokolov and Wagner postulate a descending inhibitory system activated by the memory trace that inhibits the arousal system. The dual-process theory postulates no such inhibitory system. Instead, both the S–R pathway and the state system activated by stimulus repetition will habituate. Another difference is that Sokolov, and particularly Wagner–Konorski, focus on long-term or between-session habituation, whereas much of the focus in the dual-process theory is on short-term or within-session habituation.

Mechanisms of Habituation

In animals with nervous systems, the underlying processes yielding behavioral habituation are due to alterations in neurons and synapses (see below). However, single-cell animals that behave, for example, ameba and paramecium, also show at least some phenomena of habituation, and

they, of course, have no neurons. Perhaps the most simplified or reduced preparation used for habituation is the PC12 cell line, studied by Daniel Koshland and associates. The cells in this cell culture do not behave, but they do secrete. The PC12 cell culture is a pheochromocytoma (cancer) cell line from the adrenal medulla. The cells are immortal in that they stop dividing until activated by the nerve growth factor. When appropriately stimulated, they secrete norepinephrine (NE) and other neurotransmitters. Koshland stimulated PC12 cells by pulses of potassium ions (K^+) and measured release of ^{3}HNE from the culture. The amount released showed clear habituation and exhibited both the frequency effect (more rapid and pronounced habituation with more rapid frequency of stimulation) and the intensity effect (the weaker the stimulus, the more rapid and pronounced the habituation). Further, they showed both within-session and between-session habituation, that is, both short- and long-term memory. The PC12 cell culture appears to be a most promising model for the analysis of the physical–chemical bases of habituation-like phenomena.

In animals with nervous systems, a consistent picture appeared to emerge in the 1970s of the basic synaptic mechanism of habituation. In their work on spinal reflexes, Spencer and Thompson argued that synaptic depression seemed a likely mechanism. In a series of elegant experiments, Eric Kandel and associates analyzed within-session habituation of the monosynaptic gill-withdrawal response in *Aplysia*. In brief, repeated stimulation resulted in a decrease in the probability of neurotransmitter release at the sensory–motor synapse, a presynaptic process, due in turn to a decrease in calcium ion influx at the sensory nerve terminals. Therefore, a clear picture seemed to emerge, at least for within-session habituation. Unfortunately, more recent work, for example, by Glanzman and by Rankin suggests that the mechanisms of both short- and long-term habituation are far more complex than earlier believed.

See also: Amnesia.

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Learning and Memory: Computational Models

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Glossary

Episodic memory – Memory for specific events in the past.

Mental context – Any information that is actively represented in a person's brain at the time they are processing a particular stimulus.

Semantic memory – Memory for meanings.

The goal of learning and memory research is to understand how we store and retrieve information based on our experiences. Toward this end, computational models provide formal implementations of memory theories; these formal implementations facilitate hypothesis testing and the generation of novel predictions. Computational models of memory are constrained in two directions. One goal of memory modeling research is to capture the behaviors that participants exhibit during memory tasks. Another goal is to explain how the brain gives rise to these behaviors. High-level, abstract models focus on reproducing behavior but not neural data, whereas biologically based models attempt to explain both neural and behavioral data. This article focuses on models of declarative memory, which can be divided into two components: episodic and semantic memory. The first section of the article describes abstract models of episodic memory, our ability to remember specific, previously experienced events. The second section describes abstract models of semantic memory – our ability to learn and remember the meanings of stimuli. The final section describes the Complementary Learning Systems (CLS) model, which seeks to account for both semantic and episodic memory phenomena within a single, biologically plausible computational framework.

Abstract Models of Episodic Memory

Episodic memory experiments typically consist of a study phase (where subjects are exposed to a set of stimuli) followed by a test phase. The test phase takes the form of either a recognition-memory test (where subjects have to discriminate between studied and nonstudied stimuli) or a recall test (where subjects have to retrieve specific details from the study phase of the experiment). Abstract models of episodic memory try to describe the mental algorithms that support performance on recognition and

recall tests, without specifically addressing how these algorithms might be implemented in the brain. Although there is considerable diversity within the realm of abstract episodic memory models, most of the abstract models that are currently in use share a common set of properties: Individual memories, commonly referred to as memory traces, are typically represented as vectors – where each element of that vector represents a particular feature of the memory. At study, memory traces are placed separately in a long-term store. Because of this ‘separate storage’ postulate, acquiring new memory traces does not affect the integrity of previously stored memory traces. At test, the model computes the match between the test cue and all of the items stored in memory. This item-by-item match information can be summed across all items to compute a ‘global-match’ familiarity signal. Most abstract models posit that subjects make recognition-memory judgments based on the strength of the global-match familiarity signal (i.e., the stronger the match, the more likely it is that the item was studied). Some abstract models that conform to this overall structure are Search of Associative Memory (SAM) model (first implemented by Raaijmakers and Shiffrin), Retrieving Effectively from Memory (REM) model (first implemented by Shiffrin and Steyvers), and MINERVA 2 (developed by Hintzman).

In abstract models, the same ‘match’ rule that is used to compute the global-match familiarity signal is also used when simulating recall tasks, although the specific way in which the match rule is used during recall differs from model to model. For example, MINERVA 2 simulates recall by computing a weighted sum of all of the items stored in memory, where each item is weighted by how well it matches the test cue. In contrast, models like SAM and REM use the individual match scores to determine which (individual) memory trace will be recalled.

Collectively, abstract models have been very successful in explaining behavioral recall and recognition data from normal subjects. They have also been used to explain data from memory-impaired subjects, by finding a set of parameter changes that lead to the desired pattern of memory deficits. The remaining part of this section presents a detailed description of the REM model. REM is highlighted because of its principled mathematical foundation, and because (of all of the models mentioned above) it is the abstract model that is being developed and applied most actively.

The REM Model of Recognition and Recall

The REM model, first published by Shiffrin and Steyvers in 1997, is the most recent iteration of a line of models that dates back to the SAM model that was published by Raaijmakers and Shiffrin in 1981. One of the main differences between REM and previous models like SAM and MINERVA 2 is that REM implements a principled Bayesian calculation of the likelihood that the cue ‘matches’ (i.e., corresponds to the same item as) a particular stored memory trace, whereas the match calculation was not defined in Bayesian terms in previous models.

In REM, items are vectors of features whose values are geometrically distributed integers. The primary consequence of feature values being distributed geometrically is that high-feature values are less common than low-feature values. When an item is studied, the features of that item are copied into an episodic trace for that item via a two-step process. First, each feature is stored with a specified probability that is a parameter of the model. If a feature is stored, then a second probability determines whether that feature is stored correctly or whether it is replaced by a value sampled from the geometric distribution. A zero value means that no value is stored for the feature.

At test, the retrieval cue is compared to each stored memory trace. For each trace, the model calculates the likelihood that the cue and the trace match (i.e., they correspond to the same item). This likelihood is based on two probabilities: the probability of obtaining the observed pattern of matching and mismatching features – assuming that the cue and trace correspond to the same item – divided by the probability of obtaining the observed pattern of matching and mismatching features, assuming that the cue and trace correspond to different items.

The same core ‘match’ calculation is used for both recognition and cued recall in REM. The model is applied to recognition by computing the overall odds that the item is old (vs. new), calculated as the average of the likelihood values from the match calculations. If this odds value exceeds a preset criterion then the item is called ‘old.’ The fact that the effects of individual feature matches (and mismatches) are combined multiplicatively within individual trace comparisons and additively across traces ensures that multiple matches to a single trace have a larger effect on the odds that an item is old than the same number of feature-matches spread across multiple traces.

Recall (i.e., retrieval of specific stored details) in REM has both a sampling component (which picks a single trace out from the memory store) and a recovery component (which determines whether the sampled memory trace is retrieved successfully). The sampling probability for each item is based on the match between the memory cue and the item, scaled by the sum of the matches to all items. Once an item is sampled, the probability that the image will be recovered is based on the proportion of

correctly stored item features. Thus, in REM, well-encoded items are more likely to be recovered than poorly encoded items.

Recently, Malmberg and colleagues developed a dual-process version of REM that utilizes both the ‘global-match’ familiarity signal and the recall process described above. When a test item is presented, the model computes the global match and it also attempts to retrieve a specific stored memory trace that matches the test item. The key benefit of using recall is that it helps the model reject lures that closely resemble studied items. For example, if subjects study ‘rats’ but are tested with ‘rat,’ this test item will trigger a strong global-match signal, but it may also trigger recall that ‘rats’ was studied (not ‘rat’). This mismatch between the test item and the retrieved memory can be used to identify the item as a related lure. Malmberg argues that subjects primarily use this recall process to reject related lures, and that it does not play a significant role in recognizing actually studied items (this view is controversial).

Representative REM results

Researchers have demonstrated that REM can explain a wide range of episodic memory findings. For example, Shiffrin and Steyvers demonstrated that the ‘global-match’ familiarity mechanism described above can account for the list-length, list-strength, and word-frequency effects in recognition memory.

The list-length effect refers to the finding that recognition-memory performance tends to be lower for longer versus shorter study lists. REM explains this effect because adding words to the study list increases the odds that the test item will spuriously match a stored memory trace from the study list (i.e., the model will conclude that the test item matches a stored memory trace when in fact it does not). The (null) list-strength effect refers to the finding that strengthening some list items (by repeating the items or presenting them for longer periods of time) does not impair recognition of other, nonstrengthened items. REM explains this effect because strong items have more features stored (i.e., they have more ‘differentiated’ memory representations) and thus are less likely to be confused with other test items. The word-frequency effect refers to the finding that words that occur with high frequency in natural language are recognized less well than low-frequency words. In REM, high-frequency words have more common feature-values than low-frequency words, which makes them more likely to be confused with other test items.

Context and Episodic Memory

While the basic REM model provides a mechanism for how the memory system responds to a particular cue, it does not describe how the memory system behaves when

external cues are less well specified, and subjects have to generate their own cues in order to target a particular memory (or set of memories). Take the scenario of trying to remember where you left your keys. The most common advice in this situation is to reinstate your mental context as a means of prompting recall – if you succeed in remembering what you were doing and what you were thinking earlier in the day, this will boost the probability of recalling where you left the keys. This idea of reinstating mental context plays a key role in theories of strategic memory search. For the purposes of this article, mental context can be defined broadly as any other information that is actively represented in a person's brain at the time they are processing a particular stimulus.

Multiple laboratory paradigms have been developed to examine strategic memory search. The most commonly used paradigm is free recall, where subjects are given a word list and are then asked to retrieve the studied word list in any order. REM can be extended to simulate free recall by adding a set of contextual features to each memory trace. For example, all of the items in the study list could be given a shared set of contextual features (effectively, a 'context tag') that signify membership in the study list. To simulate free recall, we can cue with this 'list-context' representation and sample items that were paired with the list-context representation at study. However, while this simple context-tag representation gives REM the ability to simulate free recall, it does not allow REM to simulate more nuanced features of free recall data. To fit detailed patterns of free recall data, it is necessary to specify in more detail how context changes over time, and how context is used to cue memory at retrieval.

The Temporal Context Model

The Temporal Context Model (TCM; first published by Howard and Kahana) is the most recent in a long succession of models that use a drifting mental context to explain our ability to selectively target memories from particular time periods. The basic idea behind these models is that the subject's inner mental context (comprising the constellation of thoughts that are active at a particular moment) changes gradually over time. Early models viewed context as a vector that evolves as a function of random noise when each item is presented, with a drift-rate parameter governing the overlap of context from time-step to time-step. The main difference between TCM and previous contextual-drift models is that context does not drift randomly in TCM. Rather, contextual updating is driven by the features of the items being studied and recalled.

During the study phase of a memory experiment, two things happen when an item is presented: first, the item is associated with the state of the context vector at the time of presentation; second, context is updated by averaging together the current state of the context vector with the semantic features of the just-studied item. At test, the recall process is initiated by cuing with the current state of the context vector, which (in turn) triggers retrieval of items that were associated with these contextual elements at study. Specifically, each item is activated to the degree that the current state of context overlaps with the context that was present when that item was studied. In the most recent version of TCM, called TCM-A, these activated items compete with one another via a set of accumulators that add up evidence for each item over time (based on that item's level of activation and the activation levels of all the other items). If the level of evidence for an item reaches a prespecified threshold level, that item is recalled, and the current state of context is updated in two ways: first, by averaging in the semantic features of the just-recalled item, and second, by averaging in the state of the context vector that was present when the item was studied. This latter updating operation can be construed as 'mentally jumping back in time' to the moment when the (just-retrieved) item was studied. Once the context vector is updated, it is used to cue for additional items, which leads to additional updating of the context vector, and so on.

How TCM accounts for recall data

The drifting context vector in TCM explains a number of findings in episodic recall, including both recency and contiguity effects. In TCM, the current state of context acts as the cue for memory retrieval via context-to-item associations. Because context changes gradually, the state of context at the time of test will overlap most strongly with the contexts associated with recent items. This gives rise to the recency effect seen in all episodic memory tasks. TCM can also explain the temporal contiguity effect: the finding that, when a subject recalls a particular item from the study list, they show an increased probability of (subsequently) recalling items from nearby time points in the list. TCM shows this effect because recalling an item (at test) also triggers recall of the contextual state that was present when the item was studied. This retrieved context will closely match contextual states associated with temporally proximal items, thereby making it easier to retrieve these items. For example, the contextual state associated with the fourth item on the study list will closely match the contextual states associated with the third and fifth items; thus, recalling the 'fourth-item' context will make it easier to access the third and fifth items.

Abstract Models of Semantic Memory

Earlier, we defined semantic memory as the ability to learn and remember the meanings of stimuli. More concretely, semantic memory is our ability to construct an internal representation of the world that allows us to make predictions about ‘unseen’ aspects of stimuli. For example, the semantic memory system allows us to categorize the pig we see at the petting zoo as a mammal and to generalize that it has a brain and gives birth to its children alive following a gestation period, without having to take a magnetic resonance image of the pig’s head or watch it give birth. The semantic memory system also allows our friends to retrieve these basic features of a pig when we recount seeing a pig at the petting zoo.

Rumelhart Model of Semantic Cognition

We focus on the model of semantic memory developed by David Rumelhart and colleagues, because it is the simplest extant model that explains how we develop internal representations and make predictions using these representations. Our discussion here draws heavily on recent work using this model by Rogers and McClelland.

The goal of the Rumelhart model is to activate the proper set of attributes when probed with an item (e.g., ‘pig’) and relation (e.g., ‘can’). The basic structure of the Rumelhart model (see schematic in **Figure 1**) consists of multiple layers of units connected in a feedforward fashion. The item units specify the objects that are being observed, the relation units specify the contexts in which we observe these objects, and the attribute units specify

the ‘unseen’ aspects of the objects that we are trying to predict. When the network is probed by activating an item unit and a relation unit, activation spreads forward in the network (through the representational layer and the hidden layer) until it reaches the attribute layer. The spread of activation is governed by the strengths of connections between units, and the resulting pattern of activity in the attribute layer constitutes the model’s prediction (e.g., ‘pig’ + ‘can’ should yield ‘walk’ in the attribute layer). A critical aspect of the model is that it does not prespecify what patterns should appear in the representational and hidden layers. Rather, the network learns to generate patterns in these layers (by adjusting weights) that help it predict the correct attributes. The pattern of activity in the representational layer serves as the primary representation of the item’s meaning, whereas the pattern of activity in the hidden layer represents the meaning of the item in the context of a particular relation.

A central aspect of the Rumelhart model is that it learns to make better predictions by adjusting connection strengths. Learning takes place incrementally over many presentations of item, relational, and attributional patterns (i.e., seeing a fish swim in water would be represented by activating the ‘fish’ item unit, the ‘can’ relation unit, and the ‘swim’ attribute unit). On each trial, after generating its prediction, the network receives feedback on which attributes are actually observed for that item/relation combination. Learning in the network is driven by prediction error, that is, the discrepancy between attributes that are predicted to be present, and the attributes that are actually present. On each trial, after prediction error is computed, and weights are changed

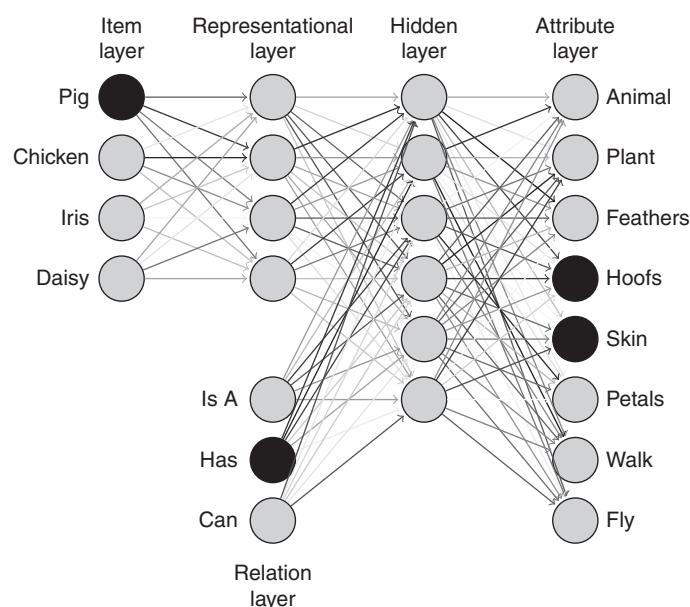


Figure 1 Simplified diagram of the Rumelhart model.

throughout the network in order to reduce prediction error.

Specifically, Rumelhart used the backpropagation neural network learning algorithm to adjust network weights. For each unit in the network (except for item and relation units), backpropagation computes a delta (δ)-value for that unit that indicates whether that unit was too active ($+\delta$) or not active enough ($-\delta$). First, backpropagation computes δ -values for attribute units; then it computes δ -values for each preceding layer (in turn) by multiplying δ -values by network weights. For example, if an attribute unit has a positive δ -value (i.e., it is too strongly active), active hidden units with positive connections to the overly active attributional unit are also assigned positive δ -values (indicating that they are ‘at fault’ for the prediction error, and that prediction error can be reduced by reducing the activity of hidden units). After δ -values have been assigned to all of the attribute-layer, hidden-layer, and representation-layer units, backpropagation changes network weights based on these δ -values: if a unit is too active, weights coming into that unit (from active sending units) are reduced, and if a unit is not active enough, weights coming into that unit (from active sending units) are increased.

Successes of the Rumelhart model

After a sufficient degree of training, Rumelhart showed that the model learns context-sensitive mappings between items and attributes. For example, after training, activating the ‘pig’ item and the ‘has’ relation will activate the ‘skin’ and ‘hoofs’ attributes. If instead we activate the ‘pig’ and the ‘can’ attribute, the ‘walk’ attribute will activate (and the ‘fly’ attribute will certainly not activate). This context sensitivity arises because the relation units modify the activation in the hidden layer, which is responsible for combining the activity in the representational and relational layers before activating the attribute units.

The most interesting aspect of the Rumelhart model is how internal representations (i.e., the patterns of activity in the representation and hidden layers elicited by different items) change during learning. Items with similar attributes come to elicit similar patterns of activity in the representation and hidden layers, and items with distinct attributes come to elicit distinct patterns of activity in the representational and hidden layers. For example, when training the sample Rumelhart model in **Figure 1**, the representations for ‘pig’ and ‘chicken’ start to converge, and these representations diverge together from the representations for ‘iris’ and ‘daisy’ because (for any given relation) ‘pig’ and ‘chicken’ are more likely to share attributes than, say, ‘pig’ and ‘daisy.’ In this way, the model learns just as a child would – by first forming a coarse representation of the environment that is refined over time based on experience.

A key property of the model is its ability to generalize to new stimuli based on their similarity to previously encountered stimuli. For example, after learning the various attributes of pigs and chickens, the Rumelhart network will be able to predict basic properties of a new animal, such as a cheetah, just by learning that it is an animal. This is because training the network to predict animal given cheetah will push the cheetah’s internal representation closer to the representations of other items that predict animal (e.g., pig and chicken). This overlap in internal representations will lead to cheetah predicting other attributes that were associated with pigs and chickens (e.g., that cheetahs have skin and can walk).

Temporal Context and Semantic Relationships

The Rumelhart model modifies its internal representations based on explicit instruction concerning which attributes should be active in a given context. Recently, several researchers have argued that meaning representations can also be acquired without explicit instruction, if the model keeps track of temporal context (i.e., it learns which items tend to be presented close in time to a given item). The key idea here is that items with similar meanings tend to occur in similar temporal contexts (e.g., couch and sofa both tend to occur close in time to other words like rug, lamp, and cushion). Given this premise, it should be possible to learn that couch and sofa have similar meanings by learning about which other words tend to co-occur with couch and sofa.

The Latent Semantic Analysis (LSA) algorithm developed by Landauer and colleagues provides a large-scale proof of the relationship between meaning and temporal context. Landauer and colleagues took a massive corpus of English texts and computed how often each word co-occurred in the same paragraph as every other word (normalized to account for differences in overall word frequency). The net product is a matrix of size $N \times N$, where N is the number of distinct words in the text corpus. One way of thinking about this matrix is that each word in the matrix is represented by a ‘temporal context vector’ of length N , listing how often that word occurred with every other word. In principle, it should be possible to estimate the similarity of word meanings by looking at the similarity of the N -dimensional temporal context vectors associated with each word. However, Landauer also had the insight that there is considerable redundancy in the $N \times N$ co-occurrence matrix, and that (because of this redundancy) words could be represented by vectors with many fewer-than- N elements. To eliminate this redundancy, LSA applies a technique called singular value decomposition (SVD) to the $N \times N$ matrix. SVD returns a set of N orthogonal temporal context vectors (each of size N), ranked by how much variance they explain (across words) in the original matrix

(technically, these are the eigenvectors of the original matrix). Landauer found that the first 300 or so of these vectors accounted for almost all of the variance in the original matrix. As such, he discarded the remaining vectors, and re-expressed each word's temporal context vector in terms of a weighted combination of these 300 'basis vectors.' The net result of this process to go from an N -dimensional representation for each word to a (much more manageable) 300-dimensional representation. Using these 300-dimensional vectors, Landauer and colleagues found that the cosine distances between these vectors map quite well onto the similarity values that people assign to pairs of words. For example, the cat and dog vectors are quite similar to each other (i.e., their cosine distance is very small), whereas the chicken and daisy vectors are not.

Importantly, while LSA substantiates the idea that temporal context provides information about stimulus meaning, it does not provide a mechanistic account of how the brain exploits temporal context to acquire semantic representations. Recently, Howard and colleagues argued that the TCM (described earlier) can meet these desiderata. The previous section discussed how TCM can account for episodic memory phenomena (by rapidly binding items with coactive contextual features, such that items can trigger recall of associated contexts and vice versa). TCM can be extended to address semantic learning by supplementing this rapid binding process with another learning process that gradually (across trials) learns which contextual features tend to be associated with a given item.

Another important point is that, while temporal context provides some information about word meanings, LSA-like algorithms are not meant to be a substitute for the kinds of error-driven learning that are built into the Rumelhart model. Meanings learned by LSA do not always coincide perfectly with human semantic judgments (e.g., LSA tends to give antonyms very similar meaning representations because they occur in similar contexts). To remedy these misconceptions, models of semantic memory need to receive feedback on how well they are predicting the item's attributes, and they need to be able to learn based on prediction errors. One way to update the Rumelhart model to take advantage of both error-driven learning and temporal context information would be (1) to include a representation of temporal context (akin to the representation generated by TCM) and (2) to train the model to predict the current state of the temporal context representation (plus other relevant attributes) when an item is presented. Forcing the model to predict temporal context will bias the model to assign similar internal representations to items with similar temporal contexts (just as forcing the model to predict attributes like 'animal' causes the model to assign similar representations to all of the 'animal' items).

Finally, while the above discussion focused on temporal context, other kinds of context also provide information about stimulus meaning. For example, knowing that two items tend to appear at similar spatial locations provides some information about their meanings, irrespective of whether they appear close in time to one another. The general principle of training models to predict contextual information (be it temporal, spatial, or some other type of information) will allow models of semantic memory to leverage all of these regularities when learning about meanings.

Learning, Memory, and the Brain

The previous sections focused on abstract models of episodic and semantic memory. This section describes the CLS model, which intertwines episodic and semantic memory into a single, neurally plausible computational framework.

The Complementary Learning Systems (CLS) Model

The CLS model (first outlined by McClelland, McNaughton, and O'Reilly) incorporates several widely held ideas about the division of labor between hippocampus and neocortex that have been developed over many years by many different researchers. (It is important to note that the CLS model is only one of many biologically-plausible models of the hippocampal role in learning and memory. For example, see Further reading for other influential hippocampal models by Gluck, Rolls, Hasselmo, and Eichenbaum.) According to the CLS model, the neocortex forms the substrate of our internal model of the structure of the environment. In contrast, the hippocampus is specialized for rapidly and automatically memorizing patterns of cortical activity, so they can be recalled later (based on partial cues). This characterization makes it clear that neocortex is the key substrate for semantic memory and that hippocampus is crucial for episodic memory, although (as discussed below) both structures contribute to both kinds of memory.

The model posits that the neocortex learns incrementally; each training trial results in relatively small adaptive changes in synaptic weights. These small changes allow the cortex to adjust its internal model of the environment gradually in response to new information. The other key property of neocortex (according to the model) is that it assigns similar representations to similar stimuli. Use of overlapping representations allows cortex to represent the shared structure of events, and therefore makes it possible for cortex to generalize to novel stimuli based on their similarity to previously experienced stimuli. In contrast, the model posits that hippocampus assigns

distinct, pattern-separated representations to stimuli, irrespective of their similarity. This property allows the hippocampus to memorize arbitrary patterns of cortical activity associated with particular events rapidly without suffering from unacceptably high (catastrophic) levels of interference.

Norman and O'Reilly model of episodic memory

Norman and O'Reilly constructed hippocampal and cortical networks that instantiate the CLS principles outlined above, and applied these networks to simulating episodic memory data. In both the hippocampal and cortical networks, to-be-memorized items are represented by patterns of excitatory activity that are distributed across multiple units (simulated neurons) in the network. Excitatory activity spreads from unit to unit via positive-valued synaptic weights. The overall level of excitatory activity in the network is controlled by a feedback-inhibition mechanism that samples the amount of excitatory activity in a particular subregion of the model, and sends back a proportional amount of inhibition.

The architecture of the model (illustrated in **Figure 2**) reflects a broad consensus concerning key anatomical and physiological characteristics of different hippocampal and cortical subregions, and how these subregions contribute to the overall goal of memorizing cortical patterns. The entorhinal cortex (EC) contains a compressed representation of information represented elsewhere in cortex. The hippocampal network memorizes patterns of EC activity

by linking these patterns to a set of units (an 'episodic representation') in region CA3, which is then linked back to EC via region CA1. When a pattern is presented, connections are strengthened between active EC and CA3 units, between active units within CA3, and between active CA3 and CA1 units; collectively, these synaptic modifications allow the network to recall entire stored EC patterns based on partial cues (pattern completion). To minimize interference, the network has a built-in bias to assign relatively nonoverlapping (pattern separated) CA3 representations to different episodes. Pattern separation occurs because of strong feedback inhibition in CA3, which leads to sparse representations (i.e., representations with relatively few neurons active). The dentate gyrus (DG) assists in the pattern separation process by forming an even more sparse representation of the EC pattern, which then projects into region CA3.

The cortical component of the CLS model consists of an input layer (corresponding to lower regions of the cortical hierarchy) which projects in a feedforward fashion to a hidden layer (corresponding to regions further up in the hierarchy, including perirhinal cortex (PC) and EC). As mentioned earlier, the main function of cortex is to extract statistical regularities in the environment. The two-layer CLS cortical network (where hidden units compete to encode regularities that are present in the input layer) is meant to capture this idea in the simplest possible fashion. In the Norman and O'Reilly simulations, learning in both the cortical and hippocampal subregions of the model was implemented by means of a simple Hebbian learning rule that strengthens connections between active sending and receiving neurons and weakens connections between active receiving neurons and inactive sending neurons.

As described by Norman and O'Reilly, the hippocampal and cortical networks constitute a biologically based dual-process model of recognition memory. As with the dual-process REM model described earlier, the CLS model posits that familiarity (i.e., global match) and recall of specific details both contribute to recognition memory. In the CLS model, hippocampus supports recall of specific studied details, but (because of its tendency to assign distinct CA3 representations to stimuli, regardless of their similarity) it is not well suited for computing the global match of the test cue to studied items. The cortex, on the other hand, does not learn quickly enough to support recall of details from specific events, but it can compute a scalar familiarity signal that tracks how well the test cue matches studied items. As items are presented repeatedly, their representations in the hidden layer of the cortical network become sharper: novel stimuli weakly activate a large number of hidden units, whereas previously presented stimuli strongly activate a relatively small number of units. Sharpening occurs in the cortical model because Hebbian learning specifically tunes some hidden units to

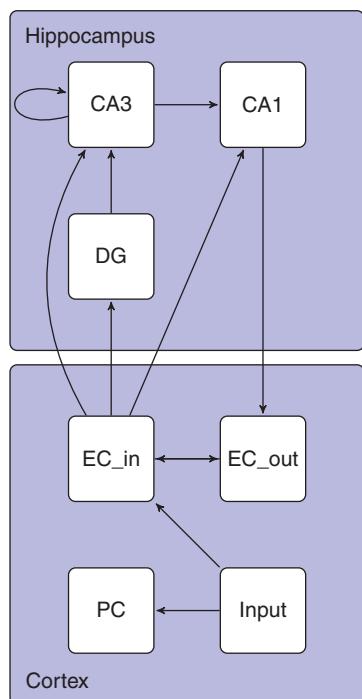


Figure 2 Architecture of the Norman and O'Reilly Complementary Learning Systems model of episodic memory.

represent the stimulus, and these units suppress the activity of other units (via the feedback-inhibition mechanism). Furthermore, since the cortex assigns similar hidden representations to similar stimuli, these sharpening effects generalize smoothly to other stimuli based on their similarity to the original stimulus (so sharpening tracks global match).

Norman and O'Reilly showed how, taken together, the hippocampal network and cortical network can explain a wide range of behavioral findings from recognition and recall list-learning experiments. For example, the model can account for the null-recognition list-strength effect described earlier, and it also makes the prediction that list-strength effects should be observed when recognition memory is driven by recall of specific details as opposed to familiarity. Furthermore, because the CLS model maps clearly onto the brain, it is possible to use the model to address neuroscientific data in addition to (purely) behavioral data. For example, the model correctly predicts that focal hippocampal lesions should differentially impair recognition performance on tests where distractors are very similar to studied items (because these tests benefit from the hippocampus' ability to assign distinct representations to similar patterns). The model also successfully predicts that lesioned patients' deficit on these tests can be ameliorated by giving subjects a forced choice between studied items and corresponding related lures (because the forced-choice procedure allows subjects to leverage small but reliable differences in cortical familiarity between studied items and corresponding lures).

The original form of the CLS episodic memory model also has some serious flaws. In particular, Bogacz and Brown showed that the cortical network's capacity for familiarity discrimination (i.e., the number of studied patterns that it can discriminate from nonstudied patterns with 99% accuracy) falls far below the documented capacity of human recognition memory. This problem can be traced back to the Hebbian learning rule, which is insufficiently judicious in how it adjusts synaptic strengths: it strengthens synapses between co-active units even if the memory is already strong enough to support recall, and it weakens synapses between active receiving units and other inactive units, even if those other units are not interfering with recall of the sought-after memory. This excess synaptic modification greatly increases the extent to which new learning interferes with stored knowledge. The solution to this problem is to switch to an error-driven learning rule that compares top-down expectations (generated by cortex's internal representation of the environment) to sensory inputs, and only modifies synapses when the model's expectations are incorrect. The backpropagation rule described earlier fits this description, but this rule is widely believed to be biologically implausible. Determining biologically plausible methods of enacting error-driven learning in cortex has

been a major focus of computational modeling research, and researchers have devised a wide range of potential solutions to this problem (see, e.g., the work of Carpenter and Grossberg). Recently, Norman and colleagues swapped out the Hebbian learning rule for a more judicious, biologically plausible rule that uses neural oscillations to probe for 'weak points' in cortical representations; this new learning rule greatly improves the cortical model's familiarity discrimination capacity.

Toward a full episodic/semantic CLS model

The Norman and O'Reilly CLS simulations focused on episodic memory. However, the CLS cortical model (equipped with an error-driven learning rule) should be able to account for all of the semantic learning phenomena that were discussed in the Rumelhart model section above; the key prerequisites for simulating these results are a learning rule that is driven by prediction error, and the ability to re-represent inputs in order to minimize prediction error. CLS can also be used to explore how hippocampo-cortical interactions shape semantic memory. One of the key claims made in the original formulation of CLS was that hippocampus could play back significant, once-presented events to the cortex during sleep, thereby allowing the slow-learning cortical network to absorb these events into its semantic network. Recent modeling work by Norman, Newman, and Perotte explored other aspects of learning during sleep (e.g., the possibility that learning during REM sleep could help to repair cortical memories that are damaged by new learning).

Another feature missing from most CLS models is a representation of context. Some CLS models have included a simple context layer (akin to the one used in the Rumelhart model), but none of these models have seriously explored how temporal context is represented in the brain. It seems likely that prefrontal cortex (PFC) will play a key role in temporal context memory (by virtue of its ability to actively maintain patterns of neural firing over time). Other researchers have noted that the EC also has some intrinsic ability to maintain information over time. Future work using the CLS framework will explore the contributions of both PFC and EC to representing temporal context.

Key Challenges

Over the past several decades, a consensus has emerged among computational modelers regarding certain key aspects of memory functioning (e.g., how the hippocampus supports episodic memory). However, there is still extensive work to be done in specifying extant computational models of learning and memory. For example, while there is widespread agreement that cortical learning

is driven by a comparison of top-down expectations versus bottom-up inputs, there is still extensive debate concerning precisely how the brain implements this learning process. In addition, extant models have focused on specifying basic encoding and retrieval processes, and have not yet addressed fundamental issues with regard to strategic influences on memory (e.g., how much to rely on recall of specific details vs. familiarity when making recognition decisions; how to strategically construct retrieval cues during memory search). This latter issue might benefit from a normative approach (i.e., mathematically deriving how subjects should be cuing memory and making decisions in order to maximize performance, and then exploring whether subjects actually use these 'optimal' strategies).

Importantly, the models of episodic and semantic memory described above constitute only a small portion of the total space of memory models. Other models have been developed to account for data from other kinds of memory tasks, such as working memory tasks (which ask subjects to actively maintain stimulus information in the face of distraction), conditioning tasks, spatial learning tasks, and motor-learning tasks. These other models leverage some of the same computational principles and neural systems described above, but they also describe important ideas that were not reviewed in the preceding sections. For example, one topic that has received extensive attention in recent years is how the brain leverages simple reinforcement signals (rewards and punishments) to improve behavior. Another topic that has received extensive attention is the control of working memory (i.e., how does the brain learn when to 'gate' information into working memory and when to release it from working memory; see the work of Frank, O'Reilly, and colleagues).

The key challenge, moving forward, will be to integrate insights gleaned from all of these models while still keeping model complexity within manageable limits. Models like CLS are quite complex as they stand, and the extensions proposed above (e.g., adding a PFC network to CLS to help it maintain temporal context) will make the models even more complex. The saving grace here is that modern-day memory models can be used to address an enormous range of findings – using a complex model to explain a single result may not be all that meaningful, but simultaneously accounting for behavioral and neural data from multiple types of learning experiments is still a worthy challenge. So long as modelers continue to apply all available constraints to theory development, we should continue to see steady progress toward a complete computational account of learning and memory data.

See also: Declarative Memory; Episodic and Autobiographical Memory: Psychological and Neural Aspects; Learning and Memory: Computational Models; Memory and Aging, Neural Basis of; Memory Consolidation.

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Motor Learning in the Vestibulo-Ocular Reflex

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Glossary

AMPA receptor – A type of glutamate receptor containing a cation channel. α -Amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptor current depolarizes the postsynaptic membrane, mediating most excitatory synaptic transmission in the brain.

Compensation – See the glossary entry ‘vestibular compensation.’

Consolidation – The conversion of a memory from a short-term, labile form to a long-term, more stable form.

Flocculus – A region of the cerebellar cortex that is reciprocally connected with the vestibular nuclei and which modulates the vestibulo-ocular reflex (VOR).

Flocculus target neurons – The neurons in the vestibular nuclei that receive direct input from the flocculus.

Gain – The ratio of output to input of a system; in the case of the VOR, the ratio of the eye velocity generated by the reflex to the head velocity that triggers it.

Inferior olive – A brainstem nucleus that projects to the cerebellar cortex and is the source of climbing fibers.

Long-term depression (LTD) – A persistent (45 min or more) decrease in the efficacy of transmission by a synapse.

Metabotropic glutamate receptors (mGluRs) – The glutamate receptors that do not contain ion channels, but interact with second-messenger systems through G proteins.

Motor learning – A change in the motor response to a sensory stimulus that is acquired by experience and that can be retained without further experience.

Parallel fibers – The long, unmyelinated axons of granule cells that make up the molecular layer of the cerebellar cortex, and that provide excitatory input to Purkinje cells.

Purkinje cells – Large inhibitory neurons, located in the cerebellar cortex, that project out of the cortex to the cerebellar nuclei and vestibular nuclei.

Retinal slip – The motion of an image with respect to the retina.

Vestibular compensation – The process by which vestibular function, including VOR function, is restored following damage to the vestibular sensory organ or vestibular nerve.

Vestibulo-ocular reflex (VOR) – A reflex that stabilizes gaze by moving the eyes in the opposite direction to the

head, and at the same speed, utilizing sensory signals that arise from the vestibular labyrinth.

VOR adaptation – A change in vestibulo-ocular reflex performance that occurs as a result of visual and vestibular experience and that persists in the absence of visual input.

The Vestibulo-Ocular Reflex

The vestibulo-ocular reflex (VOR) uses information from the vestibular labyrinth of the inner ear to generate eye movements that stabilize gaze during head movements. Without the VOR, when walking down the street, it is impossible to read signs or even recognize faces. Even an inaccurate VOR can cause the visual image to slip with respect to photoreceptors, blurring images. The VOR responds both to rotation and to translation, using signals arising from different receptors in the inner ear. During rotation, the VOR can stabilize the eyes accurately even at angular velocities of over 300° s^{-1} and frequencies above 20 Hz. This is because the VOR pathway is relatively short (see [Figure 1\(a\)](#)) and uses only vestibular sensory information to activate motoneurons. Precisely because it is so quick, the VOR must be intrinsically accurate; on this timescale, vision is too slow to be useful as a corrective mechanism.

Functional Significance of Learning in the VOR

Since vision cannot be used as feedback, how can the VOR ensure good visual stability? One solution is to calibrate the movement by trial and error, that is, by motor learning. Motor learning in the VOR is also called VOR adaptation or plasticity. [Figure 1\(b\)](#) shows an example of the VOR’s response to sinusoidal rotation in the horizontal plane. Although the VOR also responds to rotation in other planes as well as to translation and tilt, most studies of motor learning have been carried out using horizontal rotation. The eye movement generated by the VOR is opposite in direction and approximately equal in speed to the head movement. The ratio of eye to head speed is called the ‘gain’ of the reflex. A gain of 1.0 would describe a perfect VOR for rotation under normal conditions.

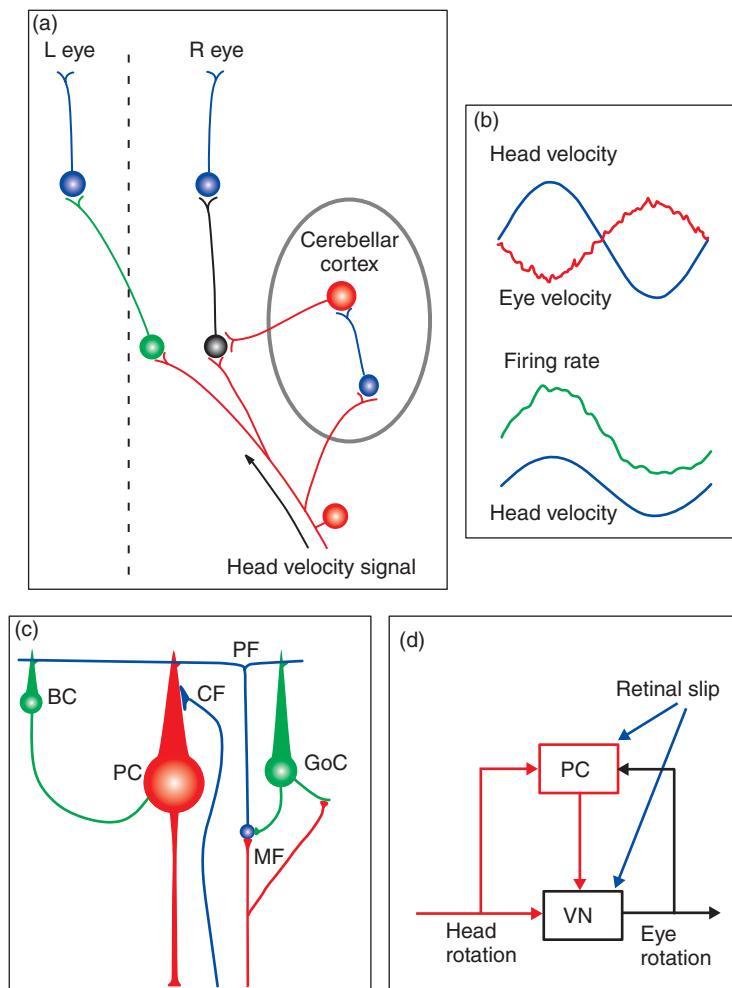


Figure 1 Anatomy of the vestibulo-ocular reflex. (a) Circuitry of the horizontal rotatory VOR. Primary afferents in the vestibular nerve (red) provide a head velocity signal to excitatory (green) and inhibitory (black) secondary vestibular neurons in the vestibular nuclei, which in turn project to motoneurons (blue) innervating the lateral rectus muscles. Because the axon of the excitatory interneuron crosses the midline (dashed line) to the contralateral side, the excitatory and inhibitory pathways are synergistic. Additional pathways (not shown) innervate the medial rectus muscles. An inhibitory side loop modulates the VOR in situations where its gain is too high or too low for perfect stabilization. The side loop includes granule cells (blue) and Purkinje cells (red) in the cerebellar cortex. The Purkinje cell output signal inhibits the inhibitory VOR interneuron. (b) Response of the VOR to rotation. Head (blue) and eye (red) movements are opposite in direction and approximately equal in velocity. Data from alert cat (gain = 0.8). The firing rate of a secondary neuron (green) closely follows head velocity. (c) Actual neural circuit of the cerebellar cortex. Input signals arrive in the cortex through mossy fibers (MF, red) and climbing fibers (CF, blue). Granule cell axons (blue, also called parallel fibers) excite dendritic spines of Purkinje cells (PC), Golgi cells (GoC), and inhibitory interneurons (BC or basket cell). Thus, excitatory inputs from climbing fibers and parallel fibers converge on the Purkinje cell dendrites. (d) Signal flow in the cerebellar cortex and the VOR. Primary afferent fibers from the semicircular canals send head velocity signals both to Purkinje cells and to VOR interneurons in the vestibular nuclei (VN). The Purkinje cell, in turn, inhibits the VOR interneuron. Climbing fibers (blue) carrying retinal slip signals excite Purkinje cells and send collaterals to the vestibular nuclei as well. Finally, an appropriate eye velocity signal is sent to the extraocular muscles. A feedback loop provides a copy of the eye velocity signal (through mossy fibers) to the Purkinje cells. (a, b, d) with permission from Broussard DM and Kassardjian CD (2004) Learning in a simple motor system. *Learning and Memory* 11: 127–136, Cold Spring Harbor Press; (c) with permission from Eccles JC, Ito M, and Szenthagothai J (1967) *The Cerebellum as a Neuronal Machine*. Springer: Berlin.

If the VOR gain is inappropriate for the existing conditions, then learning is needed to improve its accuracy and, hence, visual stability. In everyday life, the VOR gain becomes inappropriate as we age and lose vestibular sensitivity, whenever we get a new pair of prescription glasses, and when we suffer from vestibular damage. Each of these situations provides a situation where motor learning is beneficial. For example, when a near-

sighted person puts on a new pair of glasses with a stronger prescription, the visual field is reduced and the eyes do not need to move as far or as fast to stabilize gaze. Initially, then, the VOR is inaccurate when wearing the new glasses. After wearing them for a while, motor learning reduces the VOR gain and gaze is stable again. In the lab, learning can be demonstrated by measuring the gain of the VOR in darkness after the subject has been exposed

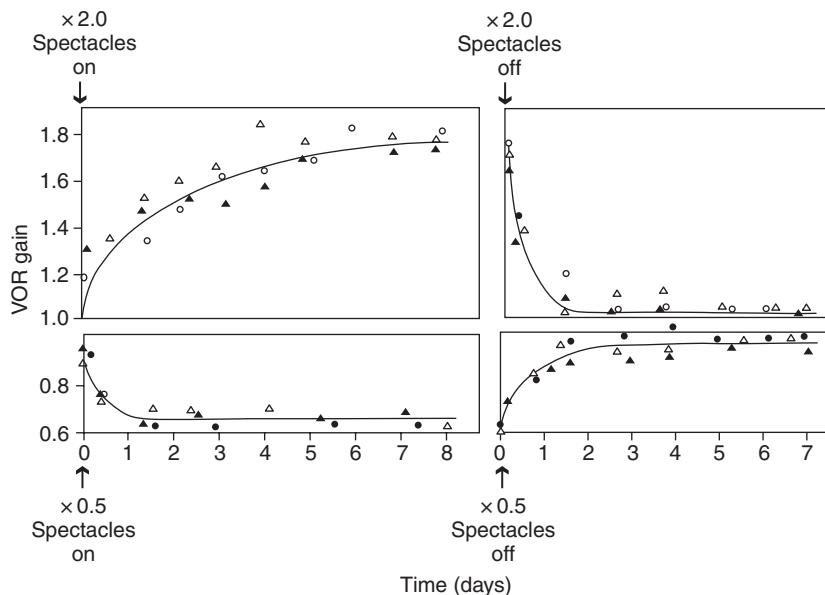


Figure 2 The time course of motor learning. Data are shown from monkeys wearing magnifying ($\times 2.0$, top two rectangles) or miniaturizing ($\times 0.5$, bottom two rectangles) telescopic spectacles. Head velocity was provided by the monkey's own movements. Different symbols are from different animals. The change in gain was fit by an exponential decay. In the right-hand panels, the spectacles were removed and the VOR gain was allowed to return to normal. The return to normal had a different time constant for high as compared to low gain. Reproduced with permission from Miles FA and Eighmy BB (1980) Long-term adaptive changes in primate vestibuloocular reflex. I. Behavioral observations. *Journal of Neuroscience* 43: 1406–1425, The American Physiological Society.

to particular combinations of visual magnification and head movement (see **Figure 2**).

In motor learning research, various reflexes and more complex systems are studied in the hope of defining the underlying principles and common mechanisms. The VOR is a popular choice because its short anatomical pathway means that the number of possible sites and mechanisms for learning is comparatively small. In addition, eye movements can be treated as pure rotations, which make them easy to describe quantitatively. As eye movements are kinematically simpler than limb movements, it is easier to establish a close correspondence between their parameters and neurophysiological measurements than it is for any other type of movement. The oculomotor system can be mathematically modeled as a simple linear control system, and the model is fairly accurate within a moderate range of stimulus parameters. The modeling approach has been used successfully to solve important problems in motor learning.

Anatomy and Physiology of the VOR

In **Figure 1(a)**, primary afferent fibers from the horizontal semicircular canal (shown in red) provide a signal, representing the angular speed of rotation, to secondary neurons in the vestibular nuclei. Majority of secondary

neurons increase their discharge rates as the head is rotated toward the side they are on (**Figure 1(b)**). Secondary neurons, also known as interneurons, project to the motoneurons, located in the abducens nuclei, which innervate the lateral rectus muscles. The direct projections are excitatory (green) to the contralateral and inhibitory (black) to the ipsilateral abducens. Therefore, the shortest VOR pathway is a three-neuron arc within the brainstem.

In addition to their excitatory inputs from the vestibular nerve, secondary neurons also receive both inhibitory and excitatory inputs from the vestibular commissure, which connects the vestibular nuclei on the two sides of the brainstem. Some secondary neurons also receive inhibitory inputs from Purkinje cells (PCs) in the cerebellar cortex (a region known as the 'flocculus') (**Figure 1(a)**). These secondary neurons are known as the 'flocculus target neurons.' Another vestibular pathway involves the circuitry of the flocculus itself, which receives vestibular input both from primary vestibular afferents and secondary vestibular neurons. **Figure 1(c)** illustrates the basic anatomy of the cerebellar cortex, consisting of mossy fiber (MF) and climbing fiber (CF) inputs, excitatory (granule cell, GrC) and inhibitory (basket cell, BC) interneurons, and PCs. PCs of the lateral vestibulocerebellum project directly back to the vestibular nuclei (**Figure 1(a)**), this simple arrangement reflecting the early phylogenetic origin of the VOR circuitry.

Sites and Mechanisms of Motor Learning

VOR motor learning is assumed to be due to some change in the VOR circuitry, either synaptic plasticity, a change in neuronal excitability, or another similar mechanism. In order to investigate this question using the techniques of neurophysiology and molecular biology, we first need to know where exactly the change occurs. The possibilities can be narrowed down by considering how and where the needed sensory information is transmitted. Signals encoding head speed and visual-image motion are clearly necessary for VOR motor learning, and ideally would converge on a common site so that they could interact directly. Such convergence actually occurs both in the cerebellar cortex and in the vestibular nuclei. The cerebellar cortical circuit is integrated into the VOR circuitry in a feedback arrangement similar to that shown in **Figure 1(d)**. Information about head velocity arrives at the vestibular nuclei from the vestibular primary afferents and at the cerebellar PCs by way of the MF/parallel fiber pathway. At the same time, the PCs receive CF input from the inferior olive nucleus that encodes retinal slip. Eye movement signals, including smooth pursuit and VOR-generated signals, return to the cerebellum by the feedback pathway (black), as shown in **Figure 1(d)**. These pathways provide information about ongoing eye movements, which could also participate in motor learning. The feedback signal arrives at the cerebellar cortex through MFs from the dorsolateral pontine and vestibular nuclei.

In the 1970s, Masao Ito proposed that during motor learning, CLs from the inferior olive provide a visual teacher signal that adjusts the sensitivity of PCs to particular inputs from parallel fibers. These fibers were assumed to provide a head velocity signal. In thinking about Ito's hypothesis it is important to keep in mind that the cerebellum is not the only site of convergence of visual and vestibular signals. However, leaving aside the possibility of other sites for now, according to Ito's hypothesis, the synapse between the parallel fiber and the PC becomes less effective in a process known as 'long-term depression,' or LTD. As PCs inhibit their target neurons in the VOR pathway, LTD of the synapses from parallel fibers firing preferentially for one direction of rotation would cause an increase in VOR gain for rotation in that direction. LTD of a different set of parallel fibers with different on-directions could decrease the gain. In this model, the reverse of LTD, LTP, is used strictly to adjust overall levels of activity. In a more recent theory of cerebellar motor learning, gain increases and decreases in the VOR rely on different plasticity mechanisms. While LTD is involved with learned gain increases of the VOR, LTP may be involved with learned gain decreases.

On the cellular level, the initial stages of cerebellar LTD and LTP are thought to be implemented by insertion and removal, respectively, of neurotransmitter receptor molecules in the postsynaptic membrane. Specifically, α -amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA)-type glutamate receptors are shifted in or out of the plasma membrane, depending on their phosphorylation state (**Figure 3**). The phosphorylation of AMPA receptors is thought to be regulated by the balance of activities of protein kinase C and various protein phosphatases. Protein kinase C, indirectly activated by mGluR1 receptors (metabotropic glutamate receptor 1, mGluR1), phosphorylates the AMPA receptor. At the same time, another pathway inhibits protein phosphatases, preventing them from dephosphorylating AMPA receptors. This kinase–phosphatase switch, in turn, is believed to be under the control of calcium levels. Thus, a high level of calcium in the PC flips the switch toward LTD, while a lower concentration of calcium results in LTP.

The strong depolarization evoked by CLs activates voltage-gated channels in the PC, increasing the influx of calcium, whereas parallel fibers are thought to activate AMPA and metabotropic glutamate receptors. Parallel-fiber stimulation alone can induce LTP. However, coactivation of parallel fibers and CLs is thought to be necessary to achieve the greater calcium threshold needed for the induction of LTD. The LTD mechanism is known to be dependent on both N-methyl-D-aspartic acid (NMDA) receptors and nitric oxide. Neither PCs nor parallel fibers express NMDA receptors in most species, but evidence suggests that these may be located on stellate and BCs.

In an experimental setting, learning can be imposed on the VOR by manipulating the visual image to induce apparent errors in the stabilization of gaze. Initially, reversing prisms were used as part of a portable headset. Reversal of vision induces tremendous image motion on the retina, which in turn provides a strong (and quite disorienting) stimulus for motor learning and induces learning rapidly and effectively. A less-disorienting visual stimulus is provided by miniaturizing and magnifying spectacles, or telescopes. **Figure 2** illustrates the exponential time course of motor learning. In monkeys wearing telescopic spectacles, the time constant of the exponential change in gain is different for increases (top left) and decreases in gain (bottom left), starting from the normal gain. This observation and others led to the consensus that learned increases in gain are fundamentally different from learned decreases.

Cerebellar and Brainstem Sites of Learning

In evaluating potential sites and mechanisms for VOR motor learning, it is helpful to remember a few principles that are generally agreed upon. These may be briefly

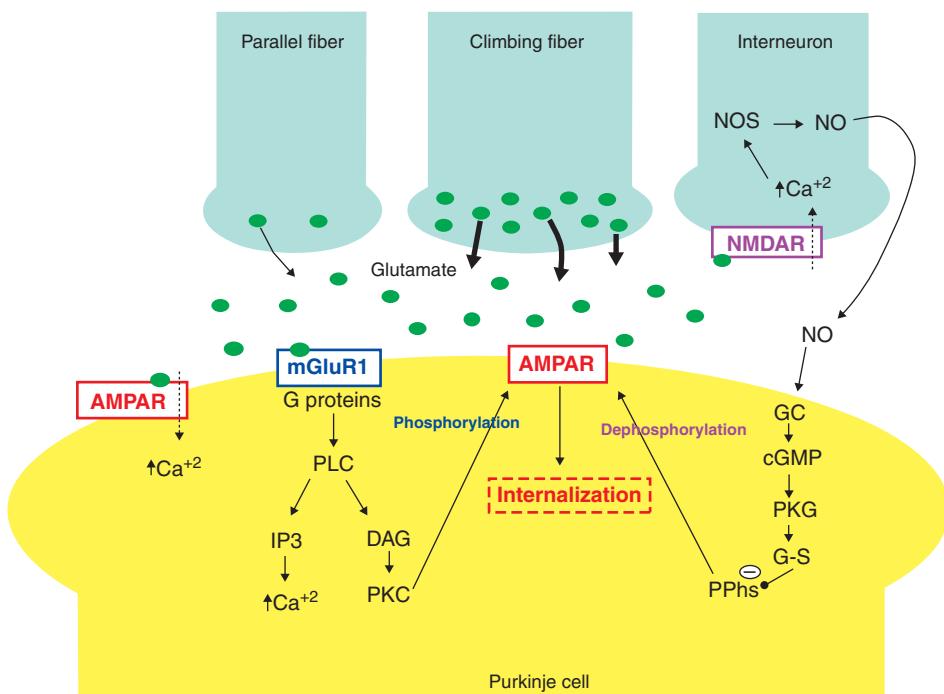


Figure 3 The cellular mechanisms of synaptic plasticity in the cerebellar cortex. During both long-term potentiation (LTP) and long-term depression (LTD), calcium enters the cell through AMPA receptors that are activated by glutamate from parallel fibers. During LTD, additional glutamate released from climbing fibers activates the metabotropic glutamate receptors (mGluR1), which cause calcium release from internal stores through phospholipase C (PLC) and inositol triphosphate (IP3). Protein kinase C (PKC) then phosphorylates the AMPA receptor and clathrin-mediated endocytosis removes phosphorylated AMPA receptors from the Purkinje cell membrane, depressing the postsynaptic response. Concurrently, another pathway inhibits protein phosphatases (PPhs) from dephosphorylating the AMPA receptors, under the control of both NMDA receptors and nitric oxide (NO) in local interneurons. Both the NO and the G-protein pathway must be active for LTD to occur. If the calcium concentration is too low because one of these pathways is inactivated, the phosphorylation balance shifts and more AMPA receptors become dephosphorylated. Then, they are actively inserted into the plasma membrane and LTP occurs instead.

summarized as follows: (1) an intact, functioning cerebellar cortex is required; (2) vision, in combination with another signal, is required; (3) the second signal can be either vestibular or oculomotor; and (4) both the lability and reversibility of VOR motor learning depend on the exact conditions and on the time course of learning and memory. VOR motor learning requires visual sensory input paired with either head or eye movement. Specifically, the gain of the VOR will be increased if either (1) the head movement and retinal slip are in opposite directions, or (2) the eye movement and retinal slip are in the same direction. In the reverse of each of these situations, the gain of the VOR will be decreased. This rule can be translated into specific pairings of pre- and postsynaptic activity that could occur either in the cerebellum or in the vestibular nuclei. The visual stimulus for learning need not be full-field retinal slip; smooth pursuit alone, in which a moving spot is tracked against a dark background, can change the gain of the VOR. Apparently, visual signals from the small pursuit target are adequate for motor learning. A strobe signal can even substitute for the pursuit target,

suggesting that visual motion *per se* is not necessary for learning.

Early lesion studies unequivocally showed that the flocculus and the nearby ventral paraflocculus are required for motor learning. A thorough investigation of ventral parafloccular PC responses to rotation concluded that although PCs do change their sensitivities when motor learning occurs, they change them in the wrong direction to support learning. In the long term, PCs decreased their sensitivity to rotation with a learned decrease in the VOR gain. This should have the effect of increasing the sensitivity of the VOR interneurons, which would be expected to lead to the opposite effect on VOR gain (i.e., an increase). This result led to the proposal by Fred Miles that the flocculus is not the site of learning; instead, the interneurons themselves (by default) are the sites where motor memory is stored. The Miles hypothesis introduced the idea that the cerebellar cortex, or the inferior olive, can provide a teacher signal to bring about modifications that are stored at a brainstem site. For example, direct transmission by the three-neuron arc is modified slightly during motor learning. The sensitivity of vestibular neurons to inputs from the vestibular

commissure, or to polysynaptic as well as monosynaptic inputs from the ipsilateral vestibular nerve, could also be altered by a Hebbian mechanism.

The flocculus target neurons in the brainstem modify their discharge patterns dramatically during motor learning; in fact, they change the polarity of their responses to rotation. In other words, when the VOR learns a lower gain, these neurons begin to increase their discharge rates during rotation away from the side they are on. Notably, this difference, similar to the difference in the VOR's response, is evident at short latencies after the start of a sudden rotation in contrast to the differences in PC responses, which are evident only at longer latencies. The flocculus target neurons are believed to be inhibitory VOR interneurons that project to the ipsilateral abducens nucleus (**Figure 1**). The excitatory, contralaterally projecting secondary neurons probably remain unmodified during motor learning.

Shifting Memory Storage and Consolidation

Why does VOR motor learning require the cerebellum, and yet apparently fail to encode the memory in the cerebellum? Why does the memory instead appear to be located in the brainstem? What, then, is the function of cerebellar plasticity mechanisms? Some results seem to indicate that the memory does initially form in the cerebellar cortex. In order to understand all of the apparently contradictory data, we must take into account the passage of time both during and after the learning process. In the VOR, if learning has occurred recently, it can be reversed by rotation in darkness alone, without any requirement for visual input. Over time, VOR memory becomes more resistant to disruption. The increased resistance to disruption indicates that VOR memory becomes consolidated. Consolidation over several days appears to coincide with a shift in storage the memory from the cerebellar cortex to a more distributed representation that includes the vestibular nuclei. However, compared with learning itself, the processes underlying consolidation of newly formed motor memories in the VOR are not well understood, and may (like learning) be different depending on the direction of the gain change.

So far, the results indicate that cerebellar plasticity processes are responsible for motor learning, and the brainstem becomes involved in long-term memory storage. In the case of learned gain decreases, existing evidence supports long-term synaptic potentiation as a likely mechanism and the cerebellar cortex as the initial site of learning. However, consolidation may also occur within the cerebellar cortex. Rapid consolidation of VOR motor memory requires just 1 h after the end of learning. Recent results suggest that rapid consolidation may

reinforce the memory trace at its original location, and the shift to the brainstem may occur subsequently. To understand this evidence, we must examine the way in which motor learning depends on the exact stimulus conditions, such as rotation frequency. VOR motor learning is selective for the particular rotation frequency that is used during training. This selectivity is thought to be conferred by the localization of changes to a particular set of synapses that are operational under the learning conditions, and cerebellar LTD and LTP could confer the necessary selectivity.

As described above, newly formed memory in the VOR can be reversed by rotation in darkness. Such disruption might reverse LTD or LTP that has not yet been stabilized. Disruption of gain decreases is most effective when measured at test frequencies that are different from the rotation frequency used during disruption. In contrast, gain-up learning is highly selective for the training frequency. Gain-up learning is, therefore, not a likely mechanism for disruption. However, these results would be expected if the rule during disruption is simply reversal, on the molecular level, of any changes that have recently occurred. Disruption in the VOR may be analogous to early extinction of fear memories, which has been attributed to depotentiation of recently potentiated synapses. Similarly, rapid consolidation does not show any frequency selectivity, suggesting that during rapid consolidation, synaptic changes that have recently occurred are simply converted to a more permanent form.

Motor Learning after Vestibular Damage

One situation in which motor learning might be useful is following the loss of peripheral vestibular function, for example, following damage to the inner ear. When this happens, the VOR gain is reduced due to the loss of part of the sensory signal, and a mechanism is needed for increasing the gain. Within hours after acute damage, VOR gain starts to increase by a process known as 'vestibular compensation' and, eventually, VOR gain reaches a near-normal value. In thinking about whether motor learning is responsible for this recovery, there is a major pitfall to be avoided. Compensation has several problems to solve, including the loss of balance, ongoing dizziness, and continuous eye movements (nystagmus). Some of these problems can be solved by changes in excitability or in tonic input to vestibular neurons, and such changes could even be under cerebellar control. As a result, we cannot simply compare changes (such as gene expression) during compensation and during motor learning, as two or more processes may be occurring simultaneously. Instead, we must attempt to sort out what affects VOR performance following vestibular damage. It is also helpful to look at studies where semicircular canals are plugged, since other symptoms are minimized in this situation.

Following a canal plug, the initial problem for the VOR may in fact be similar to what we would encounter when wearing magnifying lenses, and motor learning mechanisms should automatically be brought into play.

A few studies have specifically addressed the issue of motor learning during compensation (in all such studies, the changes in VOR function are long term, over days to weeks). Following ablation of the labyrinth, the flocculus is necessary for VOR recovery. Both the CF pathway to the cerebellar cortex and vision are necessary for compensation of VOR function. Following canal plugs, the excitatory commissural inputs to secondary vestibular neurons appear to be modified in a way that could account for the restoration of VOR gain. Flocculus target neurons (but not other secondary neurons) receive excitatory input from the commissure, suggesting that the commissural changes may be under cerebellar control, as we would expect in a motor-learning mechanism. On the other hand, cerebellar LTD does not seem to contribute to recovery of the VOR during compensation. Following compensation, the compensatory changes in the short brainstem pathway (the three-neuron arc) of the reflex are more significant than they are in long-term motor memory. These results suggest that brainstem sites may be more important in changing VOR gain if damage has occurred than if it has not.

To summarize the evidence so far, it appears that the mechanisms that allow VOR gain to recover following vestibular damage include, but are not restricted to, motor learning. The occurrence of damage may trigger mechanisms that are not available during motor learning in intact subjects. In particular, brainstem memory storage may take place without the necessity for cerebellar cortical plasticity, if damage has occurred. Since nystagmus, acute vertigo, and (in most cases) surgical stress are inextricably linked with vestibular damage, we must consider the possibility that stress following vestibular damage makes additional mechanisms available for brainstem plasticity. In support of this idea, stress hormones (corticosteroids) are known to act on vestibular neurons, and to contribute to some compensatory processes. In the absence of acute stress, when the damage occurs gradually (as it does during aging), these processes may not be available.

Summary

Several decades of research on motor learning in the VOR have yielded abundant information about the mechanisms

involved in motor learning and in long-term memory storage. As the rotatory VOR is so simple, it has been possible to localize the initial formation of the memory to the cerebellar cortex. Cellular mechanisms of plasticity in the cerebellar cortex are known to exist and appear likely to be a basis for the initial memory storage. In the long term, the exact mechanisms involved in memory storage are less clear but may include brainstem plasticity, which may also occur following vestibular damage.

See also: Active Avoidance and Escape Learning; Animal Models of Learning and Memory; Cerebellum: Associative Learning; Eyelid Classical Conditioning; Fear Conditioning; Fear: Potentiation and Startle; Gaze Stabilization and the VOR; Learning and Memory: Computational Models; Memory Consolidation; Navigation in Virtual Space: Psychological and Neural Aspects; Neural Representations of Direction (Head Direction Cells); Neural Substrates of Conditioned Fear and Anxiety; Neuron Excitability and Learning; Synaptic Mechanisms for Encoding Memory.

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Neural Plasticity of Spinal Reflexes

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Introduction

The concept of neural plasticity has been studied for many years. Indeed, the concept of alterations of neural activity is recognized as the basis for learning and memory. The activity of the nervous system in both sensory and motor function is now known to be based partially on its past activity and sensory inputs. However, until about 25 years ago, the spinal cord was thought to play little to no part in this plasticity. It was thought of as a passive transmitter of impulses to and from the periphery and brainstem, the brain being the site where neural plasticity occurred. The reflex patterns that could be readily shown in the spinal cord were viewed as essentially hard-wired patterns of input/output relationships that served as the first line of defense against tissue damage, organizers of movement patterns, and visceral activity. This dogma was despite the fact that Sherrington, at the turn of the twentieth century, studied spinal reflex alterations such as sensitization and habituation extensively, as did his student, Eccles. As will be seen later, several lines of study in the mid-1900s showed that spinal circuits could undergo activity changes with various inputs and experiences, but the general thought in neurophysiology and neuropsychology was that spinal cord activity patterns did not show persistent or event-dependent alterations so characteristic of higher brain centers. This can be viewed as the first era of spinal cord research.

This view began to change with various demonstrations in the 1960s and 1970s from several laboratories that spinal reflex properties could be altered by several non-associative and associative paradigms that markedly resembled memory and simple learning well known in the intact organism. This second era of spinal research led to demonstrations of spinal excitability changes that mirrored associative conditioning, such as Pavlovian and instrumental learning, as well as nonassociative long-term activity changes that in many ways mirrored memory. Of course, the associative changes had to be underlain by memory-like changes as well.

The third era of spinal plasticity began in the 1980s with the application of model systems approaches to spinal plasticity and produced evidence that the spinal reflex pathways functioned in many ways like full-brain systems and could be used to identify underlying neural processes that might underlie similar whole animal learning and memory systems. The fourth era of spinal

plasticity developed from the third and entails employing many different technologies and behavioral techniques to define processes in the spinal cord that underlie processes of nociception (pain), locomotion, simple memory, and learning. This era has produced a wealth of information useful in understanding chronic pain, spinal cord injury rehabilitation, and even motor control. The spinal cord and its neural machinery are a very complex and plastic place, a far cry from the dogma of a simple hard-wired reflex machine and bundle of transmission lines.

Nonassociative Plasticity

Beginning with the studies by Sherrington and his students, short-term alterations of spinal reflex excitability have consistently shown that with repeated unaltered inputs, the output of a spinal reflex can either reliably increase or decrease. These changes are known as 'sensitization' if they increase and 'habituation' if they decrease. The behavioral properties of sensitization and habituation are well known in that these processes are relatively short lasting, appear quickly with stimulation, and are shown by almost all animals from simple, multicellular organisms to humans. In addition, many types of reflex patterns show both behavioral changes, with the exception in mammals of the stretch reflex, a monosynaptic reflex pattern from the muscle spindles to the motoneurons. Importantly, the spinal reflexes have been shown to undergo these alterations independent of the sensory receptors and that the changes are independent of muscle fatigue or spasticity. Thus, the phenomena of spinal sensitization and habituation are true properties of the spinal reflex circuits and dependent on the interposition of interneurons (small processing neurons) between the afferent inputs and the motoneurons (no sensitization or habituation in monosynaptic circuits), mainly in the dorsal horn of the spinal gray matter. The difference between the two alterations has to do primarily with stimulus intensity and frequency. With more intense and/or more frequent stimuli, sensitization is manifest and habituation is manifest with less intense and/or infrequent stimulation. One of the most used reflexes in studying these effects is the withdrawal or defensive reflex, which readily shows both changes. It appears that at least one of the underlying mechanisms for sensitization and habituation is an alteration in amount of

neurotransmitter released with each neural impulse at the primary afferent or input terminals from sensory neuron to interneuron. More transmitter release increases the probability of depolarization to threshold of the interneurons, hence a greater output to the motoneurons, and vice versa for habituation. The effect of stimulation in increasing the output of a simple reflex circuit can be seen in as little as one intense stimulus input, but usually shows increases over several seconds to minutes of stimulation, eventually reaching a plateau. Once the habituating or sensitizing stimulus is terminated, reflex excitability gradually returns to normal levels over several minutes.

A related phenomenon to sensitization is long-term sensitization, in which the increased response of the reflex circuit does not fall to its prestimulus levels for as long as 24 h. This longer-term excitability alteration is apparently due to alterations in the membranes of the interneurons that become more sensitive to transmitter released from the sensory input neurons. This effect has more in common with short-term memory phenomena than with the short-term effects of sensitization and habituation, and seems to increase with the length and, possibly, intensity of stimulation.

A longer-lasting alteration has been termed 'fixation' in the behavioral literature and central sensitization/excitation or long-term potentiation (LTP) and its analog, long-term depression (LTD). These changes have been extensively studied especially in the nociception (pain) literature and are thought to underlie altered pain states such as secondary hyperalgesia and allodynia. Fixation refers to an alteration of spinal reflex excitability that can be produced by things such as a cerebellar lesion, spinal cord partial transections, and direct peripheral stimulation, all of which cause a flexion usually of a hind limb. If the anesthetized animal is left in that position for 30–45 min, depending on amount of flexion and stimulus intensity, stopping the input to the spinal cord by spinal transection or stimulus cessation leaves the limb in an actively flexed posture. This effect can be shown to last for weeks. It is an active process in the spinal reflex centers that apparently causes sufficiently increased neural excitability such that impulses are spontaneously generated, causing the active motor activity without peripheral or descending inputs.

Fixation appears to be closely related to central sensitization or LTP. It is these long-term central excitatory states, especially LTP that are thought to be the basis for mammalian memory processes, having first been described in the hippocampus. A great deal of work has looked at these processes in the dorsal horn neurons of the spinal cord; in reality, the interneurons mentioned above, as a major site for alterations involved in nociceptive information processing. Primary nociceptive inputs, mainly unmyelinated C-fibers primarily subserving

deep pain senses, synapse on the dorsal horn interneurons and given appropriate circumstances, appear to alter, at times dramatically, the excitability characteristics of the nociceptive pathways underlying pain perception at cortical levels. Many of these studies have involved the induction of inflammation of a joint, such as a knee, and the effects of the resulting nociceptor inputs on the dorsal horn interneurons. While several types of interneurons have been identified, the production of a central excitatory state seems to involve the same basic processes such as sensitization, long-term sensitization, and fixation. Initially, an increase occurs in transmitter release, followed by an increase in membrane excitability of the interneurons. After continued C-fiber inputs, the interneurons begin to show an alteration in certain genetic functions, with genes such as *c-fos* being upregulated, resulting in an increased production of receptors on the cell surface. This produces an increased excitability of the interneuron even with a reduced amount of transmitter release. It seems that there are multiple neurotransmitters involved in the process, but N-methyl-D-aspartate (NMDA) and substance P seem central to the process. This process has been identified as one of the bases for the development of chronic and neuropathic pain states. It should be noted that C-fiber activation seems to be a central component of producing the central excitatory states, although some evidence suggests that fixation may be possible with lower-intensity stimuli, but then it is produced only by quite long stimulation periods.

There is some evidence to show that concurrent with the development of LTD or the central excitatory state, over a period of days, some interneurons in involved areas of the dorsal horn may actually die. These are apparently mainly inhibitory interneurons that would downregulate the total excitability of the reflex system. Inhibitory cell death would lead to an almost permanent change in excitability characteristics of a reflex pathway and potentially allow for sprouting of new connections from other healthy neurons, possibly from lower levels of the dorsal horn. Since the lower lamina inputs are primarily from mechano- and proprioceptors, sprouts from these areas connecting to nociceptive pathways would be expected to produce nociceptive inputs to the brainstem when movement occurred. Such a situation is often reported by chronic and neuropathic pain patients, and is a truly disabling and perhaps permanent situation.

In addition to importance for information processing and nociception, the nonassociative alterations described here are important for rehabilitation in spinal injuries and transactions. Ongoing studies on the effects of training on incomplete and even complete spinally transected animals and humans are showing often-dramatic improvements in functional capacity. Especially in incomplete spinal transactions, the individual can often regain a large degree of coordinated movement with

intensive training. These data suggest that signals from the brain that reach the spinal reflexes can be utilized to trigger motor patterns that are provided by the stepping movements during the rehabilitation periods. It appears that the proprioceptive inputs induced by the forced stepping or other exercises can reestablish sufficient background activity for the limited commands from the higher centers to use in coordinating movements. It seems likely that such training induces long-term excitability changes in interneurons involved in the activity patterns, allowing for new functional pathways to coordinate basic movement patterns. Thus, rehabilitation strategies for paralyzed patients can increasingly take advantage of spinal neural plasticity to enhance recovery after spinal injuries.

Associative Plasticity

The nonassociative changes in spinal reflexes reviewed above are obviously important in regulation of reflex excitability and in the processing of nociceptive and other sensory inputs to the spinal cord and ultimately to the brain. While some may call such changes a form of learning, they certainly represent a highly sophisticated form of short- and long-term memory. However, for most psychologists and neuroscientists, to be classified as learned alterations, behaviors must have an associative component.

The question of the capabilities of the spinal reflex circuits to learn began with the studies in the mid-1930s. It was generally assumed that learning was reserved for the cerebral cortex and perhaps subcortical regions of the mammalian system and that the spinal cord reflexes as hard-wired input–output relationships would not support such alterations as even the simplest of learning or associative changes. In fact, the progenitor of classical conditioning, Ivan Pavlov, assumed that the great cerebral cortices of the mammalian brain were the seat of all learning. However, studies in the late 1920s and 1930s strongly suggested that dogs could show behavioral changes consistent with learned behavior after complete or near-complete decerebration. The responses learned did not, however, show the fine-tuning associated with conditioning of motor responses in normal dogs. The question then became whether the neural organization at lower or spinal levels of the neuraxis could sustain any comparable behavioral changes that could be reasonably classed as learned or associative changes.

Classical Conditioning

Pavlovian classical conditioning procedures had become well known by the early 1930s, having been introduced by Ivan Pavlov in the late 1920s. The typical Pavlovian

conditioning or learning paradigm involved selecting an unconditioned stimulus (UCS), such as a paw shock that reliably and without training elicited an unconditioned response (UCR), such as a paw withdrawal. The UCS was then paired temporally with a conditioned stimulus (CS), which did not produce a response in the target motor system, such as a tone. After a variable number of pairings of the tone and shock (usually with the CS beginning 0.5–2 s prior to the UCS), the tone would begin to elicit a paw withdrawal (a conditioned response or CR) prior to the shock onset. If the same two stimuli were presented but not paired in time (e.g., CS presented 30 s before the UCS), no such response to the CS would develop. This type of learning is considered to be the simplest form of associative learning.

The first studies of classical conditioning in spinal reflex systems were performed in spinal dogs in late 1930s. The results suggested that a CR would develop to properly paired CS–UCS pairings and that the response declined when the CS was presented alone (extinction). However, the results were soon challenged with the argument from other studies that there had not been a new response formed, but only the modification of an existing one. By the mid-1960s, the question of spinal conditioning was an open one.

In the next set of studies, an appropriate control condition, the unpaired control group, was used in spinal cat preparations given a light-shock CS to the thigh and a UCS of a strong shock to the toe pads, producing a leg withdrawal. The results showed that there was no change in the response to the CS in the unpaired animals and a large increase in the paired group. Admittedly, no new response was formed, but the pairing had a definite effect on the response properties. In the late 1960s, spinal conditioning in cats was established as a model preparation for this type of associative learning. The preparation generally consisted of a spinalized animal with the CS delivered to the thigh skin and later to a toe sensory nerve directly, and the UCS delivered to the thigh skin. The CR was recorded from the deep peroneal motor nerve. This preparation provided for maximal stability of stimulus and response parameters, and also allowed for later neural studies within the spinal cord.

Over the 1970s and 1980s, several groups used this model to examine various aspects of classical conditioning in the spinal preparation and began examining the neural substrates of the process. In the behavioral studies, results showed that in many ways, the spinal conditioning preparation paralleled the findings in intact animal conditioning. For example, the optimal CS–UCS interval for conditioned increases in response during paired trials was found to be about 250 ms, with decreasing response increases with shorter or longer intervals. There was no response increase found with backward pairings (UCS–CS) or with CS-alone or unpaired trials. The CR

once established did not spontaneously decay, but showed the usual extinction decreases with CS-alone trials. Other findings showed that the usual discriminative or differential conditioning paradigm in which a CS⁺ is paired with a UCS and a CS⁻ is not paired in the same trials was not generally successful, suggesting that the behavioral parallels between spinal and intact animal conditioning could not be complete. It appeared that there was sufficient cross talk between the CS⁺ and CS⁻ neural pathways that robust differential conditioning was not possible. It remains to be seen whether the use of other CS inputs could show differential conditioning.

The neural underpinnings of spinal conditioning were examined in several studies. Here it was found that there was no change in afferent terminals of the CS input neurons in the dorsal horn during the conditioning process, suggesting that the changes were within the interneurons of the reflex pathways. Likewise, the motoneuron excitability was found to remain stable during conditioning procedures, although it was found that more motoneuron firings occurred as the CR increased. It was not possible to determine whether more motoneurons were recruited or there were more spikes from the same population of motoneurons. In either event, the data suggested an increased drive onto the involved motoneurons from the interneuron pools. Other work using a decerebrate cat suggested a form of unstable backward conditioning could occur, but that this was not a robust effect. It was also shown that the major pathways involved in the conditioning reflexes seemed to be disynaptic and that blocking the NMDA receptors of the interneurons during conditioning suppressed the CR increases. These data suggest that the spinal conditioning may be similar to LTP in the brain and other spinal areas. In addition, some data suggest that good conditioning occurs in acute preparations but not robustly in 2–3-week chronic spinal preparations, but reappears at chronicity of 3 months. This would suggest that as the cord undergoes changes and reorganization after injury, plasticity is decreased until a later time when the reorganization has taken place. These findings could have implication for rehabilitation of spinal-injured patients.

Other investigators have used rat models for classical conditioning, presenting the CS to the leg and the UCS to the tail. This preparation has shown that the CS–UCS pairing can produce less tail-flick habituation later. The implication is that the pairing strengthens the ability of the CS to cause a response, not unlike the results of the cat preparation.

Overall, the results of classical conditioning of spinal reflexes have shown remarkable similarities to classical conditioning in intact animals. However, as noted, there appears to be a limit to the amount of behavioral complexity available in the spinal cord, as would be expected from the relatively simple neural networks involved when

compared to the full brain. The changes during conditioning appear to occur in the interneuron pathways of the cord and not in the afferent terminals or motoneurons and may well involve neural mechanisms similar to LTP and other similar memory-like changes within the brain. Spinal conditioning serves as a well-controlled model for ongoing studies of simple behavioral plasticity.

Instrumental Conditioning

Instrumental conditioning utilizes a different paradigm from classical conditioning. Instrumental conditioning involves establishing a relationship between the response and an outcome. In the instrumental paradigm, the response thus generates some change in the situation that can be sensed by the organism and results in either an increase or decrease in response probability. In the 1960s, a behavioral paradigm was established in cockroaches that became known as the Horridge preparation. Headless cockroaches were suspended above a water dish and a hind leg was allowed to fall into the water. A shock was delivered by water contact and the leg would withdraw. The measure of learning was whether the leg learned to maintain withdrawal to avoid the shock. A yoked animal was used that received a shock whenever the master animal did, but the yoked shock was independent of leg position. The master headless animal learned to keep the leg flexed, whereas the yoked animal did not. This was taken as learning in the headless cockroach. While the paradigm generated controversy in the psychological learning community over what the appropriate control actually showed learning, the basic paradigm has become a very useful one in looking at various aspects of learning and now, neural mechanisms of behavioral change. In the 1970s, the paradigm was adapted to the spinal rat, and this is the paradigm generally currently in use. Here, the acute or chronic spinal rat is suspended with one hind leg over a dish of saline. A metal rod is anchored to the leg and the height of the water is raised to an appropriate level such that the rod is in the water. A record of shocks delivered through the water–rod interface, and of the number and durations of leg flexions is made. Learning is shown by an increase in the duration of time spent with the rod out of the water, the number of leg flexions, and the time of rod in the solution. Early results using the yoked control showed that the master animal reduced number of flexions, that is, had more time out of the saline, whereas the control did not. In addition, when control rats were given contingent shocks, they did not learn. In fact, if a noncontingent shock is delivered for a few minutes to a naive rat then the rat is put on a learning schedule, a severe deficit in learning results, much as if the rat had been a yoked control. Interestingly, a study of time between when the electrode

rod touched the water and the shock was given showed that if there was no delay, robust learning occurred, but decreased with intervals of 50, 100, and 200 m, a result indicating that strict contingency between response and outcome is best for this type of learning. In addition, exposure to continuous stimulation did not produce a deficit, whereas intermittent burst or single-pulse regular or irregular stimulation shocks did produce a deficit. These results suggest a sensitivity to temporal relations.

Subsequent studies have abundantly confirmed that this instrumental learning is robust in spinal rats and that it occurs fairly rapidly; does not spontaneously decay but is subject to the usual extinction procedures. In addition, data show that the effect transfers readily from the trained to the untrained leg, suggesting the possibility of common neural elements between the two sides.

Studies of the underlying neural mechanisms have shown that the effect is clearly dependent on spinal reflex pathways. In addition, studies have implicated NMDA in the formation of the effect, suggesting a link with LTP and central sensitization processes in the brain. There is also some evidence that there exists some dependency of this learning on *de novo* protein synthesis, suggesting a functional basis of neural development during learning. Other studies have suggested that spinal instrumental learning is impaired by neonatal hind-limb injury, possibly due to alterations in nociceptive processing in the cord. Recent studies have implicated neurokinin 1 and 2 receptors as important in the formation of the learning as well as the NMDA receptors and that γ -aminobutyric acid-A (GABA_A) receptors may be active in the inhibition produced by noncontingent shock that inhibits learning during contingent shock. Interestingly, the region of the rat cord involved in the hind-limb learning appears to be between L4 and S1. Relevant to human spinal cord injury, it has been found that neonatal spinal rats could alter the motion characteristics of walking imposed during treadmill exercise. Thus, the spinal cord could learn to respond adaptively to externally imposed forces.

These data suggest that the instrumental spinal learning is a very useful tool in elucidating the plasticity of spinal reflexes and ongoing work is uncovering many of the underlying neural mechanisms involved. It will continue to be useful in uncovering the inherent mechanisms and potentials of spinal reflexes.

Effects of Higher Centers on Spinal Plasticity

It is well known that the higher brain centers have profound effects on spinal reflex excitability and function. In fact, many of the effects seen in spinalized preparations are difficult, if not impossible, to observe in intact preparations due to these higher influences. Work has shown that higher centers can also cause spinal plasticity.

Examples of such plasticity are studies of the effects of higher centers on operant conditioning of the H-reflex that provide task-dependent changes from supraspinal centers onto the spinal stretch reflex or its analog, the H-reflex. If the mouse, rat, or monkey is rewarded for increasing the H-reflex amplitude, it reliably increases and if the reward is for lowering the amplitude, it goes down. The changes are slow compared with spinal conditioning, occurring over days, weeks, and months of training. The plasticity is formed in the cord by continuous control from the higher centers, not by direct stimulation of the spinal reflex. Since the reflex is a monosynaptic one, the influence must be either on the alpha motoneuron or on the 1a afferent terminal from the muscle spindle. In any event, the alteration is at the spinal level as it outlasts spinal transection. Recently, the effect has been shown in humans, indicating that the H-reflex can be altered by operant conditioning procedures.

These data, while not as advanced as the instrumental or classical conditioning data, suggest that long-term alterations of descending influences can alter spinal function independent of nociceptive inputs generally needed for the more rapid spinal reflex alterations. There are very important implications for human rehabilitation procedures in the discovery of higher-center-driven spinal plasticity that are only beginning to be recognized.

Summary

Neural plasticity is a fundamental process at all levels of the nervous system. While it is only recently that functional and perhaps structural plasticity have been widely recognized at the spinal level, such recognition opens a tremendous opportunity to develop new and exciting opportunities for rehabilitation of human spinal cord injuries and function. In addition, the deeper understanding of the plasticity involved in nociceptive processing in the dorsal horn is providing greater insights into chronic and neuropathic pain mechanisms and will ultimately provide new pathways to treat these devastating conditions. The spinal cord is a very active processor of information, and its effects on locomotion, visceral function, and pain are only beginning to be understood.

See also: Animal Models of Learning and Memory; Drug Sensitization and Drug Abuse; Neural Basis of Classical Conditioning; Synapse Formation and Memory.

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Sleep: Learning and Memory

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Glossary

Dendritic spines – Dendritic spines are contiguous with dendrites and continuously sense the local dendritic membrane potential. Majority of synapses in the central nervous system and almost all excitatory synapses in the hippocampus are at this specialized structure. These spines are small well-circumscribed biochemical compartments that localize proteins and signaling molecules to a specific postsynaptic compartment.

Hippocampal theta rhythm – The theta rhythm consists of regular, sinusoidal oscillations of waves in the range of 5–8 Hz that normally appear in the hippocampal electroencephalogram (EEG). The appearance of the hippocampal theta rhythm in the EEG is one of the most prominent electrophysiological characteristics of rapid eye movement (REM) sleep in mammals. Theta rhythm could also occur in the wake state when animals perform ecologically relevant species-specific behaviors. Theta waves are involved in the acquisition and processing of meaningful environmental information.

Learning – Learning is an altered behavioral and/or individual neuronal response produced by the individual's experience with a novel environmental stimulus. Normally, to assess learning, participants are exposed to repeated acquisition experiences and progressive changes in performance are observed. This activity provides an acquisition curve – an assessment of learning as a function of acquisition trials and time. Traditionally, measuring behavioral performance has been a gauge of learning. More recently, evaluation of learning has been possible by direct examination of physiological and cellular changes in the brain (neuronal plasticity). The term learning has thus taken on a broader meaning that now includes observed cellular changes that can also be used as indices of learning. Such changes include any number of cellular and/or subcellular neuronal alterations that result in improved performance.

Long-term potentiation (LTP) – LTP is an increase in synaptic excitability that persists for hours or more than days after the delivery of a few seconds of brief tetanic stimulus (100–500 Hz) to certain afferent pathways. LTP is now widely accepted as a neuronal model of memory.

Memory – In the experimental neuroscience literature, a concept related to learning is memory (the ability of the organism to retain acquired information over a period of

time). Learning is believed to include the process of forming memory (memory consolidation). Memory is believed to be a store of acquired information made more or less permanent by plastic changes in the brain's cellular structure at some level. In contrast, the philosopher and psychologist William James believed that there is a fine distinction between learning (explained as habits) and memory. He viewed memory as the conscious recollection of events and facts and this definition of memory still remains popular with many contemporary scientists and philosophers. There are different taxonomies for the classification of human and animal memory types. The most widely accepted taxonomy divides human memories first into declarative and nondeclarative, based on their accessibility to conscious recall, and then into finer and finer subdivisions of these categories.

Memory trace – A subset of neuronal elements that exhibit the learning-training-induced plasticity essential for such learned behavior.

P-wave generator – The P-wave generator is a collection of glutamatergic cells in the pons. P-wave generating cells discharge high-frequency (>500 Hz) spike bursts (3–5 spikes/burst) in the background of tonically increased firing rates (30–40 Hz) during the transition between nonrapid eye movement (NREM) and REM sleep. These cells remain silent during wake and NREM sleep. P-wave generating cells project to the hippocampus, amygdala, entorhinal cortex, visual cortex, and many other regions of the brain known to be involved in memory processing. The P-wave in the rat is equivalent to the pontine component of the ponto-geniculo-occipital (PGO) waves in other mammals.

Sleep Stages in Human and Other Mammals

Sleep in mammals is not a homogenous behavioral state; rather it is a continuum of mixed states that differ in their physiology, chemistry, and phenomenological experiences. The details of sleep–wake stages and their specific physiological, neurochemical, and phenomenological signatures are described below. Because some human sleep stages are named differently from those in animals, and since the present description on the

mechanisms of sleep stage-specific memory formation is based on both human and animal research, the following section describes the similarities and differences between human sleep stages and those of animals.

Using behavioral and physiological signs, sleep in mammals has been broadly divided into two major states: nonrapid eye movement (NREM) and rapid eye movement (REM) sleep. Human NREM and REM sleep alternate throughout each of the four to six sleep cycles that occur every night. The most common, and preferred, animal models for sleep research include the mouse, rat, and cat; in these animals, NREM–REM sleep cycles are much shorter than in human and nonhuman primates. These cyclic NREM–REM sleep epochs in rodents and cats continue throughout sleep during day and night, except when the animal is engaged in activities that require wakefulness. In a human, NREM sleep is further subdivided into four stages (I, II, III, and IV), each corresponding to an increasing depth of sleep. The deepest stages of NREM sleep, III and IV, are collectively called slow-wave sleep (SWS or delta sleep). Distinctions between stages of NREM sleep in animal models differ slightly from those in humans. In the aforementioned animals, NREM sleep is normally subdivided into two stages (SWS-I and SWS-II). SWS-I is identified by the presence of sleep spindles in the cortical EEG. SWS-II is considered deep sleep and is identified by the presence of high-amplitude, low-frequency waves in the cortical EEG (delta sleep). Human NREM-II sleep is comparable to mouse, rat, and cat SWS-I, whereas stages III and IV NREM sleep in humans are comparable to the animal SWS-II. Physiological signs of REM sleep in humans are comparable to physiological signs of REM sleep in the rat and cat. However, another sleep stage, one between SWS-II and REM sleep, is identified mainly in rats and cats and is called the transitional sleep stage (tS-R). During this transitional stage, the appearance of cortical EEG signs is similar to the cortical EEG of SWS-I and hippocampal and pontine EEG signs are similar in appearance to those during REM sleep, but with less intensity. Since most animal sleep and learning studies have not recorded hippocampal and/or pontine EEG, such studies have often considered this stage to be SWS-I. Similarly, in sleep studies of healthy humans, hippocampal and/or pontine EEG cannot be recorded to help identify sleep stages. Therefore, based on EEG signs in humans, this transitional sleep stage has always been identified as stage II NREM sleep. In reality, at least 80% of stage II NREM sleep periods in humans are the transitional sleep stage between SWS and REM sleep.

Stages of Memory Development

The development and maturation of memory is a complex process occurring in several distinct stages over time. The two major stages of memory formation are: (1) acquisition of information (learning or encoding) and (2) consolidation of memory trace. There is now evidence that the different stages of memory development are influenced by different stages of sleep. The first stage, acquisition, is when memories are initially formed or encoded by engaging with an object or performing an action, leading to the formation of a representation of the object or action within the brain. This initial encoding of a memory is a relatively rapid process requiring only a few milliseconds. At this stage, memory remains in a short-term store. This short-term memory store has very limited capacity and, in the absence of rehearsal, persists for only minutes at most. If this encoded information persists in the form of reverberating activity in the neuronal circuits, then another process transforms this short-term memory into an intermediate form. This intermediate form of memory is relatively more stable than the short-term memory and can last for several hours. However, at this stage, memory still remains sensitive to interference from competing or disrupting factors. Acquisition of information occurs only during the awake state, but the formation of the intermediate form of memory can occur during both waking and light sleep.

Following acquisition, the consolidation stage begins. The original definition of the term memory consolidation refers to a process whereby a memory trace, through the simple passage of time, becomes increasingly resistant to interference from competing or disrupting factors in the absence of further practice. At the end of the consolidation stage, a memory has become stable and resistant to even extreme disruptions, such as electroconvulsive shock or the application of neuronal gene and protein activation inhibitors. Following consolidation, a memory can be retained for days to years, during which time it can be recalled from long-term memory storage. But the act of memory recall itself is now suggested to destabilize the memory representation, making it again labile and subject to potential degradation. Subsequent to recall and destabilization of a memory, passage of time in a suitable neurochemical and molecular state of the brain can again consolidate this destabilized memory and return it to long-term memory storage. This process has been termed memory reconsolidation and is an automatic process that can occur without conscious awareness. This reconsolidated memory becomes more stable and easier to retrieve than was its original long-term memory trace. However, it is important to mention that, at this time,

some of the major cognitive neuroscientists do not believe that there exists such a stage as memory reconsolidation.

Sleep and Memory Encoding

At present, there are no physiological and/or structural measures that directly quantify or assess the levels of memory encoding. Normally, in animal studies, the level of memory encoding is measured by analyzing a learning acquisition curve over a training session. This method measures the rate of improvements in performance between individual trials within a single session of learning training. This method has also been used for measuring short-term memory. The optimal neurophysiological, neurochemical, and molecular conditions of the brain for memory encoding is illustrated in **Figure 1**.

Although there is no evidence to indicate that encoding of memory occurs during sleep, a number of recent human and animal studies have described the detrimental consequence of inadequate pretraining sleep on successful memory encoding. Human studies, using a variety of declarative memory paradigms (temporal, verbal, word association, episodic), have demonstrated that 24–36 h of pretraining sleep deprivation severely impairs the encoding of declarative memories, resulting in poor retention 24–48 h later. Such impairments have been demonstrated for both emotionally positive and neutral types of

declarative memory. Similarly, a number of animal studies have demonstrated that pretraining deprivation of total sleep or only REM sleep impairs encoding of memory in a variety of learning paradigms, including spatial, contextual, one-way and two-way active avoidance, taste aversion, and passive avoidance protocols. However, unlike encoding of emotionally positive and neutral stimuli, memory encoding of emotionally negative stimuli has been shown to be resistant to pretraining sleep deprivation in both humans and animals.

In addition to attention impairments resulting from sleep deprivation-induced physical and mental tiredness, there are additional physiological explanations as to why pretraining sleep deprivation is detrimental for the successful encoding of memory. Human brain imaging studies over the past decade have shown that baseline activity in the prefrontal cortex (PFC), hippocampus, and parahippocampal areas is significantly less following one night of sleep deprivation compared to that following a night with adequate sleep. Similarly, animal studies have shown that deprivation of total sleep or REM sleep only, over the range of 6–72 h, reduces the baseline excitability of dorsal hippocampal neurons. Thus, it appears that certain levels of baseline activity in the PFC, hippocampus, and parahippocampal areas are critical for memory encoding (**Figure 1**).

Neurophysiological and neurochemical evidence show that sleep provides a window of rest (reduced

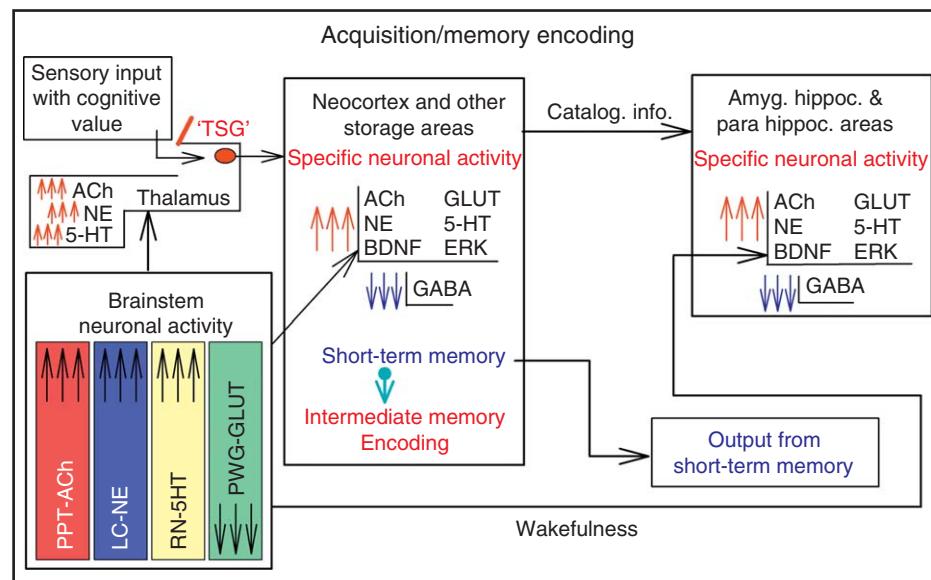


Figure 1 Physiological and biochemical conditions of the brain during wakefulness are conducive for memory encoding. Black arrows with solid lines between the blocks of brain structures: possible direction and flow of cognitive information and/or neurotransmitter; three upward arrows: highest levels of neuronal activity (PPT-ACh, LC-NE, and RN-5HT), extracellular level of neurotransmitters (ACh, NE, 5-HT, and GLUT), and intracellular proteins (BDNF and ERK); three downward arrows: lowest levels or absent of neuronal activity (PWG-GLUT) and extracellular level of neurotransmitter (GABA). 5-HT, 5-hydroxy tryptamine (serotonin); ACh, acetylcholine; BDNF, Brain-derived neurotrophic factor; ERK, extracellular signal regulated kinase; GABA, gamma amino butyric acid; GLUT, glutamate; LC-NE, noradrenergic cells in the locus coeruleus; NE, norepinephrine; PPT-ACh, cholinergic cells in the pedunculopontine tegmental nucleus; PWG-GLUT, glutamatergic cells in the pontine-wave generator; RN-5HT, serotonergic cells in the raphe nucleus; TSG, thalamic sensory gate.

neuronal activities and receptor sensitivities) to both aminergic (noradrenergic and serotonergic) and cholinergic neurons and their receptors in the brain. This well-deserved rest allows these neurons and their postsynaptic receptors to be optimally reenergized for activity during the subsequent waking period. A number of studies have shown that successful acquisition of information (a prerequisite of memory formation) requires that the aminergic and cholinergic postsynaptic receptors in the cerebral cortex, hippocampus, and amygdala are both optimally sensitive and surrounded by sufficient amounts of norepinephrine (NE), serotonin (5-HT), and acetylcholine (ACh). Thus, the memory encoding deficits resulting from inadequate amounts of sleep before training may result from altered sensitivities of postsynaptic aminergic and cholinergic receptors in the cerebral cortex, hippocampus, and amygdala and/or from neurochemical imbalances of aminergic and cholinergic neurotransmitters. Similar to brain-activity and neurotransmitter-release studies, molecular studies in animals have shown that 3–6 h of selective REM sleep deprivation reduces the levels of brain-derived neurotrophic factor (BDNF) and extracellular signal-regulated kinase (ERK) in the hippocampus. BDNF and ERK in the dorsal hippocampus are both involved in the development of memory. Thus, the memory encoding deficits in animals and humans with inadequate pretraining sleep may be due to altered baseline levels of BDNF and ERK. Based on this neurophysiological, neurochemical, and molecular evidence, it appears that adequate sleep during the pre-training period allows the brain to make the necessary physiological as well as chemical preparations for the successful encoding of memory during the subsequent wake period (**Figure 1**).

Sleep and Memory Consolidation

The current views of memory consolidation have their roots in the perseveration-consolidation hypothesis originally proposed by Mueller and Pilzecker in 1900. This hypothesis suggested that the neural processes underlying memory persevere in a labile form following an experience and subsequently become fixed or consolidated over passage of time. However, the past 50 years of sleep and memory research have revealed that not simply the passage of time, but also an adequate amount of sleep during this time is required for memory to successfully consolidate. Sleep and memory researcher Carlyle Smith has termed this time period the ‘REM sleep window’ for memory consolidation.

The hypothesis that sleep states play an important role in the consolidation of memory has had a long history even before scientists learned to record EEG activities to objectively identify sleep. Observational findings have

suggested that memory retention was better following a night of sleep than after an equivalent amount of time awake. Since the discovery of REM sleep, a huge number of human and animal studies have demonstrated that intact and/or undisturbed sleep following a learning session is critical for memory consolidation. Behavioral evidence that links sleep with consolidation of different categories of memories suggests that the specific homeostatic need for additional amounts of SWS, stage II NREM sleep, and/or REM sleep for the successful consolidation of both declarative and nondeclarative memory, depends mostly on the type of learning task. Contrary to those studies that have shown the beneficial role of sleep in learning and memory, there have been some animal, as well as human studies, that did not find any changes in amounts of sleep during the postraining sleep period. Normally, the results of those studies are interpreted to suggest that sleep may, in fact, not be involved in memory processing. If, however, the training has no effect on the amounts of sleep stage(s), one cannot necessarily conclude that the sleep stage(s) in question plays no role in processing of that type of memory. Indeed, some studies have also shown that normal and/or reduced duration of sleep stage(s) following training is sufficient for memory consolidation following many different types of tasks.

Mechanisms of Sleep Stage-Specific Memory Consolidation

Different parts of the brain are responsible for the formation of different categories of memory. Despite the differential role of distinct brain regions in different types of memory formation, recent experimental evidence suggests that all types of memory undergo a consolidation process in the period following training. The behavioral evidence on sleep and memory suggests that all four stages of sleep (animal/human: SWS-I/stage II NREM, SWS-II/SWS, tS-R/transitional stage II NREM, and REM sleep) are involved in consolidation of all types of memory.

Memory consolidation is not a single-step process; rather it is a multistep process and it occurs exclusively during periods of sleep. All of these processes occur over time, automatically, outside of awareness and without intent. Thus, they are specifically different from changes that result from conscious reminiscing or intentional rehearsal. Operationally, the cascade of memory consolidation processes can be divided into four stages: (1) search and readout of the intermediate form of memory; (2) elimination of unnecessary and/or redundant memory; (3) strengthening of cognitively relevant memory; and (4) transfer of stable memory to long-term storage. There is a clear consensus that neuronal plasticity (both

functional and structural changes in response to relevant stimuli/experiences) in the memory trace is the physical evidence of memory consolidation. Therefore, by quantifying those physiological and structural changes, one could measure the progress of each stage of the memory consolidation process.

Search and Readout of the Intermediate Form of Memory

The first stage of the memory consolidation process is the search and readout of the intermediate form of memory. In this stage, memory traces are sorted to identify memories that are to be stored and those that are to be deleted (or removed). For this particular step of memory consolidation, interference by sensory inflow to memory trace forming brain structures is blocked at the level of the thalamus (which normally remains open during the waking state). Yet, memory trace-forming brain structures are sufficiently conscious (active) to sort memory traces. By recording neuronal activity and measuring neurotransmitter release, researchers have shown that during the first part of NREM sleep (SWS I/stage II NREM sleep), brainstem cholinergic and glutamatergic cells are silent and stop releasing their neurotransmitters, ACh and glutamate (GLUT), in the thalamus, hippocampus, and cerebral cortex (Figure 2). Steriade and colleagues have

shown that the GABAergic cells (GABA – gamma aminobutyric acid) in the thalamus are activated when cholinergic and glutamatergic neurotransmitter levels are low in the thalamus. They have also shown that the activation of thalamic GABAergic cells generates sleep spindles that are normally recorded in the thalamus and cerebral cortex. An epiphenomenon of sleep spindle, called sharp-wave/ripple activity, could also be recorded in the hippocampus. It is known that this spindle activity prevents incoming sensory signals from reaching its targets in the cortical and subcortical structures by blocking them at the thalamus (Figure 2). Similar physiological studies have also shown that during this early part of NREM sleep (SWS-1/stage II NREM), the activities of the brainstem aminergic cells (noradrenergic cells in the locus coeruleus and serotonergic cells in the raphe nuclei) are reduced, but remain sufficiently active to release NE and 5-HT in the cerebral cortex, hippocampus, and amygdala. These reduced levels of NE and 5-HT are sufficient for maintaining neuronal activities at their targets in the cerebral cortex, dorsal hippocampus, and amygdala (Figure 2). Indeed, by recording neuronal activities in freely moving rats, Wilson and colleagues have shown that the pattern of cortical and hippocampal activities elicited while practicing a memory task is replayed in and around the time of hippocampal sharp-wave activity during this part of NREM sleep. These

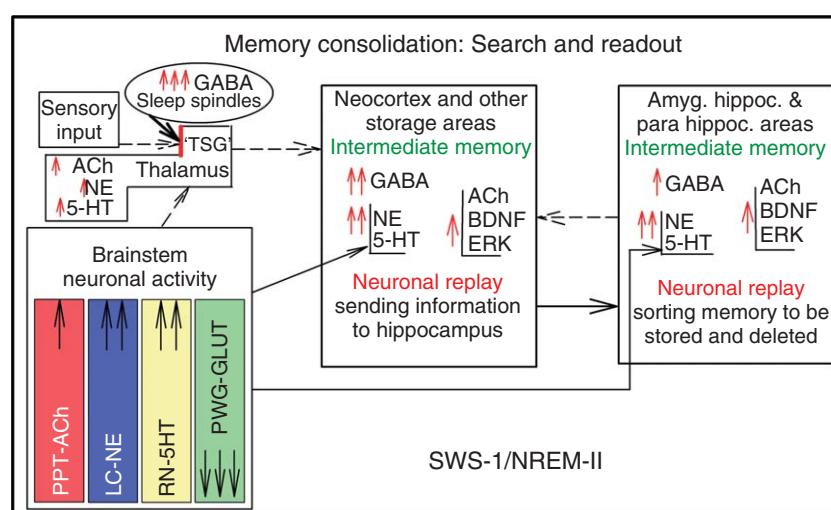


Figure 2 Physiological and biochemical conditions of the brain during SWS-1/NREM-II stage are conducive for the search and readout step of memory consolidation. Black arrows with solid lines between the blocks of brain structures: possible direction and flow of cognitive information and/or neurotransmitter; three upward arrows: highest levels of neuronal activity (PPT-ACh, LC-NE, and RN-5HT), extracellular level of neurotransmitters (ACh, NE, 5-HT, and GLUT), and intracellular proteins (BDNF and ERK); three downward arrows: lowest levels or absent of neuronal activity (PWG-GLUT) and extracellular level of neurotransmitter (GABA); black arrows with dashed lines between the blocks of brain structures: cognitive information and/or neurotransmitter flow in that direction is blocked; Arrows: levels of neuronal activity, extracellular levels of neurotransmitter, and intracellular levels of proteins: 3 arrows, highest; 2 arrows, medium; and one arrow, low. 5-HT, 5-hydroxy tryptamine (serotonin); ACh, acetylcholine; BDNF, Brain-derived neurotrophic factor; ERK, extracellular signal regulated kinase; GABA, gamma amino butyric acid; GLUT, glutamate; LC-NE, noradrenergic cells in the locus coeruleus; NE, norepinephrine; PPT-ACh, cholinergic cells in the pedunculopontine tegmental nucleus; PWG-GLUT, glutamatergic cells in the pontine-wave generator; RN-5HT, serotonergic cells in the raphe nucleus; TSG, thalamic sensory gate.

investigators have also shown that this type of learning-experience-induced off-line reactivation of a memory trace first begins in the cortex and then in the hippocampus, indicating that the cortex initiates this cortical-hippocampal interaction. Similarly, Peigneux and colleagues, using brain-imaging techniques in humans, have shown that the cortex and hippocampus are activated during the training session of spatial memory tasks, and are then reactivated during the early period of NREM sleep. These neuronal activity recordings and brain-imaging studies demonstrate that, during this early part of sleep, memory trace-forming structures are not only active, but are also replaying memory. Therefore, among all stages of the sleep-wake cycle, the early part of NREM sleep (light SWS) is the most conducive for the search and readout of the intermediate form of memory.

Elimination of Unnecessary and/or Redundant Memory

At this stage of memory consolidation, memories, which are identified as redundant, are deleted from the memory trace. Elimination of those redundant or irrelevant memory traces allows the brain to pay additional attention to memory traces that are more relevant for the next step of memory consolidation (increased signal-to-noise ratio). Since memories are stored as alterations in the strength

of synaptic connections, during this stage of the memory consolidation process, the number of synapse decreases, especially at the dendritic spines. This process is also known as synaptic pruning. Neuronal activity recording studies have shown that during the deepest part of the NREM sleep stage (SWS II/stages III and IV NREM, and deep SWS), the activities of brainstem cholinergic, and glutamatergic cells are at their lowest and that of aminergic cells is also very low (**Figure 3**). By measuring neurotransmitter release, studies have shown that the extracellular levels of NE, 5-HT, GLUT, and ACh in the cortex and in many other subcortical structures known to be involved in cognitive functions are very low during this deepest part of SWS. On the contrary, levels of inhibitory neurotransmitters, like GABA and adenosine, in the forebrain areas and cerebral cortex are at their highest level. This neurochemical state, with high levels of inhibitory neurotransmitters and low levels of excitatory neurotransmitters, is an inhibitory state of the brain. Indeed, brain-imaging and neuronal activity recording studies have shown that the deepest part of SWS is the most deactivated behavioral state of the brain. Recent molecular studies have shown that during the deepest part of SWS, hippocampal and cortical levels of immediate-early genes, early growth response 1 (Egr-1, also called zif-268, NGF1-A), and BDNF are at their lowest (**Figure 3**). Both conditions, either high levels of

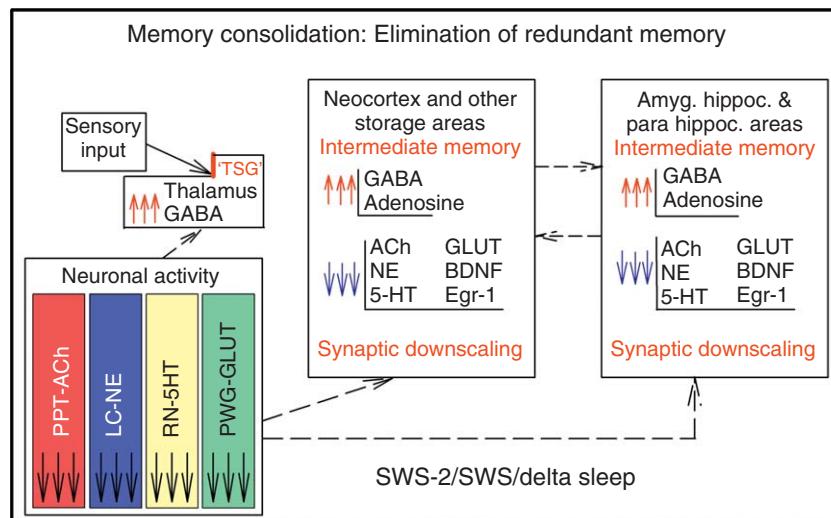


Figure 3 Physiological and biochemical conditions of the brain during the deep SWS stage are conducive for the elimination of redundant memory. Black arrows with solid lines between the blocks of brain structures: possible direction and flow of cognitive information and/or neurotransmitter; three upward arrows: highest levels of neuronal activity (PPT-ACh, LC-NE, and RN-5HT), extracellular level of neurotransmitters (ACh, NE, 5-HT, and GLUT), and intracellular proteins (BDNF and ERK); three downward arrows: lowest levels or absent of neuronal activity (PWG-GLUT) and extracellular level of neurotransmitter (GABA); black arrows with dashed lines between the blocks of brain structures: cognitive information and/or neurotransmitter flow in that direction is blocked; Arrows: levels of neuronal activity, extracellular levels of neurotransmitter, and intracellular levels of proteins: 3 arrows, highest; 2 arrows, medium; and one arrow, low. 5-HT, 5-hydroxy tryptamine (serotonin); ACh, acetylcholine; BDNF, Brain-derived neurotrophic factor; ERK, extracellular signal regulated kinase; GABA, gamma amino butyric acid; GLUT, glutamate; LC-NE, noradrenergic cells in the locus coeruleus; NE, norepinephrine; PPT-ACh, cholinergic cells in the pedunculopontine tegmental nucleus; PWG-GLUT, glutamatergic cells in the pontine-wave generator; RN-5HT, serotonergic cells in the raphe nucleus; TSG, thalamic sensory gate.

adenosine or low levels of BDNF and zif-268 are known for the reduction of dendritic spines and synaptic contacts (a process known as synaptic downscaling). Thus, the deepest part of SWS is the most conducive behavioral stage for the elimination of redundant memory.

Strengthening of Cognitively Relevant Memory

Physiologically, this is a process of synaptic strengthening in the memory trace. Over many years, pharmacological and biochemical research in cognitive neuroscience has shown that increased cholinergic and glutamatergic neurotransmission in the hippocampus, amygdala, and cerebral cortex improves this stage of the memory consolidation process. On the contrary, increased GABAergic and aminergic neurotransmissions in these areas attenuates this stage of the memory consolidation process. Studies of neuronal activity recordings in the brainstem and forebrain of rats and cats have shown that during REM sleep, cholinergic and glutamatergic neurons are very active but aminergic and GABAergic neurons are almost silent. Similarly, neurotransmitter release studies of Lydic, Baghdoyan, and many others have shown that, during REM sleep, the extracellular levels of ACh and GLUT are very high and at the same time extracellular

levels of NE, 5-HT, and GABA are very low in the hippocampus, amygdala, cerebral cortex, and many other areas of the forebrain. Thus, based on pharmacological and physiological evidence, only REM sleep neurochemistry could support this stage of memory consolidation (**Figure 4**).

Long-term potentiation (LTP) is hypothesized to be a neuronal model of learning and memory. LTP is also suggested to be the sign of synaptic strengthening in the memory trace. High-frequency (>200 Hz) electrical stimulation of an afferent pathway is important for inducing this type of LTP in the hippocampus, amygdala, and/or cerebral cortex. Datta and Hobson have identified a group of glutamatergic cells in the brainstem that discharge high-frequency (>500 Hz) spike bursts (3–5 spikes/burst) only during tS-R and REM sleep. They have also shown that the activation of these brainstem glutamatergic cells generates the pontine-wave (P-wave). Interestingly, activation of these cells facilitates hippocampal theta activity. Physiological evidence suggests that the hippocampal theta rhythm favors induction of LTP in the hippocampus as well as in many different parts of the cerebral cortex. Based upon physiological principles, neuronal reactivation is also critical for synaptic strengthening during sleep. Pioneering work by

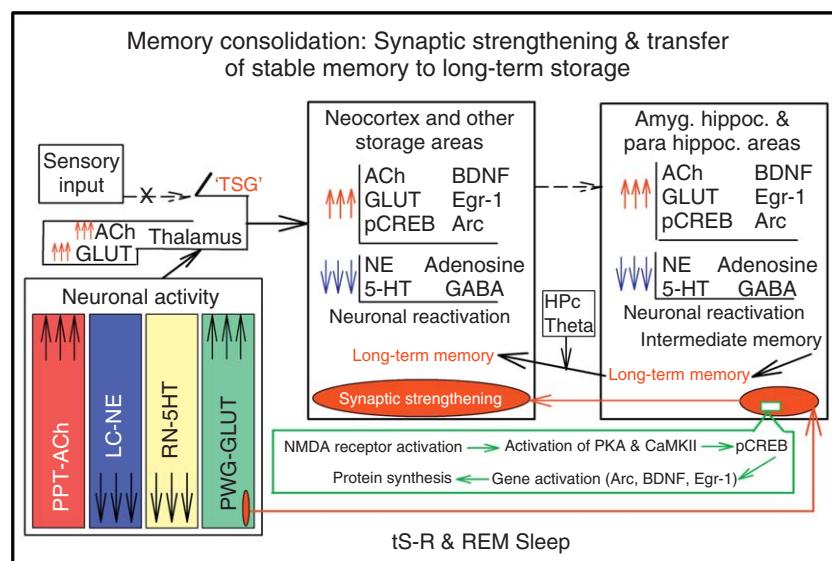


Figure 4 Physiological and biochemical conditions of the brain during tS-R and REM sleep stages are conducive for synaptic strengthening and transfer of stable memory to long-term storage. Black arrows with solid lines between the blocks of brain structures: possible direction and flow of cognitive information and/or neurotransmitter; three upward arrows: highest levels of neuronal activity (PPT-ACh, LC-NE, and RN-5HT), extracellular level of neurotransmitters (ACh, NE, 5-HT, and GLUT), and intracellular proteins (BDNF and ERK); three downward arrows: lowest levels or absent of neuronal activity (PWG-GLUT) and extracellular level of neurotransmitter (GABA); black arrows with dashed lines between the blocks of brain structures: cognitive information and/or neurotransmitter flow in that direction is blocked; Arrows: levels of neuronal activity, extracellular levels of neurotransmitter, and intracellular levels of proteins: 3 arrows, highest; 2 arrows, medium; and one arrow, low. 5-HT, 5-hydroxy tryptamine (serotonin); ACh, acetylcholine; BDNF, Brain-derived neurotrophic factor; ERK, extracellular signal regulated kinase; GABA, gamma amino butyric acid; GLUT, glutamate; LC-NE, noradrenergic cells in the locus coeruleus; NE, norepinephrine; PPT-ACh, cholinergic cells in the pedunculopontine tegmental nucleus; PWG-GLUT, glutamatergic cells in the pontine-wave generator; RN-5HT, serotonergic cells in the raphe nucleus; TSG, thalamic sensory gate.

Pavlides and Winson and, more recently, by Louie and Wilson has demonstrated that hippocampal neuronal activity expressed in place cells (neurons that fire in response to encountering specific locations) during waking is re-played (expressed as neuronal reactivation) during subsequent REM sleep. Moreover, Poe and colleagues have shown that this hippocampal place cell replay occurs at the positive phase (peak) of theta rhythm during REM sleep. Pavlides and colleagues and a number of other investigators have shown that the LTP-inducing tetanic stimuli are most effective at the positive phase of theta rhythm. Thus, the activation of P-wave generating cells during tS-R and REM sleep is capable of inducing physiological LTP in the hippocampus and amygdala. Similarly to neuronal recording in animals, in human subjects, using brain imaging, Maquet and colleagues have shown that patterns of brain activity expressed during training on a serial reaction time motor task reappear during subsequent REM sleep. Collectively, these physiological data suggest that REM sleep provides the ideal physiological environment for synaptic strengthening in the neuronal circuits of memory storage.

Over the last 25 years, cellular and molecular studies have demonstrated that *N*-methyl-D-aspartate (NMDA) glutamate receptor activation-induced, neuronal activation-dependent gene expression and protein synthesis in the dorsal hippocampus, amygdala, and cortex are necessary for synaptic plasticity and memory consolidation. However, such cellular and molecular studies have ignored the possibility that postlearning-training sleep could also positively (or negatively) influence learning-induced cellular and molecular changes. Using combinations of anatomical, physiological, pharmacological, and molecular techniques in the rat model, Datta and colleagues, during the last 10 years, have shown that the sleep stages, tS-R and REM sleep, tightly regulate this cellular and molecular cascade of memory formation. These studies have shown that after a session of learning training, P-wave generating cells increase their activity during the tS-R and REM sleep periods. Furthermore, the level of memory retention during the subsequent test session exhibited a positive relationship to the level of P-wave generating cell activation during REM sleep. They have shown that the activation of P-wave generating glutamatergic cells increases glutamate release in the dorsal hippocampus. This learning-training-induced increase in glutamate release during REM sleep in turn activates NMDA receptors in the hippocampus and amygdala. Activation of these postsynaptic NMDA receptors then activates intracellular signal transduction cascades, such as the cyclic AMP-dependent protein kinase-A (cAMP-PKA). Learning-training-induced P-wave generator activation-mediated activation of this cAMP-PKA then increases phosphorylation of cAMP response element binding protein (pCREB). This transcription factor

pCREB then increases the expression of Egr-1, activity-regulated cytoskeletal-associated protein (Arc), and BDNF messenger ribonucleic acid (mRNA) and proteins in the dorsal hippocampus and amygdala. More interestingly, they have shown that the increased REM sleep P-wave density during the posttraining sleep period is positively correlated with the increased levels of pCREB, BDNF, and Arc in the dorsal hippocampus. Collectively, these multidisciplinary studies of Datta and colleagues provided evidences to suggest that glutamate release by the activation of P-wave generating cells during tS-R and REM sleep is necessary for NMDA receptor-activation-mediated synaptic plasticity-related genes activation and protein synthesis in the hippocampus and amygdala (**Figure 4**). Thus, the above neurochemical, physiological, and molecular evidence suggests that tS-R and REM sleep stages provide a unique brain environment that is ideal for synaptic strengthening in memory-trace-forming structures of the brain.

Transfer of Stable Memory to Long-Term Storage

The last step of memory consolidation is to transfer stable memory traces to their long-term storage areas in the cerebral cortex. Based on memory testing in both hippocampus-lesioned patients and in experimental hippocampal- and amygdala-lesioned animals, it is now clear that once memory is processed in memory trace-forming structures (hippocampus, amygdala, PFC, cerebellum, and elsewhere), processed memories are then shifted from those structures to a permanent storage in the cerebral cortex. Louie and Wilson showed that, during REM sleep, the time frame of correlations between activities in different hippocampal neurons expands by two orders of magnitude resulting in the replay of waking activity patterns spanning seconds or even minutes. Such REM-related temporal expansion, relative to their original waking activity, in the time course of correlations between firing of different neurons is suggested to be the physiological sign of memory transfer from the hippocampus to its long-term storage in the cerebral cortex. Indeed, Pavlides and Ribeiro have shown that, during REM sleep but not other behavioral states, memory-consolidation-specific zif-268 activation begins in the hippocampus and then activation spreads into the cerebral cortex. Thus, this memory transfer occurs only during REM sleep.

The collective evidence from all sleep and memory studies suggests that all stages of sleep are equally important for the consolidation of all types of memory. Based on the type and amount of information to be processed and also the environment in which information was acquired, homeostatic demands for specific stages of sleep are readjusted for the completion of the memory consolidation process.

Acknowledgments

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See also: Active Avoidance and Escape Learning; Animal Models of Learning and Memory; Birdsong and Vocal Learning during Development; Cognition: Learning and Memory: Pavlovian; Cognition: Learning and Memory: Spatial; Declarative Memory; Effects of Stress on Learning and Memory; Episodic and Autobiographical Memory: Psychological and Neural Aspects; Fear Conditioning; Implicit Learning and Memory: Psychological and Neural Aspects; Knock-Outs: Learning and Memory; Learning and Memory: Computational Models; Memory and Aging, Neural Basis of; Memory Consolidation; Neural Basis of Working Memory; Neurogenesis and Memory; Neurotransmitters and Neuromodulators Regulating Sleep and Wakefulness; Sleep Genetics; Protein Synthesis and Memory; Role of Gene Transcription in Long-Term Memory Storage; Short-Term Memory: Psychological and Neural Aspects; Sleeping, Waking, and Dreaming; Synapse Formation and Memory; Synaptic Mechanisms for Encoding Memory.

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Voluntary Movement: Control, Learning and Memory

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Glossary

Attractor networks – Recurrent networks with persistent states toward which the activity state of the network is attracted; persistent states are called fixed points.

Composite action command – Command refers to the output of a motor area, action commands include commands for plans and commands for movements, and a composite command generated by a mesoscopic module refers to a set of elemental commands generated by microscopic modules.

Distributed processing module (DPM) – One motor area together with its subcortical loops through the basal ganglia and through the cerebellum forms a distributed processing module.

Microscopic versus mesoscopic modules – DPMs are mesoscopic modules because they are composed of sets of microscopic modules; the microscopic modules have essentially the same computational architecture as do mesoscopic modules.

Training signal – For supervised learning the training signal specifies how the network can improve its response whereas for reinforcement learning the training signal simply evaluates whether performance is better (reward) or worse (punishment) than previously.

synapses at one site in the brain to synapses at other sites. For example, during practice of a particular program, planning operations tend to migrate from subcortical sites to cortical sites and then from PM areas of the cortex to M1. These migrations permit more rapid and accurate implementation of automatic movements. Neural signals returning from subcortical loops regulate the process of migration.

The learning rules that mediate synaptic plasticity at different sites in the brain differ (see **Table 1** for a summary of the sites highlighted in this article). In the cerebral cortex, plasticity at excitatory synapses onto pyramidal cells utilizes a Hebb-like rule for the detection of coincidence between input and output. In the BG, plasticity at spiny neuron synapses from cortical afferents uses a trace of coincidence detection that is trained by reward signals; this learning rule is called reinforcement learning. In the cerebellum, plasticity at Purkinje cell synapses formed by parallel fiber afferents also uses a trace of coincidence detection, but in this case depressions in synaptic weight are trained (instantiated) by errors in performance; the learning rule is akin to supervised learning. Diffuse synaptic inputs modulate the above learning rules; they function as permissive factors that gate the intensity of learning. Distributed processing modules composed of one area of cerebral cortex, together with its loops through the BG and cerebellum, benefit from all of these mechanisms operating in combination. In this article, motor learning in the cerebral cortex, the BG, and the cerebellum are each treated individually; following this, the benefits of coordinated learning in entire distributed processing modules (DPMs) are discussed.

Motor programs involve both the planning and the execution of movements. For voluntary movements of the limbs, these functions are distributed among several cortical motor areas. Generally speaking, planning operations are controlled by premotor areas (PMs in **Figure 1**) whereas execution is controlled by the primary motor cortex (M1 in **Figure 1**). Although not specifically illustrated in **Figure 1**, each of these motor areas is regulated by relatively private subcortical loops through the basal ganglia (BG) and the cerebellum (CB). These subcortical loops are specialized for learning from training signals. In contrast, the cortical motor areas are specialized for learning from practice.

Cellular and molecular processes at specific synaptic sites underlie the learning and memory of motor programs. Learning modifies the weights of specific synaptic inputs to specific neurons and the persistence of these weight changes represents one form of memory. Migration is a process that transfers memories from

Hebbian Learning of Motor Programs in Cortical Motor Areas

The learning of motor skills over extended periods of practice results in enlargement of motor and sensory representations in the cerebral cortex. Initial learning relies on long-term potentiation (LTP); this Hebbian learning rule enhances the strength of synapses that participate in practice. Projections from the BG and the cerebellum force their targeted cortical neurons to practice particular firing patterns until more direct inputs from other cortical areas are strengthened enough to generate similar firing patterns without guidance from the BG and/or cerebellum. Cholinergic neuromodulatory

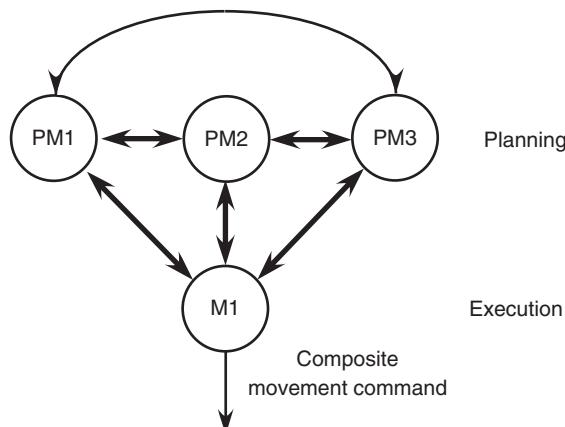


Figure 1 Cortical motor areas. M1 = primary motor cortex; PM1 = premotor area 1; PM2 = premotor area 2; PM3 = premotor area 3.

input from the nucleus basalis is a permissive factor related to attention. It permits the consolidation of cortical memories through synaptogenesis and cytogenesis. This scenario is especially important for crystallizing automatic movements.

BG Loops for the Selection of Ballpark Motor Programs

The cortical motor system's loops through the BG learn to regulate the selection of motor plans and movement commands – operations collectively referred to as action selection. The learning rule given in **Table 1** includes both coincidence detection, as in the cortex, and also training information transmitted by dopamine neurons. Brief bursts of dopamine-neuron firing signal the likelihood of future reward; these neural signals serve well as training signals for reinforcement learning. Next, we consider just what is being learned and why it is a difficult learning problem. The left side of **Figure 2** is a simplified schematic of one mesoscopic loop through the BG; it is comprised of thousands of microscopic loops originating from the same area of the cerebral cortex.

We discuss the coarse selection of a ballpark action since a small set of potential actions is selected rather than

an individual action. This imprecision results from cortex-to-striatum connections: any given spiny neuron (in the input stage of BG, called the striatum) receives a very large array of afferents from neurons in the cerebral cortex. Among several areas sending inputs, one forms a recurrent loop or module. Neighboring spiny neurons representing alternative actions thus receive a very similar, but slightly different, array of potential inputs. Spiny neurons compete with each other via prominent recurrent collaterals of their inhibitory γ -aminobutyric acid (GABA)ergic axons; the winners of this competition transmit their decisions to the next stage of BG processing. This pattern-classification operation by spiny neurons effectively decides on the relative merits of alternative actions based on the synaptic weights of its cortical inputs. These cortical-striatal synapses are trained by the dopamine input that signals the likelihood of future reward. Dopamine signals also function as a permissive factor that enhances the excitability of activated spiny neurons. The result is a powerful pattern classification operation that selects ballpark actions from thousands of alternative actions.

How do the inhibitory GABAergic outputs from selected spiny neurons instantiate their coarse decisions? This question is complicated by multiple stages of inhibition and by the presence of multiple pathways through the BG. Direct pathways learn to select context-dependent desired actions by disinhibiting positive feedback loops between the thalamus and cortex. These operations yield bursts of output from selected cortical neurons. Indirect pathways are tuned to deselect undesired actions by disinhibiting BG output neurons so that they can inhibit activity in inappropriate loops between the thalamus and cortical neurons.

To summarize, a large array of spiny neurons in the striatum receives a diverse convergent input from the area of the cerebral cortex that participates in any given mesoscopic loop. The array of spiny neurons performs a pattern-classification operation that is made competitive by the presence of collateral inhibition. Competition is biophysically mediated by both presynaptic and postsynaptic collaterals of the GABAergic spiny neurons. The superior performance of the presynaptic inhibitory mechanism appears to be augmented in phylogeny, and

Table 1 Different learning rules in the cerebral cortex, basal ganglia, and cerebellum

Brain site	Cerebral cortex	Basal ganglia striatum	Cerebellar cortex
Cellular Site	Excitatory afferents Onto pyramidal cells	Cortical afferents Onto spiny neurons	Parallel fibers Onto Purkinje cells
Learning rule Operation	Hebbian Coincidence	Reinforcement Coincidence • reward	'Supervised' Coincidence • error
Permissive factors	Cholinergic+	Dopaminergic+	Noradrenergic+

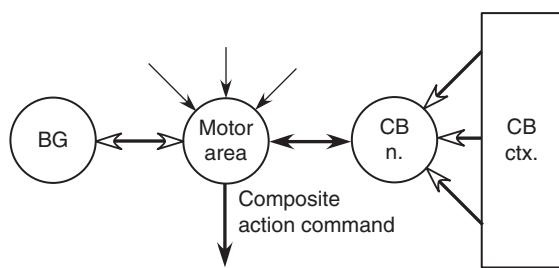


Figure 2 Subcortical loops. BG = basal ganglia; CB n. = cerebellar nucleus; CB ctx. = cerebellar cortex.

its occasional disruption associated with unfavorable epigenetic expression might help to explain schizophrenia. The selection of potential actions (by individual microscopic loops) from thousands of alternative actions is a very difficult pattern-classification task, which probably explains why it typically yields only a coarse selection of ballpark actions. The amplification and refinement of these ballpark actions into a precise action is attributed to the loop through the cerebellum.

Cerebellar Loops for the Amplification and Refinement of Motor Programs

The motor system's loops through the cerebellum (right side of **Figure 2**) are needed to amplify and refine potential action – ones that are promoted by synaptic input from other areas of the cerebral cortex and selected by loops through the BG. Amplification is mediated by positive feedback in an array of microscopic loops between a given area of the cerebral cortex and the corresponding division of a cerebellar nucleus. These loops form attractor networks that facilitate the planning and execution of motor programs. Purkinje cells in the cerebellar cortex control the fixed points of these attractor networks and, in doing so, create spatiotemporal patterns of cortical output that represent refined motor plans and motor commands. Sets of microscopic loops between primary motor cortex and cerebellum are forced by Purkinje cell inhibition to coordinate their activities in order to command movement at a selected velocity to a desired endpoint in space and time. The complete array of microscopic loops forms the composite action command for any given mesoscopic loop. The loops with the cortex thus generate composite motor plans and movement commands that control accurate movements.

Motor learning in Purkinje cells is guided by error-driven feedback from climbing fibers. These training signals signify punishment when an error is made. They instantiate long-term depression (LTD) – the ‘supervised’ form of learning in the cerebellum (**Table 1**). If, for example, a movement stops short of the target, a

corrective movement toward the target fires climbing fibers. LTD is then instantiated, and this delays the time at which Purkinje cells fire to terminate the next movement in the same direction. As a permissive factor, noradrenergic fiber discharge has two modes of action. Phasic discharge signals good outcomes and helps to consolidate LTD. Tonic discharge signals poor outcome and facilitates alternative actions. Another permissive factor, serotonergic discharge, signals the opposite of urgency; thus, pauses in discharge facilitate faster actions.

To summarize, composite action commands are initiated in loops through the cerebellar nucleus (CB n. in **Figure 2**), and they are then shaped and ultimately are terminated by prominent inhibitory input from the cerebellar cortex (CB ctx.). Arrays of Purkinje cells learn to detect input patterns signifying that the time has come to terminate elements of action plans and/or movement commands. Microscopic loops generate elemental commands and mesoscopic loops generate the several composite action commands in different motor areas. The overall result is a well-coordinated and precise composite movement command.

Coordination of Eyeblink Conditioning

Eyeblink conditioning is given special treatment here, since so much is known about how the cerebellum participates in the generation of eyeblink motor commands. The core of **Figure 3** is the same as **Figure 2**, except that the ‘red’ nucleus replaces the general label ‘motor area’ because it constitutes the main output for conditioned reflex (CR) motor commands. Conditioned stimulus (CS) signifies all of the variety of sensory inputs that can be used by the other four structures. The brainstem circuitry that mediates an unconditioned response (UR)

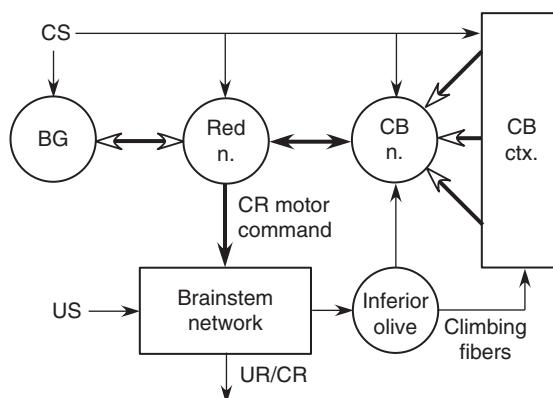


Figure 3 Eyeblink conditioning. BG = basal ganglia; CB n. = cerebellar nucleus; CB ctx. = cerebellar cortex; CR = conditioned reflex; CS = conditioned stimulus; Red n. = red nucleus; UR = unconditioned reflex; US = unconditioned stimulus.

to an unconditioned stimulus (US) and sends climbing-fiber input to CB cortex is shown at the bottom. This network also generates a CR in response to a CR motor command.

Much of the research in this field has focused on the association between a CS and the initiation of the eyeblink CR. While the BG is likely to be crucial in the discovery of potential stimuli that might fulfill a CS role, until recently the emphasis has been on where the associative link is actually made with the cerebellar circuitry that is required for CR responding. The most prominent link appears to be formed at CS synapses onto neurons in a CB nucleus – the interpositus nucleus. Once formed, this associative link is responsible for initiating positive feedback in the reciprocal loop between the red nucleus and the cerebellar nucleus. This loop forms CB modules that are analogous to the CB modules formed with motor cortex. The CB cortex then refines the amplitude and timing of the CR motor command so that it closes the eyelid in time to protect the cornea from the US.

Note that synapses at any site along the reciprocal pathway between the red nucleus and CB nucleus can fulfill the associative role ascribed to the interpositus nucleus for CRs. Viewed in this manner, eyeblink conditioning provides an excellent model system for studying learning mechanisms for motor control. The learning rule in the loop between CB nucleus and red nucleus is Hebbian as in the cerebral cortex, whereas the learning rule in CB cortex is supervised.

Coordinated Operation of DPMs

All of the operations outlined above occur in parallel in our brain, resulting in a high level of performance. Sensory and other inputs to the cerebral cortex begin the process of initiating actions. Inputs from BG regulate this process through the selection of ballpark actions and reinforcement learning that homes in on promising actions. Once competitive interactions within BG produce a ‘winner,’ that tentative action is selected and loops through cerebellum amplify and refine it into a precise composite action. Training signals from climbing fibers allow the cerebellum to optimize the action, permitting us to hit a desired endpoint with precision in both space and time. As the motor areas of the cerebral cortex practice controlling these actions, their signal-processing activities become consolidated into long-term memories, and eventually even the most elegant of actions becomes almost automatic.

Note that many factors influence motor learning. One can think of these diverse factors as different constraints on the evolutionary process that fine-tunes the

mechanisms used by the motor system. Since we live in an uncertain world, any given action may have many possible outcomes. Decision theory provides an approach to motor learning and control that can yield compact assessments of outcomes. Combining the various types of knowledge outlined in this article should help us to fathom the complexity of motor control and learning.

See also: Behavioral Planning: Neurophysiological Approach of the Frontal Lobe Function in Primates; Motor Function and Motivation; Motor Learning in the Vestibulo-Ocular Reflex.

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Genetics of Memory in *Drosophila*

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Glossary

Enhancer trap – The insertion of a P-element carrying the GAL4 coding region, resulting in GAL4 expression in a spatially and temporally defined pattern determined by a nearby enhancer.

Forward genetics – Random mutagenesis and screening (e.g., behavioral screens) to identify individuals with a phenotype of interest as a means of gene discovery.

Mushroom body – Bilateral neural structures in the insect brain required for olfactory memory functionally analogous in some ways to the vertebrate hippocampus.

Mutagenesis – The use of chemical mutagens, irradiation, or insertion of novel DNA to introduce random genomic mutations into a population of organisms to produce variant mutant alleles.

Neurogenetics – The study of genes involved in the structure and function of the nervous system.

P-element – A transposable DNA element in *Drosophila* that is flanked by inverted repeats recognized by a transposase. P-elements are often manipulated in *Drosophila* to introduce transgenes or produce mutations by disrupting endogenous genes.

Reverse genetics – The manipulation of identified genes (often as transgenes) in a system in order to test a hypothesis about the function of the gene.

Temperature-sensitive mutation – A mutation identified in a screen using temperature variations to increase detection of the mutant phenotype. Typically such mutants appear normal at the lower, permissive, temperature; a shift to an elevated, nonpermissive, temperature causes the mutant gene product to become unstable producing a change in phenotype.

neurogenetics – that complex behaviors might be genetically tractable, and in the model organism *Drosophila melanogaster*, no less – met with general skepticism and disbelief. Since then, *Drosophila* neurogenetics has resulted in pioneering studies that have elucidated the fundamentals of circadian rhythms at both the cellular and molecular levels, and has contributed significantly to greater understanding of learning and memory formation and sexual behaviors. As new tools are applied to these complex biological problems, the tiny fruit fly seems poised to bridge molecular genetics with a more systemic understanding of how neural circuits are formed and maintained in memory.

Not long after single gene mutants in phototactic and circadian behavior were isolated in Benzer's lab, confirming the promise of a neurogenetic approach, Quinn, Harris, and Benzer demonstrated that *Drosophila* could be conditioned using an operant conditioning paradigm. In this negatively reinforced assay, flies learn to avoid an odor paired with electric footshock. A forward genetic screen using this assay rapidly yielded mutants in which learning and memory was defective. The subsequent 30 years have seen the isolation and identification of numerous such mutants, some of which have been molecularly identified and examined. Many of these mutants are in highly studied cellular signaling pathways, and this has contributed to studies on learning and memory formation in other organisms.

In addition to these classical forward-genetics screens, sophisticated transgenic tools have contributed to contemporary studies. Indeed, memory research has driven the development of technology that limits transgene induction and repression both spatially and temporally. Progress has also been made in the ability to image and record neural activity in specific neurons. Most recently, it has been shown that heterologous channels can be expressed in defined neurons, allowing rapid, temporal control over their depolarization or hyperpolarization. The application of these tools to memory research will allow *Drosophila* neurobiologists to tackle heretofore unanswerable questions about neural circuitry and cellular connectivity, as well as when and where specific molecules are required for memory formation. These tools – combined with some of the genes that have been discovered – should facilitate the move from neurogenetics to the neurobiology of memory formation.

Introduction

In the postgenomic age, where the idea of genes underlying virtually every aspect of human health and function has gained wide acceptance, it can be difficult to comprehend how revolutionary neurogenetics once was. Yet when Seymour Benzer's laboratory began their pioneering work in the 1960s, the principle of

The Neurogenetics of *Drosophila* Memory Formation – the First 35 Years

Learning and Memory Paradigms in *Drosophila*

Olfactory memory paradigms have dominated *Drosophila* memory research since its inception. Tully and Quinn developed a more robust, negatively reinforced, Pavlovian (classical) conditioning assay in 1985. This olfactory classical conditioning (OCC) assay has largely replaced the original operant conditioning paradigm. In this assay, one of two odors is paired with footshock, while the other odor is unpaired. Following training, flies must choose between the paired and unpaired odors. Varying the number of training trials and their spacing produces different phases of memory analogous to those in other learning and memory models: short-term memory (STM), which is independent of transcription and translation and decays within 1 h; middle-term memory (MTM), which requires translation but not transcription and decays within 1–4 h; anesthesia-resistant memory (ARM), which is independent of translation and decays after 96 h; and long-term memory (LTM), which requires transcription and translation and lasts for at least 7 days. Single-trial or repetitive massed training (where trials are performed sequentially) produce the first three of these phases but only repetitive spaced training, where a rest period follows each training trial, produces LTM.

The other most common assay of learning and memory is an operant conditioning paradigm in which courtship is suppressed. Mated females paired with virgin males will induce courtship but reject attempts at copulation. Single, short-term exposure to mated females prevents males from subsequently courting virgin females (for 2–3 h) and fertilized females (for up to 24 h). Extended exposure to mated females (presumably resulting in many spaced rejections) results in a conditioned courtship suppression (CCS) lasting at least 9 days. While this paradigm integrates multiple sensory pathways and is based on a highly biologically relevant behavior, the underlying circuitry may be more complex due to the multimodal nature of the stimuli.

Flies are able to distinguish similar abstract visual patterns, and recall the position of items no longer present in an environment. These abilities have been exploited in a number of lesser-used memory paradigms, including appetitive sucrose-based (i.e., positively reinforced) conditioning, conditioned place-preference assays, and a disappearing-visual-landmark paradigm. Despite these alternative paradigms, the bulk of what has been learned concerning *Drosophila* memory has come from employing the OCC and CCS paradigms. Through the combined application of forward and reverse genetics, the various memory phases have been outlined, and a large number of genes that contribute to one or more of these processes

have been identified (see below). In addition, the forward genetic screens – especially those performed by Heisenberg and colleagues – identified the mushroom bodies (MBs) and central complex (CC) as key structures in olfactory memory. These discoveries have been critical in mapping the olfactory circuitry and driving recent experiments. Contemporary experimental strategies rely heavily upon the enhancer-trap screens that owed their genesis to these early screens, which identified mutations affecting the neuroanatomical architecture required for olfactory memory. The complexity of the circuitry underlying behavior is generally appreciated, but its details remain unclear, both within and outside of the MB. Recent work using temperature-sensitive dynamin (*shibire^{ts1}*) has revealed complex circuitry even for short-lived memories, providing a glimpse of the likely minimum complexity that underlies LTMs. In addition to molecular, cellular, and neurobiological issues, the first 30+ years of neurogenetics has also provided a neurocognitive perspective. Flies are able to generalize visual stimuli, and this cognitive process requires an intact MB. Prior to turning to the next 30 years of fly-memory research, we summarize some of the important genes that have been identified during the ‘golden’ years of gene discovery.

Gene Discovery in *Drosophila*

The first and best-characterized molecular pathway of memory in *Drosophila* is the cyclic adenosine monophosphate (cAMP) signaling pathway. Quinn and colleagues' original screen yielded two mutants in this pathway – *dunce* (*dnc*) and *rutabaga* (*rut*) – encoding a cAMP phosphodiesterase and a calcium ion (Ca^{2+})/calmodulin-regulated adenylate cyclase (AC). These mutants are poor learners in the operant, OCC and CCS behavioral paradigms. Subsequent experiments have shown that dysregulation of cAMP levels severely inhibits learning in OCC, indicating the cAMP signaling cascade must be intact for learning and memory to occur. Whether these genes played an architectural or acute role in memory formation, however, was unclear. For the *rut* product, temporally and spatially limited expression of the wild-type *rutabaga* in adult MB neurons rescues the learning deficit, thus demonstrating an acute requirement for *rut*. The *amnesiac* (*amn*) mutant, identified in the same screen, has recently been shown to encode a neuropeptide resembling pituitary AC-activating peptide (PACAP). Identification of the *amn* product provided an intriguing clue about the cellular connectivity of the cAMP pathway, as the gene is strongly expressed in the dorsal paired medial (DPM) neurons, which synapse onto the MB neurons and are believed to release their neurotransmitters onto the MB neurons, potentially activating the AC in the MBs.

Two other genes in the cAMP signaling cascade have been examined for their role in learning and memory: protein kinase A (PKA), a cAMP-stimulated kinase, and the transcription factor cAMP response-element-binding protein (CREB), which lies downstream of PKA activation. PKA is the principal effector of the cAMP cascade, and has been shown to up- and downregulate synaptic strength in central synapses. PKA-mediated regulation of synaptic strength can be phenocopied by *rut* expression in the postsynaptic cell. Overexpression of a regulatory subunit of PKA – creating, in effect, a dominant-negative PKA – also impairs olfactory learning and MTM, but only MTM in CCS suppression. Mutations in the catalytic domain of PKA, including *DCO*⁵⁸¹, can reduce PKA activity by 80%, and substantially impair memory. The PKA catalytic subunit is enriched in the MBs, as are the *dnc* and *rut* products; hence three principal cAMP cascade components colocalize to essential olfactory memory structures. This emphasizes the centrality of cAMP signaling in the biochemical pathways underlying learning and memory.

The role of CREB is somewhat more complex. As a transcription factor, CREB acts as a bottleneck upon which many different signaling pathways converge. This means that altering its expression is highly likely to have pleiotropic effects. In addition, it is differentially spliced in fly brains, with at least seven isoforms with presumably distinct coactivators and therefore differential transcriptional activity. In 1995, we reported that the acute expression prior to training of two different splice isoforms had opposite effects on memory: dCREB2-b, the ‘blocker’ isoform, was sufficient to prevent LTM formation following spaced training in OCC. The overexpression of dCREB2-b was also later found to selectively block LTM in CCS, illustrating the specificity of this dominant negative for consolidated memory. The expression of another isoform, dubbed dCREB2-a, was sufficient to produce enhanced memory formation following a single training trial – which would normally produce only STM and ARM. A point mutation encoding an early stop codon in dCREB2-a was discovered some years later. It has only recently become apparent that the isoform of dCREB2 responsible for memory enhancement is one whose expression is tightly regulated, in part in a circadian manner, suggesting another level of regulation in how different isoforms of CREB enact their transcriptional programs. These findings have been echoed by a report in mice that there are circadian oscillations in cAMP levels and CREB phosphorylation states and that disruption of these oscillations results in memory deficiencies. This suggests that assessing molecular components of memory pathways may require strict control of transgene expression, particularly for transcription factors.

Other Cell Signaling Molecules Implicated in Learning and Memory

The neurofibromatosis I (NF1) gene encodes a *ras* inactivating, GTPase-activating protein (GAP) and mutations in human NF1 produce a disorder marked by learning defects and benign tumors throughout the nervous system. In flies, loss of the *Drosophila* *NF1* ortholog leads to defects in learning and all later forms of memory. While dysfunctional regulation of Ras activity is the most likely cause of these deficits, *NF1* is necessary in *Drosophila* for stimulation of the *rut*-encoded AC by PACAP-like peptides. *Drosophila* *NF1* mutants display deficits in STM rescued by acute expression of a wild-type *NF1* transgene or by expression of a constitutively active PKA catalytic subunit, implying that the learning deficits in *NF1* mutants are in part related to defects in cAMP signaling. The LTM defect in *dNF1* mutants has been shown to be specifically the result of altered Ras activity, and LTM is rescued by overexpression of the GAP domain of *NF1* alone, suggesting an important role for Ras inactivation in consolidated memory. Hence, a single protein appears to be involved in mediating both learning and later forms of memory.

Two other kinases have been shown to affect learning, and indeed, to enhance some forms of memory in the fly. Temporally restricted expression of a PKC-inhibiting peptide results in normal learning but failure to suppress courtship in CCS after training – indicating a role for PKC in longer-lasting forms of memory. Based on mammalian work in hippocampal slices, we tested the atypical subfamily of PKC in learning and memory formation. The induction of a partially activated isoform of the murine or fly aPKC ζ gene, aPKM ζ or DaPKM, in a small post training window, was sufficient to selectively enhance ARM in OCC. Expression of a dominant negative form of DaPKM or use of the PKM inhibitor chelerythrine was sufficient to block ARM. Again, subsequent studies in vertebrate mammals have underscored the importance of aPKM ζ in learning and memory, confirming that such cellular signaling pathways are highly conserved.

The calcium/calmodulin-dependent protein kinase II (CaMKII) has received a great deal of attention among molecular neurobiologists. It is enriched at synapses, suggesting it may be a key player in responding to neuronal stimulation, and is capable of autophosphorylation, leading to rapid changes in its activity, and the ability maintain persistent activation. The expression of a CaMKII peptide inhibitor throughout the fly impairs learning and memory in CCS. Conversely, the expression of a constitutively active CaMKII in MB neurons and the antennal lobes – components of the olfactory learning circuitry – results in enhanced learning in the same assay.

RNA Transport and Translation Mutants

Tully, Dubnau, and colleagues paired a transposon-insertion screen for LTM-specific mutants with a DNA microarray performed in wild-type flies following spaced training. These complementary screens yielded seven genes involved in control of subcellular mRNA localization and translation. Among the mutants identified by the behavioral screen were mutations in eIF-5C, a translation initiation factor; *oskar*, a known requirement for *staufen*-mediated RNA transport; and *pumilio* (*pum*), a repressor of translation. *Pum* was also identified in the DNA microarray as a gene whose expression is increased during LTM formation. Other genes whose expression was significantly altered in the DNA microarray include *staufen* (*stau*), involved in mRNA transport within the cell; *moesin* (*moe*), an actin-binding protein that mediates *stau*-bound mRNP translocation; *orb*, the fly cytoplasmic polyadenylation element binding protein (CPEB) homolog, which is required for local translation in oocytes and presumably at synapses; and eIF-2G, another translation initiation factor. As a validation of the DNA microarray results, a temperature-sensitive *stau* mutant was used to demonstrate that learning was intact but 1-day memory specifically disrupted when flies were maintained at the elevated, nonpermissive temperature following assessment of learning. The identification of multiple genes in the well-studied messenger RNA (mRNA) transport and translation pathway provides support for the idea that LTM formation requires the transport of newly transcribed, synaptically localized mRNAs, and that local translational control is key to LTM formation.

The cytoplasmic poly(A) polymerase GLD-2, similarly to CPEB, enhances translation of its target mRNAs by extending their poly(A) tails when the polymerase is activated. *Drosophila* GLD-2 interacts with *dfmr1*, *pum*, and *eIF-4E*, placing it at the heart of the translation-regulatory machinery. Acute overexpression of a dominant-negative *Drosophila* GLD-2 prior to training has recently been shown to specifically inhibit LTM but not learning or STM, in the classical olfactory conditioning paradigm. This lends additional support to the notion that local translational activation at the time of memory formation is essential for consolidating memory.

Another gene in the translation pathway with an effect on memory is the *Drosophila* ortholog of *FMR1* in humans. Defects in *FMR1* result in fragile X mental retardation syndrome (FXS) or in fragile X tremor/ataxia syndrome, a progressive neurodegenerative disorder. *FMR1* and its orthologs normally function in the micro-RNA (miRNA) pathway, where it participates in translational repression; *fmr1* knockout models have elevated levels of mGluR activity as well. *dfmr1* mutants replicate many salient features of FXS, including memory impairment; they exhibit normal learning but defective memory in the

CCS-suppression paradigm. This memory defect is rescued by several metabotropic glutamate receptor (mGluR) antagonists, confirming that mGluR dysregulation in *dfmr1* mutants is in part responsible for the impairment of memory. More recently, *dfmr1* expression was shown to be elevated in wild-type MBs following spaced training. Overexpression of wild-type *dfmr1* following spaced training was shown to impair 1-day memory in the classical olfactory conditioning paradigm, as did a *dfmr1*-directed RNA interference (RNAi) transgene is driven in the MBs. Impairments were specific for 1-day memory after spaced training and had no effect on 1-day memory after massed training, confirming the role of *dfmr1* in consolidated memory. Notably, protein-synthesis inhibitors at low concentrations rescued the 1-day memory deficit in *dfmr1* mutants without effect on wild-type fly performance, suggesting that *dfmr1* participation in translational repression is important to its normal function in memory.

The increased expression of translational repressors *pum* and *dfmr1* after spaced training is, at first glance, counterintuitive: if local translation is required for consolidated memory, which it almost assuredly is, why then are translational repressors required as well? One possibility is that while LTM formation appears to require ongoing local translation following training in LTM formation, the mRNAs being translated likely vary at different stages of consolidation and eventual transfer of memory. If ongoing, synaptically localized translation of specific subsets of mRNAs is required, translational repressors can be thought of as essential in maintaining mRNAs in an inactive state as they are transported to distal locations in the cell, where the mRNAs may be activated and translated. One advantage of allowing the products of transcription to be delivered in a relatively defined manner would be the selective, local production of specific proteins.

Other Classes of Genes Affecting Learning and Memory in *Drosophila*

Two different genes that participate in a common pathway during neuronal development affect memory formation. Notch (N) receptors are single transmembrane proteins primarily studied in developmental cell-fate determination. Temperature-sensitive mutations in N, as well as transgenic flies carrying dominant-negative constructs, are selectively defective in LTM but not ARM in OCC. Expression of a Notch-directed RNAi transgene in the MBs alone specifically impairs LTM, demonstrating how new tools can be directed to determine the spatial requirement for genes involved in learning and memory. Overexpression of N after a single training trial enhances 1-day memory, and this enhancement is protein-synthesis-dependent.

The highly conserved E3 ubiquitin-ligase encoded by Neuralized (*neur*) enhances Notch-mediated transcription during development by ubiquitinating the Notch ligand. A recent report demonstrates that loss of a single copy of *neur* significantly reduced LTM in the OCC paradigm, whereas overexpression of wild-type *neur* in peripheral MB neurons resulted in a dosage-dependent enhancement of LTM. In neither case was learning, STM, MTM, or ARM affected. This selective effect on LTM is part of the evidence for a *neur* role in LTM formation; the requirement for *neur* in the peripheral MB neurons suggests a role in formation as opposed to consolidation as these neurons are thought to be involved in the early stages of memory.

In our discussion of genes of learning and memory in *Drosophila*, we have presented only some of the better-studied genes and pathways. This is neither an exhaustive presentation of genes implicated in learning and memory, nor a complete history of every behavioral result obtained with every gene mentioned. These represent only a fraction of the mutants, however, and many remain unidentified. Likewise, many of the mutants affect learning and STM rather than longer-lasting forms of memory. A principal challenge for *Drosophila* neurogeneticists in the future is the identification of molecules participating in the maintenance and retrieval of memory.

Novel Tools

The principal novel transgenic tools available to *Drosophila* neurogeneticists are all derived from the binary Upstream Activation Sequence (UAS)-GAL4 expression system developed by Norbert Perrimon's laboratory. This system exploits a yeast transcriptional activator (GAL4) inserted at random within the fly genome to drive the expression of transgenes that carry tandemly arranged GAL4 binding sites (the Upstream Activation Sequence, or UAS). Cell-type restriction of GAL4 permits localized expression of the transgene. Using a tyrosine hydroxylase promoter to drive GAL4 expression, for example, restricts UAS-transgene expression to dopaminergic neurons. Variants on GAL4 permit further modulation of expression, including repression or induction of GAL4 activity; such variants (GAL80^{ts} and GeneSwitch, doxycycline) give a temporal, user-controlled dimension to GAL4-UAS transgene expression. UAS-driven fluorescent reporters of intracellular Ca²⁺ activity (UAS-Cameleon, UAS-GCaMP) and synaptic transmission (synaptopHluorin) provide the means of tracking activation of defined cellular populations. Other UAS-driven tools of special interest in learning and memory include: UAS-*sbire*^{ts1} – an inducible blocker of synaptic vesicle release; UAS-ChR2 – a blue-light-activated ion channel permitting direct photostimulation of neurons; and UAS-PAC α – a photoactivatable AC

allowing direct stimulation of the cAMP pathway in neurons. The astute combination of these tools with simple behaviors in the fly has the obvious potential to answer a host of questions about learning and memory.

The size of the *Drosophila* brain is often considered a disadvantage, as it limits the use of electrophysiology. The relatively small number (approximately 100 000) neurons in the fly brain and their stereotyped nature, however, make elucidation of the circuits underlying specific behaviors an attainable goal. Resolution of neural memory circuits requires the following: (1) a defined behavior, (2) identification of the cells participating in that behavior, (3) determining how these cells are activated and how that activation is transferred or communicated throughout the circuit, and (4) finally determining what cellular and molecular mechanisms underlie the inter- and intracellular communications producing the behavior. The dissection of olfactory memory structures using genetic tools has advanced rapidly in the last decade and the gross architecture of the olfactory system circuit is now understood as are the functional roles of subsets of neurons within the circuit. Future challenges in this area include understanding just how stimuli are encoded and transformed in the circuit and how effector neurons downstream of the MBs elicit the behaviors themselves.

Summary: Tools and Genes and the New Neurobiology of Memory Formation

The last 30 years of *Drosophila* neurogenetics have revolutionized the way we understand learning and memory, forging an appreciation of single-gene function in complex behaviors and identifying biochemical pathways that have proven to be essential for many types of mammalian memory. Given the array of genetic technology now available to manipulate and dissect memory, perhaps the major challenge for the next 30 years is more conceptual than technological.

A sizable collection of memory mutants now exists, and multiple genes have been identified. Rather than striving to identify additional genes, effort might be more effectively expended in bringing coherence to those already known. The tools available to test the function of these genes in memory are formidable, but by no means have simpler assays been exhausted. The classical genetics approach of using double mutants to assess whether the mutations are in the same pathway, for example, could determine whether existing mutations represent interdependent effects on memory. A variant of this approach using the circadian clock gene period (*per*) is instructive: *Per*-null mutants are selectively impaired in LTM. The inducible expression of a wild-type *per* transgene rescued the LTM deficit when

expressed before OCC, suggesting *per* is required for LTM formation. Surprisingly, overexpression of this *per* transgene in a wild-type fly prior to conditioning produced LTM from training conditions that normal induce only STM. LTM enhancement was maintained when dCREB2-b and *per* were overexpressed simultaneously prior to training, suggesting that *per* is downstream of dCREB2.

One principle that is needed is the development of new concepts guiding the field out of the age of gene discovery. The formulation of clear hypotheses about the roles of these genes in specific aspects of learning and memory is essential; equally important is the expansion of our view of the cognitive abilities and range of experience of the organism. For example, the role that non neuronal cells play in memory formation is unclear, even though studies on the cathepsin-encoding crammer (*cer*) mutant, for example, suggest a glial role in modulating early stages of LTM. In addition, the use of UAS-*shibire^{ts1}* has already established that dopaminergic and octopaminergic neurons mediate negatively and positively reinforced behaviors, respectively. Other contributory processes, such as the behavioral or circadian state of the flies, synaptic scaling, and other neuromodulatory factors are uniquely tractable in the fly. Despite the stereotyped nature of *Drosophila* neurons, the fly-brain need not be thought of as entirely hard-wired: there is evidence that activity-dependent plasticity exists at a cellular level, and flies exhibit behavioral plasticity – modifying courtship and fighting strategies based on experience. Expanding the repertoire of behavior in *Drosophila* will provide us with a richer understanding of learning and memory in a model that has already altered the landscape of learning. The power to answer virtually any defined question about *Drosophila* learning and memory is now limited principally by the hypotheses directing the work. During the next few decades, *Drosophila* should transition from a primarily neurogenetic system to one that is fully neurobiological.

See also: Analysis of Learning in Invertebrates; Genes and Behavior: Animal Models; Protein Synthesis and Memory; Transgenic Technologies and Their Application to the Study of Senile Dementia.

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Relevant Websites

- <http://flybase.org> – Flybase, a general resource for *Drosophila* researchers.
- <http://www.ncbi.nlm.nih.gov> – NCBI *Drosophila* resources.
- <http://www.ceolas.org> – The *Drosophila* Virtual Library.
- <http://www.sdbonline.org> – The Interactive Fly, with developmental resources.

Mechanisms of Memory Formation and Storage in *Hermisenda*

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Glossary

Actin – A protein filament that is a major constituent of the cytoskeleton of all eucaryotic cells.

Cytoskeleton – The system of protein filaments in the cytoplasm of a eucaryotic cell.

ERK (Extracellular signal-regulated kinase) – A component of the signal transduction pathway that couples intracellular responses to the binding of growth factors to surface receptors.

GABA (Gamma aminobutyric acid) – An inhibitory neurotransmitter.

G protein – One of a large family of GTP-binding proteins that are important intermediaries in signaling pathways.

Hermisenda – A carnivorous nudibranch mollusk found along the Pacific coast of North America used in the studies of learning and memory.

5-HT (5-hydroxytryptamine (serotonin)) – A neurotransmitter.

Phosphorylate – The process of adding a covalently linked phosphate group to a small molecule or protein.

PKC (Protein kinase C) – A family of enzymes that control the function of other proteins by phosphorylation

Protein kinase – An enzyme that transfers a phosphate group from adenosine triphosphate to a specific amino acid of a target protein.

formation. The marine mollusk *Hermisenda crassicornis* is one preparation that has contributed to an understanding of Pavlovian conditioning at the cellular, molecular, and system levels. Classical conditioning of *Hermisenda* is well documented and follows the Pavlovian tradition where the conditioned stimulus (CS) and unconditioned stimulus (US) elicit different responses prior to training. Before conditioning light, the CS does not elicit either of the unconditioned responses (UCRs) that have been studied in *Hermisenda*: muscular foot-shortening and inhibition of forward ciliary locomotion. Stimulation of statocyst hair cells of the graviceptive system using rotation or orbital shaking as the US elicits foot-shortening and inhibition of locomotion or a reduced rate of forward ciliary locomotion. Pavlovian conditioning produces two conditioned responses (CRs): light-elicited inhibition of ciliary locomotion which results in a suppression of *Hermisenda*'s normal positive phototaxis and CS-elicited foot shortening (see **Figure 1**). In both of the CRs, a new response emerges to the CS and the response-evoking properties of the US transfer to the CS. The formation of memory in *Hermisenda* involves changes in both intrinsic cellular excitability and synaptic strength at multiple sites within the neural circuit supporting the generation of CR (see **Figure 2**). The modifications produced by Pavlovian conditioning involve the engagement of multiple cellular mechanisms that are expressed by alterations in the properties of channels in excitable membranes within identified sensory neurons and interneurons. Initial acquisition and long-term retention involve both presynaptic and postsynaptic mechanisms. The actions of several second-messenger pathways contribute to both acquisition and retention of associative memory. Short-term memory involves posttranslational modifications of proteins supported by the activation of several signaling pathways and is expressed by changes in both the strength of synaptic connections and intrinsic excitability. Intermediate-term memory requires translation and post-translational modifications, but not transcription. Long-term memory requires posttranslational modifications, new messenger ribonucleic acid (mRNA) and protein synthesis, structural modifications, and is expressed by long-term changes in intrinsic cellular excitability and the strength of synaptic connections.

Introduction

A central focus of studies on nervous system function is on how basic associations are formed and retained in memory. Pavlovian conditioning paradigms have provided the opportunity to study mechanisms underlying the formation of basic associations in memory at cellular, synaptic, and molecular levels. There is now an extensive database on Pavlovian conditioning generated from diverse species that represents sufficient complexity characteristic of the different forms of memory. The mechanistic analysis of Pavlovian conditioning has been effectively addressed in the less-complex nervous systems of higher invertebrates and has been useful in identifying cellular and molecular mechanisms underlying different stages of memory

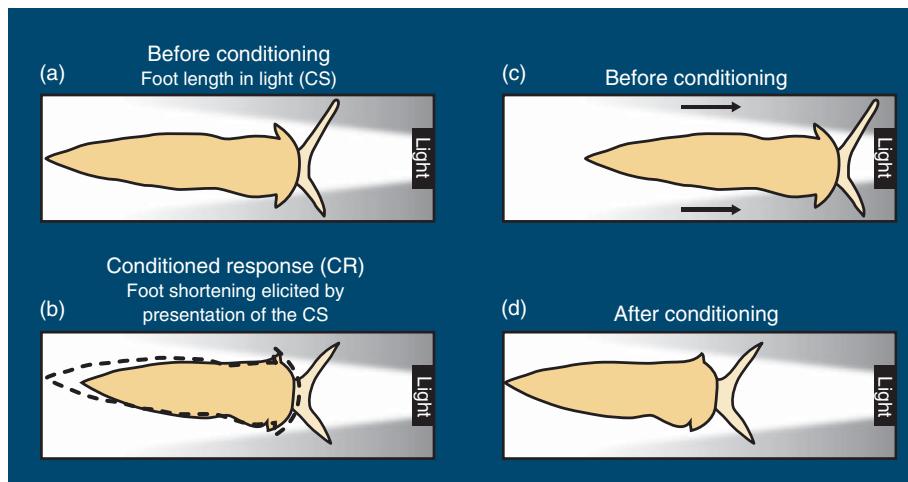


Figure 1 Pavlovian conditioning produces two CRs in *Hermissenda*: foot shortening and inhibition of locomotion. (a) Foot length measured during the presentation of the CS (light) before conditioning; (b) foot-shortening CR elicited by the CS after conditioning. The black dashed line indicates foot length measured during the presentation of the CS before conditioning; and (c) light-elicited locomotion assessed before conditioning; and (d) inhibition of light-elicited locomotion measured after Pavlovian conditioning. Random or pseudorandom presentations of the CS and US do not produce either light-elicited inhibition of locomotion or CS-elicited foot shortening. Adapted with permission from Crow T (2004) Pavlovian conditioning of *Hermissenda*: Current cellular, molecular and circuit perspectives. *Learning and Memory* 11: 229–238.

Cellular and Molecular Mechanisms Underlying Short-, Intermediate-, and Long-Term Memory Formation

Contemporary views of memory and its formation over time indicate that both declarative and nondeclarative (procedural) forms of memory involve multiple stages with different underlying mechanistic requirements. Several *in vivo* and *in vitro* procedures involving one or a few conditioning trials have been used in Pavlovian conditioning studies of *Hermissenda* to examine the initial events supporting the formation of short-, intermediate-, and long-term memory. The different protocols involving one or several conditioning trials produce behavioral changes and physiological modifications that can be detected within minutes following training. *In vitro* Pavlovian conditioning procedures can be applied to the isolated circumesophageal nervous system since the two sensory pathways mediating the CS and US are totally intact in the isolated nervous system. Pairing the CS (light) with either mechanical perturbations of the statocyst or rotation of the isolated nervous system sufficient to depolarize hair cells (US) produces electrophysiological modifications in sensory neurons that are similar to changes produced by multitrial *in vivo* procedures. In addition, a multitrial *in vitro* procedure involving pairing the CS with extrinsic current depolarization of identified statocyst hair cells (nominal US) produces modifications in sensory neurons. The relevance of these investigations to the formation of different stages of memory depends upon the different conditioning protocols, the efficacy of the US,

the number of conditioning trials, and the duration of CS-US stimulation.

One-Trial Conditioning

A one-trial *in vivo* conditioning procedure was developed to more precisely control when initial learning occurs and to not confound time after conditioning, when memory is tested, with different numbers of conditioning trials. One conditioning trial consisting of pairing the CS (light) with the direct application of one of the proposed transmitters of the US pathway (5-hydroxytryptamine (5-HT), nominal US) to the exposed nervous system of the otherwise intact *Hermissenda* produces a long-term inhibition of light-elicited ciliary locomotion (CR). An *in vitro* analog of the one-trial procedure that consists of pairing the CS (light) with the application of 5-HT to the isolated circumesophageal nervous system has been used to examine mechanisms underlying the development of short-, intermediate-, and long-term memory. The *in vitro* analog of one-trial conditioning generates a time-dependent enhancement of intrinsic excitability in sensory neurons and regulates the phosphorylation of proteins associated with memory formation (see the section titled ‘Proteins regulated by Pavlovian conditioning’ below). A variation of the one-trial *in vitro* procedure consisting of pairing the CS with mechanical perturbation of the statocyst (US) also produces a significant Ca^{2+} -dependent increase in input resistance, a correlate of enhanced excitability of sensory neurons, which is expressed within minutes postconditioning. In addition, a one-trial

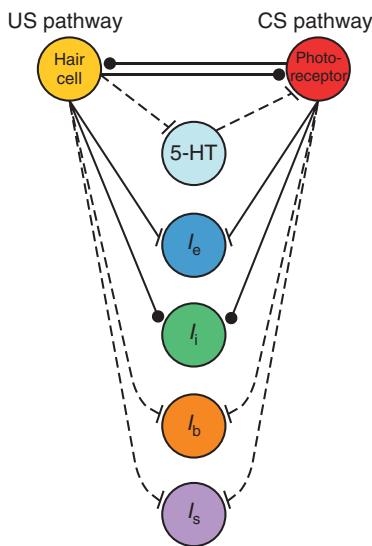


Figure 2 Diagram showing sites of synaptic convergence between identified neurons of the CS and US pathways. The formation of intrinsic enhanced cellular excitability and changes in the strength of synaptic connections between sensory neurons (photoreceptors) and sensory neurons and type I interneurons support the generation of light-evoked inhibition of locomotion. Proposed modifications in type I_b and I_s interneurons may also contribute to the development of the foot-shortening CR. Statocyst hair cells form monosynaptic GABAergic and putative polysynaptic serotonergic projections to identified sensory neurons (photoreceptors). Caudal hair cells inhibit photoreceptors and cephalic hair cells are inhibited by type B photoreceptors. Hair cells of the graviceptive system and photoreceptors of the visual system form monosynaptic connections with identified type I interneurons and polysynaptic connections with identified type I_b and I_s interneurons. (●) Inhibitory synaptic connections; (↑) excitatory synaptic connections. The neural circuit shows solid lines that represent established monosynaptic connections and dashed lines indicating polysynaptic connections to identified I_b and I_s interneurons that may involve potential interneurons not yet identified.

in vitro procedure involving gamma aminobutyric acid (GABA) application to the region of the sensory neuron terminal branches (nominal US) paired with a 10-s depolarization of the sensory neuron (nominal CS) produces an increase in the input resistance of the sensory neurons that persists for at least 10 min. These studies indicate that the application of a neurotransmitter (nominal US) when paired with depolarization (nominal CS), resulting in a brief period of Ca^{2+} elevation, is sufficient to produce short-term enhanced excitability in sensory neurons.

Multitrial Conditioning

The analysis of electrophysiological and biophysical modifications detected following multitrial Pavlovian conditioning has focused upon two sites of convergence

between the CS and US pathways (see **Figure 2**). The first site is in the sensory neurons (type A and B photoreceptors) of the pathway mediating the CS. Modifications in the sensory neurons of conditioned animals involve both intrinsic enhanced excitability and facilitation of synaptic connections between identified sensory neurons, and between identified sensory neurons and interneurons. The intrinsic modifications in sensory neurons are expressed by enhancement of the amplitude of CS-elicited generator potentials, a concomitant increase in CS-elicited spike frequency, increased spike activity evoked by depolarizing extrinsic current, decreased spike frequency accommodation, and a reduction in the peak amplitude of voltage-dependent (I_A, I_{Ca}) and Ca^{2+} -dependent ($I_{\text{K(Ca)}}$) membrane currents.

The increase in the amplitude of CS-elicited generator potentials is, in part, the result of a reduction in both I_A and $I_{\text{K(Ca)}}$. In sensory neurons of conditioned animals, the peak amplitude of I_A is significantly reduced and exhibits more rapid inactivation as compared to controls. However, both the delayed rectifier (I_K) and inward rectifier (I_h) may also play a role in conditioning-dependent enhanced excitability. The application of 5-HT to the isolated nervous system enhances the peak amplitude of I_h and decreases the peak amplitude of I_K and I_A in sensory neurons. In addition, 5-HT reduces the amplitude of $I_{\text{K(Ca)}}$ and decreases I_{Ca} in sensory neurons. The reduction in $I_{\text{K(Ca)}}$ produced by 5-HT is a consequence of the decrease in I_{Ca} by 5-HT rather than a direct effect of 5-HT on $I_{\text{K(Ca)}}$. In addition to intrinsic enhanced excitability in sensory neurons produced by multitrial conditioning, changes in the strength of synaptic connections between identified sensory neurons and interneurons occur following conditioning. The amplitude of the monosynaptic inhibitory postsynaptic potential (IPSP) between the medial type B photoreceptor and medial type A photoreceptor is significantly enhanced in conditioned animals. The second convergence site between the CS and US pathways is the monosynaptic connection between identified sensory neurons and type I interneurons (**Figure 2**). Multitrial conditioning produces facilitation of monosynaptic and complex PSPs in identified type I_e and I_i interneurons. In addition to conditioning-dependent synaptic facilitation, type I interneurons also express intrinsic enhanced excitability with conditioning. Extrinsic current pulses elicit significantly more spikes in type I_e interneurons of conditioned animals as compared to pseudorandom controls. Therefore, long-term memory in *Hermisenda* involves both presynaptic and postsynaptic modifications. The enhanced excitability of sensory neurons, expressed by an increase in both the amplitude of CS-elicited generator potentials and the number of action potentials elicited by the CS, may be a major contributor to changes in the duration and amplitude of CS-elicited complex PSPs and

increased CS-elicited spike activity in type I interneurons of conditioned animals. However, facilitation of the amplitude of the monosynaptic IPSP between sensory neurons and type I_i interneurons, and the monosynaptic EPSP between sensory neurons and type I_e interneurons of conditioned animals may involve both pre- and post-synaptic mechanisms.

Second Messengers

Studies of diverse species have shown that many different second-messenger systems are activated by procedures that produce cellular and synaptic plasticity. Multiple pathways exhibit convergence and divergence when activated and specific second-messenger cascades express both positive and negative regulation by cross talk with other pathways. Therefore, it is unlikely that a single second-messenger supports the formation of memory in the nervous system.

Protein Kinase C

Protein kinase C (PKC) activation contributes to enhanced excitability and synaptic facilitation underlying the formation of short- and long-term memory in *Hermissenda*. PKC is translocated from the cytosol to the membrane in the nervous system of *Hermissenda* by treatment with a phorbol ester (12-O-tetradecanoylphorbol-13-acetate (TPA)). The bath application of a phorbol ester (phorbol 12,13-dibutyrate (PDB)), or injection of PKC into sensory neurons, results in a reduction in the peak amplitude of two K^+ currents contributing to enhanced excitability, I_A and $I_{K,(Ca)}$.

PKC activation contributes to the development of cellular and synaptic modifications supporting both of the CRs that have been examined with Pavlovian conditioning (see **Figure 1**). Nine conditioning trials produce a foot-shortening CR elicited by the CS that is detected within minutes after the last conditioning trial. An *in vitro* conditioning procedure consisting of nine training trials of the CS paired with rotation of the isolated nervous system (US) enhances sensory neurons excitability. The *in vitro* conditioning-dependent change in the excitability of sensory neurons is blocked by the broad-spectrum kinase inhibitor H-7. A second *in vitro* procedure involving pairing the CS with extrinsic current depolarization of the sensory neurons (nominal US) produces enhanced excitability which is also blocked by the preconditioning application of the kinase inhibitors H-7 or sphingosine. One-trial *in vivo* conditioning consisting of pairing the CS with the application of 5-HT to the exposed, but otherwise intact, nervous system produces short-, intermediate-, and long-term enhanced excitability of sensory neurons. The induction of short-term enhanced excitability following one-trial conditioning is blocked by

H-7 and sphingosine, and by the downregulation of PKC produced by pretreatment with TPA. However, while H-7, sphingosine, or the downregulation of PKC by TPA blocks short-term enhanced excitability, the same treatments do not block long-term enhanced excitability produced by one-trial conditioning. The expression of long-term memory produced by one-trial conditioning does not depend upon the induction of short-term memory. Therefore short- and long-term enhanced excitability produced by one-trial *in vivo* conditioning involves independent or parallel processes and differential contributions of second messengers. Consistent with previous studies, the induction of enhanced excitability in sensory neurons produced by five *in vitro* conditioning trials involving the CS paired with depolarizing current stimulation of a statocyst hair cell is blocked by pretreatment with PKC inhibitors. However, the contribution of PKC to the expression of long-term enhanced excitability depends upon the conditioning protocol and the number of conditioning trials. Previously established long-term enhanced excitability produced by one-trial *in vivo* conditioning is not reversed by the broad spectrum kinase inhibitor H-7, or the PKC inhibitors sphingosine or staurosporine. In contrast, long-term enhanced excitability in sensory neurons produced by multitrail Pavlovian conditioning is attenuated by H-7, or sphingosine, suggesting that it is dependent upon persistent kinase activity. Moreover, injection of the PKC inhibitor peptide PKC(19–36) into sensory neurons 24–48 h following multitrail conditioning reverses enhanced excitability within 16 min postinjection, suggesting that either a long-lived activator or a constitutively active kinase contributes to the expression of enhanced excitability. Injection of the control noninhibitory peptide [glu²⁷] PKC(19–36) does not reverse enhanced excitability in the sensory neurons of conditioned animals. PKC activation also contributes to the induction of 5-HT-dependent synaptic facilitation, but persistent PKC activity is not required for the maintenance of long-term synaptic facilitation. Short-term synaptic facilitation of the connection between identified sensory neurons (type B and type A photoreceptors) is produced by the bath application of 5-HT. Injection of the PKC inhibitor peptide PKC(19–36) into medial type B photoreceptors blocks 5-HT-induced short-term synaptic facilitation of the IPSP recorded in the medial type A photoreceptor. However, injection of PKC(19–36) into medial type B photoreceptors following multitrail Pavlovian conditioning does not reduce or reverse established synaptic facilitation of the IPSP recorded in the medial type A photoreceptors.

Extracellular Signal-Regulated Protein Kinase

One trial *in vitro* conditioning of the isolated nervous system involving the CS paired with 5-HT results in the

increased ^{32}P labeling of a protein with an apparent molecular weight consistent with extracellular signal-regulated kinase (ERK). The increased phosphorylation of the protein following one-trial conditioning is blocked by pretreatment with the map kinase kinase (MEK1) inhibitor PD098059. Assays of ERK activity with the brain myelin basic protein as a substrate show greater ERK activity for nervous systems from one-trial *in vitro* conditioned animals as compared to unpaired controls. In addition, western blot analysis of phosphorylated ERK with a phospho ERK antibody showed a significant increase in ERK phosphorylation after one-trial conditioning as compared with unpaired controls. The increased phosphorylation is blocked by pretreatment with a MEK1 inhibitor PD098059. Following a multitrial conditioning procedure consisting of 10–15 trials, nervous systems from conditioned animals exhibited significantly greater ERK phosphorylation as compared with pseudorandom controls. PKC contributes to the 5-HT-dependent activation of the ERK pathway. The phorbol ester TPA increases ERK phosphorylation that is blocked by pretreatment with PKC inhibitors. TPA-dependent ERK phosphorylation is also blocked by the MEK1 inhibitors PD0988059 or U0126. The increased phosphorylation of ERK by 5-HT is attenuated, but not blocked, by pretreatment with the Ca^{2+} chelator 1,2-bis-(*o*-Aminophenoxy)-ethane-N,N,N',N'-tetracetic acid, tetraacetoxy methyl ester (BAPTA-AM) or pretreatment with PKC inhibitors Gö6976 or GF109203X. This suggests that Ca^{2+} -dependent PKC activation contributes to ERK phosphorylation, although a PKC-independent pathway also contributes to 5-HT-dependent ERK phosphorylation.

Ca^{2+}

The sensory neurons (photoreceptors) in the eyes of *Hermisenda* exhibit a spatial segregation of function. Phototransduction takes place in the apical region where the rhabdomere abuts the lens and spike generation occurs near the distal end of the axon close to the location of synapses on the terminal processes. Therefore, the CS (light) and the US (rotation) have spatially separated physiological consequences in photoreceptors. Both light and depolarization increase cytosolic Ca^{2+} levels in photoreceptors. Light activates phospholipase C (PLC) to produce an increase in inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 opens rhabdomeric Na^+ and Ca^{2+} channels which result in a depolarizing generator potential and Ca^{2+} influx. IP_3 also binds to its receptor (IP_3R), which triggers Ca^{2+} release from the endoplasmic reticulum (ER). The Ca^{2+} influx from the rhabdomere and the IP_3R -gated storage compartment can cause Ca^{2+} release from the ryanodine receptor (RyR)-gated compartment. Rotation (US) produces a depolarizing generator potential in identified statocyst hair cells and

elicits a monosynaptic GABAergic IPSP in the sensory neurons. The US is also proposed to activate a polysynaptic serotonergic pathway that projects to the sensory neurons (see Figure 2). Both 5-HT and GABA are linked to a pertussis-toxin sensitive G protein. These proteins can activate second-messenger systems that contribute to memory formation underlying one- and multitrial classical conditioning (see Figure 3). The induction of 5-HT-dependent enhanced excitability in sensory neurons is Ca^{2+} dependent since BAPTA loading before 5-HT application blocks the induction of enhanced excitability. In addition, 5-HT has been shown to modulate several membrane conductances contributing to enhanced excitability in sensory neurons (see ‘Multitrial conditioning’). With regard to the second neurotransmitter in the US pathway, it is proposed that GABA binding to G-protein-coupled receptors on sensory neurons activates phospholipase A₂ (PLA₂) to liberate arachidonic acid (AA) that interacts with Ca^{2+} to synergistically stimulate PKC and create a back-propagating wave of Ca^{2+} released from intracellular stores. When the CS and US are repeatedly paired, the Ca^{2+} influx due to light, IP_3R stores, RyR stores, and voltage-gated Ca^{2+} channels sums together. The large increase in cytosolic Ca^{2+} combined with DAG and AA acts to synergistically activate PKC by translocating it to the membrane. Each pairing of the CS and US has been proposed to incrementally increase the proportion of PKC translocated to the membrane that would contribute to the phosphorylation of K^+ channels.

Long-Term Memory Depends upon Translation and Transcription

Mechanistic differences between short- and long-term enhanced excitability are illustrated by studies showing that the inhibition of protein synthesis during one-trial *in vivo* conditioning blocks long-term enhanced excitability without affecting the induction or expression of short-term enhanced excitability. Moreover, long-term enhanced excitability produced by one-trial conditioning is blocked by the inhibition of mRNA synthesis which does not affect the induction of short-term enhanced excitability. This indicates that long-term memory following one-trial *in vivo* conditioning is dependent upon both translation and transcription.

The time-dependent development of enhanced excitability in sensory neurons following one-trial conditioning is biphasic; enhancement reaches a peak at 3 h postconditioning, decreases toward baseline control levels at 5–6 h, and increases to a plateau at 16–24 h postconditioning. Enhanced excitability following one-trial conditioning also involves an intermediate phase of memory consolidation that requires protein synthesis, but not mRNA synthesis.

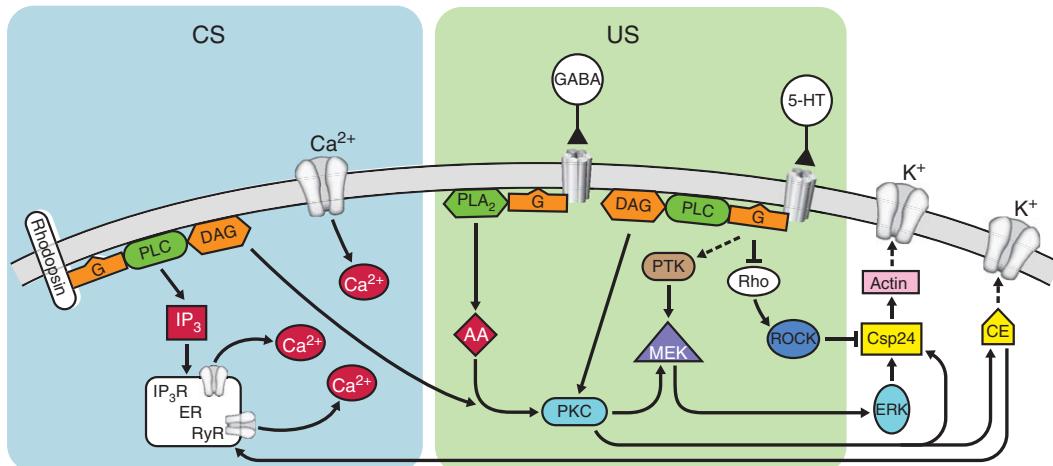


Figure 3 Mechanisms of memory formation produced by Pavlovian conditioning in *Hermisenda*. Acquisition involves the interaction of Ca^{2+} with second-messenger pathways regulated by neurotransmitter release in the US pathway. Light (CS) activates phospholipase C (PLC) to produce and increase inositol triphosphate (IP_3) and diacylglycerol (DAG). The depolarizing generator potential and IP_3 effects on endoplasmic reticulum (ER) result in an increase in intracellular Ca^{2+} . Transmitters in the US pathway bind to G-protein-coupled receptors (G) to activate phospholipase A₂ (PLA₂); increase arachidonic acid (AA); and activate protein kinase C (PKC), nonreceptor protein tyrosine kinases (PTK), extracellular signal-regulated kinase (ERK), and the Rho/ROCK pathway. The PKC pathway can couple directly to Raf that leads to the phosphorylation and activation of MEK and ERK. Two phosphoproteins that have been characterized are regulated by the second-messenger pathways. Csp24 phosphorylation is regulated by PKC, ERK, and the Rho/ROCK pathway. CE phosphorylation is regulated by the PKC pathway. Csp24 binds actin and may support cytoskeletal reorganization underlying the formation of long-term memory in sensory neurons and interneurons. Enhanced excitability is a consequence of the long-term modification of K^+ channels (dashed lines) by CE and Csp24. Adapted with permission from Crow et al. (2008) Molecular mechanisms of associative learning in *Hermisenda*. In: David Sweatt J (ed.) *Molecular Mechanisms of Memory*, vol. 4, pp. 119–132. Oxford: Elsevier, copyright 2008 by Elsevier.

The conditioned foot contraction CR is expressed at a retention interval of 5 min following two or nine conditioning trials. However, nine conditioning trials are required for 90-min retention. *In vivo* incubation of animals with the protein synthesis inhibitor anisomycin during conditioning does not affect the expression of the CR at the 5-min retention interval, but attenuates conditioning assessed at the 90-min interval for the group that received nine conditioning trials. A protocol involving *in vitro* conditioning of the isolated nervous system produces similar results to the effects of anisomycin on conditioned behavior. Two conditioning trials produce a short-term protein synthesis-independent increase in excitability that decrements within 45 min and nine conditioning trials produces a persistent protein synthesis-dependent increase in sensory neuron excitability detected at 90 min. Applying anisomycin 5 min after the ninth conditioning trial does not affect the retention of enhanced excitability.

However, under some experimental conditions, protein synthesis occurring during or shortly after learning is not necessary for the formation of long-term memory. PKC activation produced by a potent agonist of PKC, bryostatin, on days before conditioning leads to the expression of proteins that can support long-term

memory produced by later Pavlovian conditioning. Two conditioning trials typically result in a short-term (~7 min) foot-shortening CR. A 4-h exposure to bryostatin on 2 days preceding conditioning results in a long-term (>1 week) CR produced by two conditioning trials that is not blocked by anisomycin.

Proteins Regulated by Pavlovian Conditioning

Different *in vivo* and *in vitro* conditioning protocols have been used to study proteins regulated by conditioning. Multitrial conditioning produces posttranslational modifications in a number of proteins; however, only a few of the full-length complementary deoxyribonucleic acids (cDNAs) have been cloned and the phosphoproteins fully characterized. Calexcitin (CE) is a GTP- and Ca^{2+} -binding protein found in *Hermisenda* photoreceptors. CE is activated by elevated Ca^{2+} and binds to the RyR to increase cytosolic Ca^{2+} concentrations. CE is phosphorylated by PKC, which results in the translocation of CE to membrane compartments, where it modulates K^+ currents. Phosphorylation of CE also results in binding to the Ca^{2+} -ATPase transporter to increase the rate of Ca^{2+} removal from the cytosol.

Multitrial conditioning increases the phosphorylation of CE, and increases CE in B photoreceptors, specifically in Ca^{2+} sequestering organelles such as ER and within mitochondria and photopigments. The increased CE levels in B photoreceptors of conditioned animals result in increased excitability via K^+ -channel inactivation and internal Ca^{2+} release from ER due to increased CE binding to RyRs.

One- and multitrial conditioning regulate other proteins found in the CS pathway and nervous system. The phosphorylation of CS pathway protein 24 (Csp24) is regulated by Pavlovian conditioning and contributes to the formation of both intermediate- and long-term memory. One- and multitrial conditioning result in a significant increase in the phosphorylation of Csp24 that can be detected at times associated with the intermediate- and long-term phase of memory formation, but not the short-term phase. Modifying the efficacy of the US by reducing the concentration of 5-HT used in one-trial conditioning produces a short-term (<1 h) associative enhancement of sensory neuron excitability that does not result in phosphorylation of Csp24. Csp24 is a cytoskeleton-related protein that is homologous to members of the family of multirepeat β -thymosins that contain multiple actin-binding domains. Actin co-immunoprecipitates with Csp24 and is co-localized with Csp24 in the cytosol of sensory neuron cell bodies. In addition, recombinant Csp24 binds to and sequesters G actin *in vitro*, and phosphorylation of Csp24 by one-trial *in vitro* conditioning increases the co-immunoprecipitation of actin with anti-Csp24. Mass spectrometric analysis of Csp24 has identified phosphorylation sites at Ser-49 and Ser-122. One-trial *in vitro* conditioning results in an increase in the phosphorylation of Ser-122, but not Ser-49 of Csp24. Several signaling pathways regulate Csp24 phosphorylation; thus, it can integrate a number of signals that contribute to cytoskeletal remodeling. Inhibitors of PKC and MEK1 reduce Csp24 phosphorylation produced by *in vitro* conditioning. In addition to PKC and ERK regulation of Csp24, Rho GTPase activity and its downstream target ROCK contribute to the posttranslational regulation Csp24 through an inhibitory pathway. The ROCK inhibitor Y-27632 significantly increases Csp24 phosphorylation and the Rho activator lysophosphatidic acid decreases Csp24 phosphorylation. In addition, the application of 5-HT to the isolated nervous system decreases Rho activity and increases the phosphorylation of Csp24. The inhibition of cyclin-dependent kinase 5 by butyrolactone also reduces Csp24 phosphorylation. The incubation of isolated *Hermisenda* nervous systems with *Csp* antisense oligonucleotides decreases Csp24 expression, and treatment with antisense oligonucleotides before one-trial *in vitro* conditioning blocks intermediate-term enhanced excitability without affecting the induction of short-term

enhanced excitability. Since Csp24 is associated with the actin cytoskeleton, its regulation by conditioning may influence K^+ -channel activity by the spatial and temporal control of actin dynamics. One-trial *in vitro* conditioning of isolated sensory neurons produces a significant reduction in the amplitude of I_A and a depolarized shift in the steady-state activation curve of I_A without altering the inactivation curve. The conditioning-dependent changes in I_A are blocked by incubation of the isolated sensory neurons with *Csp* antisense oligonucleotide. Therefore, Csp24 contributes to the regulation of voltage-gated channels associated with the development of intrinsic enhanced excitability underlying Pavlovian conditioning in *Hermisenda*.

Overview and Discussion

Pavlovian conditioning in *Hermisenda* results in both intrinsic enhanced cellular excitability and modifications in the strength of synaptic connections at multiple sites within the neural circuit responsible for the generation of the CR. The first site of storage for the memory of the associate experience is in the sensory neurons of the CS pathway. The modifications in the sensory neurons are spatially segregated. There are alterations in the properties of K^+ channels in the soma that result in an enhancement of the amplitude of the CS-elicited generator potential, a change in the biophysical properties of channels in the spike-generating zone that results in a decrease in spike-frequency accommodation, and a concomitant enhancement of intrinsic excitability. In addition, changes in synaptic strength result in facilitation of the monosynaptic connections between identified sensory neurons (type B and type A photoreceptors) and between identified sensory neurons and interneurons. Memory produced by Pavlovian conditioning involves both pre- and postsynaptic mechanisms since the second site of memory formation and storage is in type I interneurons.

Conventional mechanistic views of memory formation have proposed that (1) the short-term phase involves posttranslational modifications of existing cellular proteins; (2) the intermediate phase is dependent on translation, but not transcription; and (3) the long-term phase is dependent upon protein synthesis, gene transcription, and the synthesis of new mRNA. Collectively, short-, intermediate-, and long-term memory produced by Pavlovian conditioning in *Hermisenda* involves the activation of several second-messenger cascades, post-translational modification of proteins, the synthesis of proteins, transcription, and the synthesis of mRNA. However, increased phosphorylation of proteins associated with the different phases of memory may occur several hours or 24 h postconditioning. This suggests that

posttranslational modifications may contribute to the maintenance or expression of translation-transcription-dependent long-term memory. The activation of several signaling cascades that express cross talk and convergent interactions contributes to all phases of memory formation. Second-messenger pathways may exhibit differential contributions to memory formation involving enhanced excitability and changes in the strength of synaptic connections. Therefore, the mechanism(s) for intrinsic enhanced excitability may be different from the mechanisms supporting modifications in the strength of synaptic connections.

Two proteins that have been fully characterized, and are regulated by Pavlovian conditioning, are CE and Csp24. The binding of CE to the plasma membrane decreases K⁺ conductances and releases Ca²⁺ from internal stores. Csp24 phosphorylation is regulated by one and multi-trial conditioning, is associated with actin, and contributes to long-term intrinsic enhanced excitability produced by the depolarized shift in the steady-state activation of I_A and the concomitant reduction in peak I_A. Therefore, the expression of Csp24 is important in both intermediate- and long-term memory involving intrinsic enhanced excitability.

The analysis of mechanisms of memory in *Hermissenda* raises a number of questions that are important for an understanding of memory produced by Pavlovian conditioning. How are posttranslational modifications in proteins supporting short-term memory transformed into long-term memory involving both intrinsic enhanced excitability and changes in the strength of synaptic connections? What are the contributions of pre- and postsynaptic modifications to short-, intermediate-, and long-term memory? How does the regulation of CE and Csp24 by conditioning result in an alteration in the properties of K⁺ channels in excitable membranes? Finally, how are modifications in intrinsic excitability and synaptic strength at several loci integrated within a neural circuit to reconfigure the circuit and support the generation of the CR?

See also: Animal Models of Learning and Memory; Learning and Memory: Computational Models; Memory Consolidation; Memory in *Caenorhabditis elegans*; Memory in the Honeybee; Neural Basis of Classical Conditioning; Neuron Excitability and Learning; Neurotransmitters and Neuromodulators Regulating Sleep and Wakefulness; Protein Synthesis and Memory;

Short-Term Memory: Psychological and Neural Aspects; Synaptic Mechanisms for Encoding Memory; Synapse Formation and Memory.

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Protein Synthesis and Memory

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Glossary

Anisomycin (ANI) – A protein-synthesis inhibitor that has been widely used in studies of learning and memory. Anisomycin prevents translation of new protein by interfering with ribosomal subunits. This drug also has several important side effects that are not directly related to inhibition of translation.

Consolidation – It generally refers to the time-dependent process through which new memories become increasingly resistant to disruption. At the cellular level, consolidation describes the development of stable synaptic plasticity which persists indefinitely in the absence of stimulation. At the systems level, consolidation can refer to the time-limited role of certain structures such as the hippocampus as a memory ‘matures.’

Immediate early gene (IEG) – These genes are rapidly activated by stimulation and contribute to multiple cellular responses including transcriptional regulation during the formation of memory. Because they are often strongly expressed in active neurons, they can also serve as a cellular marker of recent neural activity.

Reconsolidation – When a stable long-term memory is recalled it will sometimes require *de novo* protein synthesis to persist normally after retrieval. Evidence for reconsolidation comes from the observation that post-recall administration of protein-synthesis inhibitors can selectively disrupt memory at subsequent tests.

Transcription – The process through which genetic information contained in DNA in a cell’s nucleus is copied onto a complementary strand of messenger RNA (mRNA). The mRNA strands contain the information required to make a specific protein.

Translation – The process through which amino acids are assembled into proteins. Translation occurs in the cytoplasm within ribosomes and is controlled by information contained in mRNA.

system. While this conclusion is logical, the huge number of possible underlying cellular and molecular mechanisms of change provide a real challenge to any complete understanding. Consequently, researchers have tended to look for classes of cellular events that might serve as mechanisms for synaptic plasticity and memory storage.

As our understanding of how genetic information is encoded and used within cells increased during the mid-twentieth century, the idea that newly synthesized protein might be important for forming specific ‘memory traces’ gained currency. Protein synthesis triggered by experience could provide new raw material or modify the regulation of a number of processes that determine how the functional connections between neurons can be changed.

Early studies using systemic injections of drugs that interfere with the synthesis of protein – mostly by preventing its translation from messenger RNA – supported the importance of new protein in memory formation. Generally speaking, applying broad-spectrum translation inhibitors like cycloheximide or anisomycin (ANI) around the time of training would result in severely impaired behavioral retention during a drug-free test of long-term memory. Furthermore, memory impairments were sometimes seen even when the drug was given immediately after the training experience making it much less likely that sensory/motor impairments or other unintended consequences of drug administration were responsible for the apparent effects on memory. Importantly, the effects of protein-synthesis inhibitors tended to develop only when testing of the memory was temporally separated from the training session. These drugs tended not to affect short-term forms of plasticity.

These early studies on protein synthesis and memory were not without significant confounds and conflicting results. Not surprisingly, given that protein synthesis is such a critical process for many aspects in cell biology, injections of large systemic doses of translation inhibitors would be expected to have multiple important effects on an organism beyond any direct influence on activity-dependent synaptic plasticity. Nonetheless, the overall conclusion seemed to be that *de novo* protein synthesis is important for new learning to occur.

Background and Early Studies

Scientists interested in understanding the biological substrates of memory have traditionally understood that the formation of long-lasting memories must somehow involve modification of the physical properties of the nervous

Current Working Model

More recent experimental work in the neurobiology of memory has continued to support the basic idea that *de novo* protein synthesis is a required step in the formation

of new memories. The thinking here, as well as the general approach used to investigate these issues in whole animal behavioral studies, relies heavily on pioneering work in reduced preparations like the *Aplysia* abdominal ganglion and hippocampal slice long-term potentiation (LTP) showing that short- and long-term plasticity can be separated based on the requirement for macromolecular synthesis. A very general framework linking the exposure of an organism to some environmental event to the protein-synthesis-dependent modification of synapses is given in **Figure 1**. The event to be remembered generates increased activity within populations of cells within neural circuits appropriate to the particular stimuli being used. This increased neural activity gives rise to alterations in intracellular calcium ion (Ca^{2+}) and initiation of several classes of Ca^{2+} -dependent signaling mechanisms. Some of these result in posttranslational modifications responsible for short-term changes in synaptic excitability while other consequences of elevated Ca^{2+} include the activation of transcription factors which promote the synthesis of new messenger RNA (mRNA) which interacts with ribosomal proteins outside the nucleus to enable the translation of new target protein. These new proteins are thought to make a critical contribution to synaptic plasticity by functioning as structural proteins, transcription factors, biosynthetic enzymes, ion channels, neurotransmitter receptors, etc. The creation and stabilization of memory is thus a time-dependent process that relies on a series of related intracellular events. Preventing any step in the sequence would be expected to impact the behavioral evidence for memory.

It is also important to note that protein synthesis is always occurring, even in the absence of the type of stimulation that forms a new memory. Proteins are constantly being ‘turned over’ – broken down and replaced – at synapses and elsewhere in the cell. Many manipulations applied to the question of protein synthesis and memory can affect constitutive as well as learning-evoked protein expression.

Memory and Alterations in Gene Expression

One general prediction of this model is that when animals are in the process of forming a new memory, one should be able to find new protein within appropriate brain areas. More specifically, the identity and/or quantity of these gene products should specifically relate to the learning process and not be similar in animals exposed to similar stimuli in control conditions that do not result in learning. A large number of studies conducted over the last several years have supported this basic idea.

The first studies of this type tended to focus on immediate early genes (IEGs) whose expression had previously been shown to be rapidly and strongly induced by seizure activity or intense electrical stimulation. Many IEGs function as transcriptional regulators while others can have more direct effects on plasticity through interactions with other proteins that regulate synaptic physiology (such as glutamate receptors). Other IEGs have a less well-described role in learning or plasticity

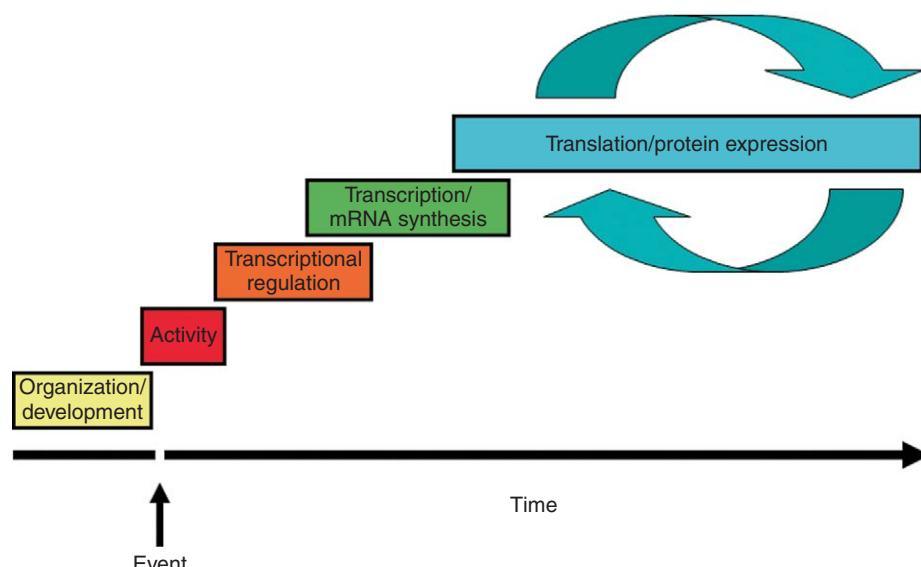


Figure 1 The formation of memory is a time-dependent process that includes multiple steps. Short-term memory and neural plasticity can rely on rapidly induced activity-dependent posttranslational modifications at synapses. Long-term memory, on the other hand, normally requires the transcription of new mRNA and the translation of these messages into new protein. Preventing either transcription or translation often results in a selective deficit in long-term memory.

per se but are often still useful as a cellular marker of neural activity.

Zif268/Egr1 is a mammalian zinc-finger transcription factor that regulates the expression of several groups of target genes and has been closely linked to learning and memory in rodents. Exposing rats or mice to standard behavioral tasks in which new memory is formed often results in increased levels of Zif268 mRNA as well as increased expression of the protein itself within brain regions known to be important for the particular task being used (e.g., the hippocampus for spatial memory or the amygdala for Pavlovian fear conditioning). Importantly, if one prevents the translation of Zif268 protein by introducing antisense oligonucleotides to critical brain areas around the time of learning this can disrupt behavioral performance in a manner consistent with a critical role for Zif-regulated proteins in memory formation. Similarly, mice lacking the Zif268 gene are selectively impaired on tests of long-term memory while general behavioral competence and tests of short-term memory appear intact. Complementary data from *in vivo* LTP studies in the hippocampus show that Zif268 may be critical for long-term (i.e., days) changes in synaptic efficacy but not baseline synaptic transmission or for short-term changes thought to require posttranslational modifications of existing proteins.

Another IEG that is closely linked to memory is the activity-regulated cytoskeletal associated protein (Arc/Arg3.1). Arc mRNA is very rapidly expressed in hippocampal neurons around the time animals are learning. A series of elegant experiments conducted by John Guzowski and his colleagues showed that Arc mRNA is induced by behavioral experience (e.g., exposure to a novel environment) in the cell's nucleus almost immediately, but after about 20 min is largely localized in the surrounding cytoplasm. This pattern of mRNA expression allowed Guzowski to identify individual hippocampal neurons that were active at two different time points in the same animal. Subsequent work by several groups has focused on how Arc protein resulting from this mRNA contributes to long-term neural plasticity and memory.

At the protein level, Arc expression also changes in a time-dependent manner in the hippocampus after learning. **Figure 2** shows the results of an experiment in which rats were exposed to a novel environment that was paired with mild shock. Animals will remember this experience and behave accordingly when tested the next day, and prior studies have shown that plasticity in hippocampal neurons is required for this form of memory. The amount of Arc protein in the dorsal hippocampus increases during the period in which the memory is consolidated and peaks at approximately 90 min after training. Other studies have shown that preventing the translation of Arc mRNA can disrupt the consolidation of some forms of memory and

that Arc protein can play an important role in regulating the function of α -amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA)-type glutamate receptors at synapses.

A number of other converging lines of evidence support the idea that new protein is being produced in neurons during the process of forming new long-term memories. The transcription factor cAMP-dependent-element binding protein (CREB) influences the expression of multiple genes related to neural plasticity. Behavioral training in several different memory tasks results in the time-dependent phosphorylation of CREB. At the level of translational control, recent evidence indicates that learning results in the activation of various enzymes that facilitate the synthesis of new protein from existing mRNAs. One example of this is the mammalian target of rapamycin (mTOR) kinase which, when activated, increases the rate at which new proteins are translated at ribosomes. **Figure 3** shows the phosphorylation of p70s6k, a downstream target of mTOR, in rat lateral amygdala neurons 1 h after training in a fear-conditioning experiment. Animals given a control treatment that did not result in new learning did not show increased translation of new protein.

Current technology offers many options for the quantification and localization of gene-expression changes related to learning and memory. One major conceptual advance has been the increased understanding that altered protein synthesis – and by inference altered synaptic plasticity – can occur throughout multiple brain regions around the time of learning even when a restricted number of structures may be considered essential for learning or behavioral performance to occur. Of course, our ability to observe changes in gene expression as they consistently co-vary with learning tells us little about the degree to which these proteins are actually required for the formation of new memories.

Manipulating Protein Synthesis *in vivo*

There are a variety of interventional strategies that more directly address the issue of whether or not a given protein or regulatory pathway is required for normal learning to occur. Transgenic approaches allow for the conditional deletion of specific genes or the targeted overexpression of selected gene products. At the transcriptional level, the literature on CREB contains numerous examples of converging approaches with multiple species and behavioral endpoints all supporting a general role for this molecule in the formation of long-term memory. While other similar examples of the systematic analysis of single molecules exist, scientists concerned with the more general question of whether or

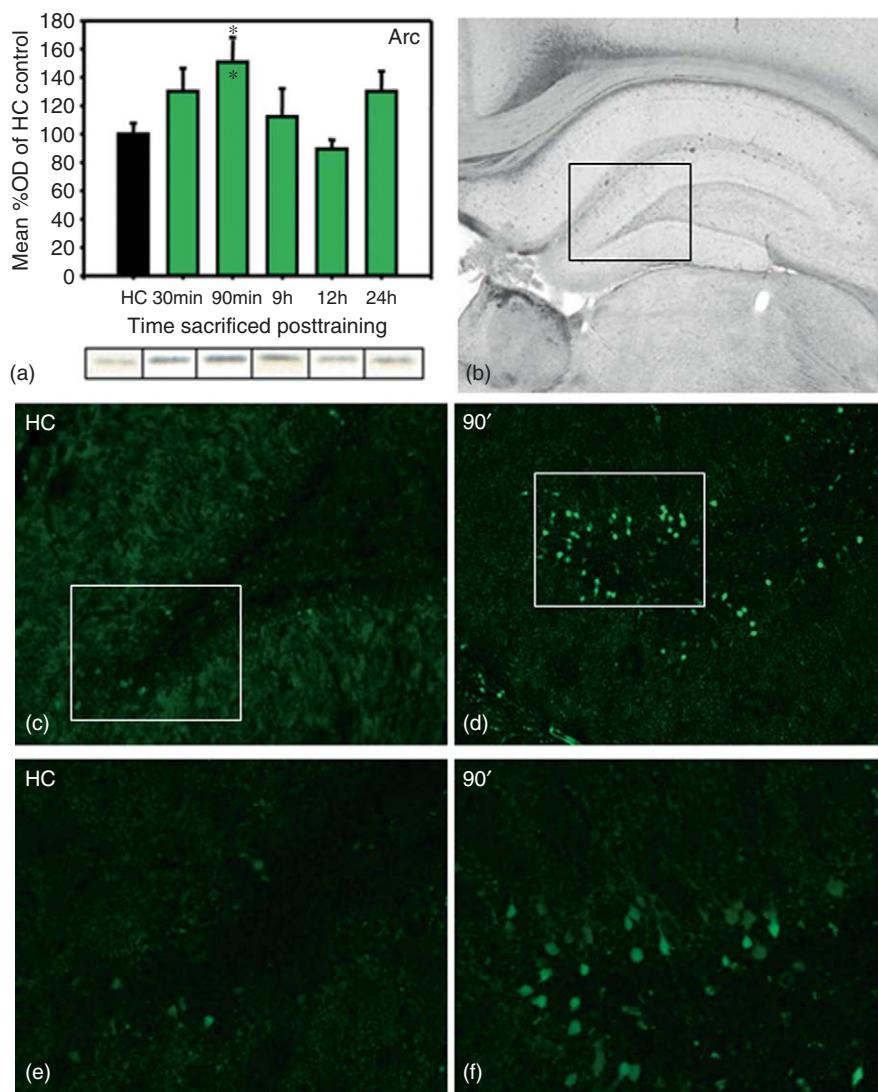


Figure 2 Immediate early genes (IEGs) were some of the first proteins to be studied during memory formation because they are rapidly induced by training and expressed at fairly high levels within key brain regions. In this experiment rats were trained by pairing a distinctive environment with shock and levels of the IEG Arc were measured. (a) Arc-protein levels in the hippocampus as measured by Western blots peaked at 90 min after training. (b–f) Arc-positive cell bodies were seen in many regions of the hippocampus including the dentate gyrus. HC = homecage control; 90' = tissue processed 90 min after training. Data are taken from a recent study by Mary Lonergan.

not new memory requires new protein have tended to employ more general manipulations.

A trend in recent behavioral studies has been to apply broad-spectrum macromolecular synthesis-inhibitors locally to target brain areas that are selected based on systems-level analysis within a particular learning paradigm. These drugs may target transcription, interfering with the synthesis of mRNA at one of several steps in the process, or may target protein translation from intact message. Using this general approach, a very large number of papers have been published all lending support to the idea that new protein is important for new learning. This conclusion appears to hold for multiple vertebrate

and invertebrate species as well as for multiple forms of memory including taste aversion, eye blink conditioning, spatial learning, fear conditioning, avoidance learning, conditioned drug seeking, and others.

Of course, using a rather nonspecific translation inhibitor like ANI (probably the most popular choice in recent studies on general requirements for protein synthesis) leaves one open to many of the same criticisms directed at the protein-synthesis-inhibition studies of the 1960s and 1970s. At doses that block memory, ANI can reduce local protein synthesis by as much as 70%. Even when small volumes are injected into restricted brain sites this level of suppression is likely to produce

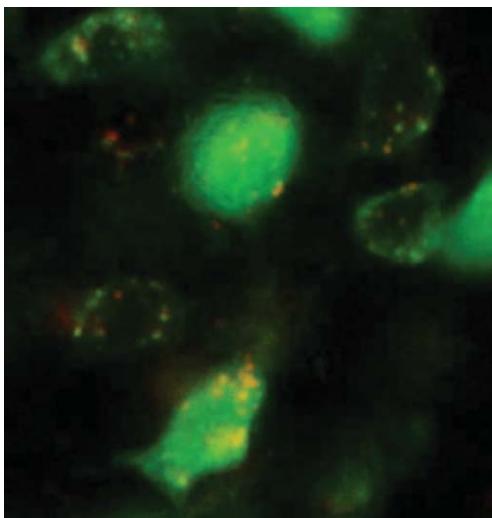


Figure 3 mTOR-dependent translation of new proteins occurs in amygdalar neurons during the period when memory is being formed. This photomicrograph shows cells in the lateral nucleus of the amygdala of a rat. The animal's brain was removed shortly after training and processed for immunohistochemistry. Neurons are shown in green through staining with the DNA-binding protein NeuN. Red immunofluorescence reflects the phosphorylation of p70s6k, a downstream target of mTOR that is activated during protein translation.

effects beyond those directly related to memory formation. Furthermore, ANI by itself will tend to ‘superinduce’ or dramatically increase the level of expression of certain IEGs as well as promote phosphorylation of at least one major protein kinase known to be involved in neural plasticity. Recently, Gold and colleagues have quantified some rather dramatic alterations in local catecholamine concentration following the application of ANI and once again called into question specific conclusions about protein synthesis made when using this drug.

While ANI may be an imperfect tool, the conclusions reached by studies that have used it may still have some merit. One alternate approach is to use inhibitors that are linked to more specific translational control pathways and that are likely to have fewer nonspecific effects when compared to ANI. Recent studies from the author's laboratory have used rapamycin (RAP) which selectively targets the mammalian target of rapamycin (mTOR) pathway. RAP prevents the phosphorylation of mTOR and its downstream targets which results in suppressed translation of some, but not all, mRNAs. **Figure 4** depicts some of the results from a recent study by Parsons and colleagues showing that RAP applied to the amygdala at a dose that results in significant memory impairment produced an ~10% reduction in local protein synthesis. For comparison, the commonly used behaviorally effective dose of ANI produced a nearly 60% decrease in protein synthesis. This observation suggests that only a small fraction of the proteins affected by ANI are actually

related to the formation of memory. It also provides a slightly more subtle demonstration of the importance of translational control and *de novo* protein synthesis in new learning.

Protein Synthesis and Memory Stability

One of the more exciting recent lines of work on protein synthesis and memory has grown out of some observations made nearly 10 years ago by Karim Nader and colleagues. They showed that ANI was effective at disrupting the storage of a memory not only when the drug was given after the initial learning, but also when it was given following the retrieval of the memory. This effect, which they termed ‘reconsolidation,’ implied that new protein synthesis is required every time a memory is brought from storage into an active state. It also suggests that some of the same mechanisms used to form a memory are also used to maintain the stability of that memory over time.

A number of studies have since replicated Nader's observation and added to our understanding of how the cellular events that follow the use or retrieval of a memory correspond to those that participate in its original formation and storage. Many of the same pathways and regulatory processes appear to be involved. It is important to note that protein synthesis at the time of retrieval does not appear to be required for the behavioral expression of a previously established memory, but rather relates to a process by which that memory is preserved and stabilized after use. Theoretically, the ‘reconsolidation’ of memory provides a mechanism through which information can be updated and new elements incorporated into particular representations.

Figure 5 illustrates how retrieval of a specific memory triggers the translation of new protein in hippocampal neurons. In this study conducted by Gafford and colleagues rats were trained to recognize a distinctive environment that was paired with shock. After this memory had been established, the animals were either returned to the original environment (where they showed behavioral evidence of retention) or to a different environment not related to shock (where they did not show any memory). Animals that retrieved and expressed the memory showed significantly higher levels of phosphorylation of two downstream targets of mTOR: p70s6k and 4E-BP1. These findings indicate that memory retrieval activated a period of protein translation that was similar in many respects to that normally seen after original training. These retrieval-induced proteins are presumably what is affected by ANI or other manipulations in reconsolidation experiments.

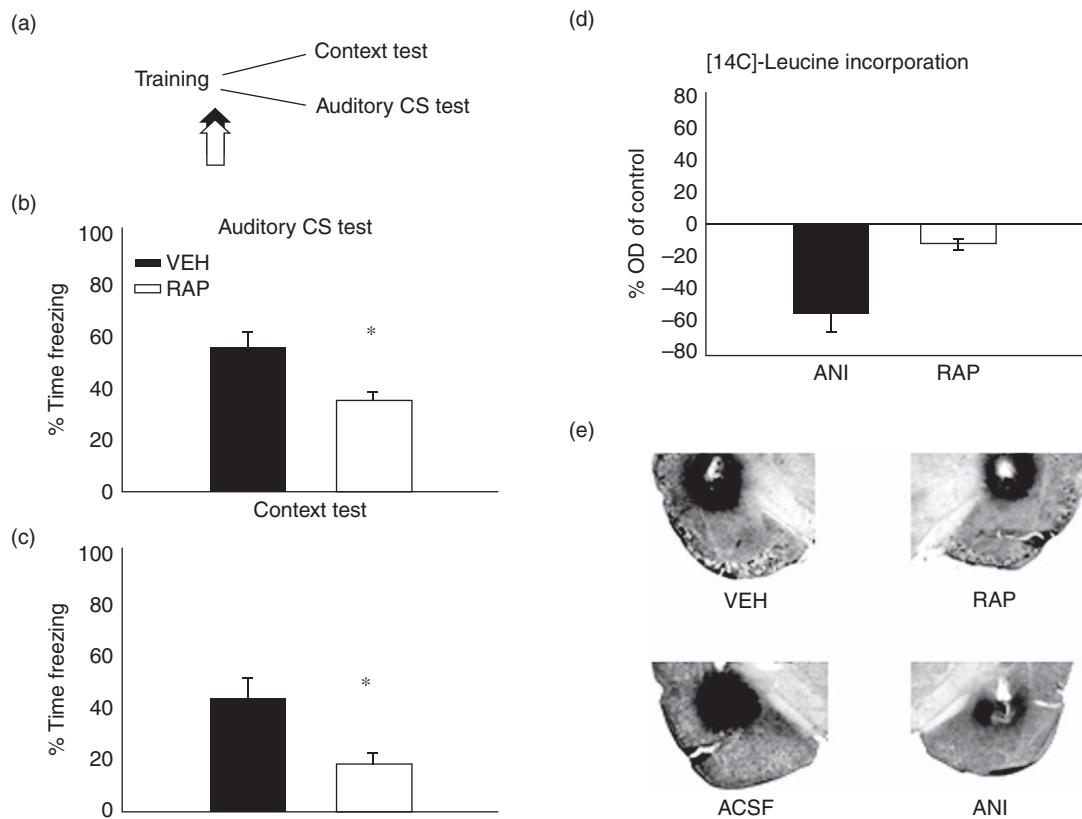


Figure 4 While translation inhibitors like anisomycin can produce large decreases in local protein synthesis along with several unintended effects, other related approaches are less affected by these limitations. Rapamycin (RAP) is a selective inhibitor of protein translation. (a) RAP was injected into the amygdala immediately after auditory fear conditioning. One day later, RAP-treated animals showed impaired memory for the (b) tone paired with shock as well as for the (c) environment in which training took place. (d) Even though RAP was effective in disrupting memory for the training experience, injections into the amygdala result in a relatively modest decrease in local protein synthesis when compared to ANI. (e) Three-dimensional reconstructions of the area using autoradiographs made with [¹⁴C]-leucine give an estimate of the area in and around the amygdala that is affected. Data are redrawn from Parsons RG, Gafford GM, and Helmstetter FJ (2006) Translational control via the mammalian target of rapamycin pathway is critical for the formation and stability of long-term fear memory in amygdala neurons. *Journal of Neuroscience*, 26:12977–12983.

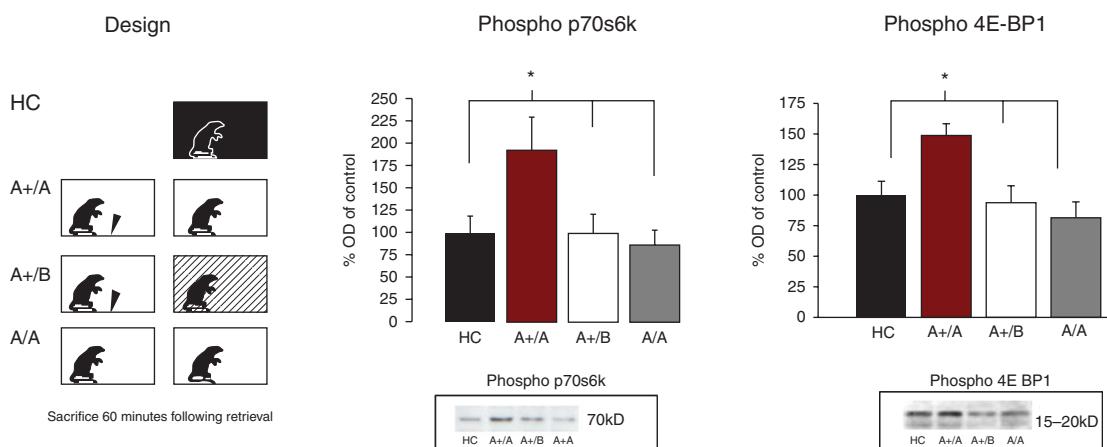


Figure 5 The retrieval of an established memory triggers a new wave of protein synthesis. Rats trained to associate shock with a distinctive environment were re-exposed to that environment or to one of several control conditions. Increased protein translation, as indicated by increased phosphorylation of p70s6k and 4E-BP1, was only seen in the group in which the original memory was retrieved. These data add support to the idea that protein synthesis is required for multiple aspects of memory encoding and maintenance. Data are taken from a recent study by Georgette Gafford.

See also: Genes and Behavior: Animal Models; Memory Consolidation; Synapse Formation and Memory; Transgenic Technologies and Their Application to the Study of Senile Dementia.

Further Reading

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Synaptic Mechanisms for Encoding Memory

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Glossary

Acute hippocampal slice preparation – An experimental preparation wherein the living hippocampus is rapidly removed from the brain and sectioned perpendicular to its long axis to create 300–400- μ m thick slices containing a great proportion of the intrinsic hippocampal circuitry within the plane of the slice. The slices are maintained in a heated, oxygenated, artificial cerebral spinal fluid bath and can be used for electrophysiological analysis of synaptic responses for hours after preparation. Such slices can subsequently be harvested for biochemical, immunocytochemical, or ultrastructural analyses of effects of stimulation or drug treatment.

Theta burst stimulation (TBS) – Patterned electrical stimulation of axonal systems designed to simulate the naturally occurring hippocampal theta rhythm. The conventionally used pattern of theta burst stimulation involves application of a train of 10 theta bursts with each burst containing four stimulation pulses applied at 100 Hz and a 200-ms interburst interval. TBS is particularly effective for inducing enduring NMDA-receptor-dependent long-term potentiation (LTP) within the hippocampus.

Theta rhythm – A spontaneous 4–8-Hz rhythm of synchronous neuronal firing typically recorded from the hippocampus, and the associated limbic regions, in freely moving animals during particular behaviors (e.g., exploratory behavior). The appearance of theta rhythm firing during what are considered learning episodes raised interest in the possibility that this firing frequency was significant for cellular mechanisms of learning and memory. Although the source of the theta rhythm generator has been a topic of debate, recent studies of hippocampal slices have shown that epochs of theta rhythm activity can arise from intrinsic activities within the hippocampus.

occurred in the nineteenth century. In his 1882 analysis of memory disorders, Ribot had a very specific argument for this conclusion and Cajal, writing at the beginning of the twentieth century, ascribed the hypothesis to earlier researchers. The arguments, then, as now, center on the immense capacity of memory: How else could the brain encode and organize the vast amount of information accumulated during even a single day other than by changing some small fraction of the trillions of connections between cortical neurons?

This point was appreciated by several prominent investigators in the middle part of the last century who used some of the better understood synaptic systems of the time (e.g., spinal cord and neuromuscular junction) to ask if protracted periods of afferent stimulation cause lasting changes in postsynaptic responses. The early results, while often intriguing, failed to meet the basic requirements of a memory mechanism. Modification of this approach by Thompson and Spencer in the 1960s resulted in the discovery that synapses in the more evolutionarily conserved parts of the nervous system do in fact possess the necessary and sufficient machinery for generating two fundamental forms of learning. Depending on the temporal patterns used, they found that repetitive presentation of a stimulus to animals results in either a gradual enhancement (sensitization) or reduction (habituation) of physiological and behavioral responses. Detailed analyses led to an extended list of features that define these opposing forms of learning and to evidence that these items are present in the synapses related to the production of the behaviors. Independent studies by Kandel and co-workers showed that invertebrate synapses also exhibit phenomena that are isomorphic with habituation and sensitization. These landmark studies, and the large literature that grew around them, provided the first descriptions of synaptic memory mechanisms.

Synaptic parallels to more elaborate forms of memory were revealed suddenly with the discovery by Bliss and Lomo of hippocampal long-term potentiation (LTP). Following on the lines of older tradition of using repetitive stimulation as a tool for engaging synaptic plasticity, these investigators found that short (20 s) trains of high-frequency afferent stimulation induced a large, and remarkably stable, enhancement of excitatory postsynaptic potentials (EPSPs) in the cortical inputs to the dentate gyrus. LTP at its introduction thus satisfied two very demanding requirements of memory: Rapid onset and extreme persistence.

Introduction: Memory Research Turns Back to the Synapse

The idea that memory is encoded by changes in the strength of synaptic connections between cortical neurons is one result of the explosion of scientific information that

Evolution of LTP research, and the search for the synaptic substrates of memory, was greatly accelerated by the introduction of the acute hippocampal slice technique in the early 1970s. This *in vitro* preparation has many advantages (e.g., ability to directly apply drugs/reagents and to visualize cell layers and afferent lamina) that vastly simplifies analysis of the cellular processes underlying the potentiation effect. Slice research from a large number of laboratories gradually led to something like a standard model for LTP and with it the detailed proposals about the synaptic chemistry of commonplace forms of memory. This material constitutes the subject of the present discussion. The size of the pertinent literature makes it necessary to restrict the discussion to events occurring during the first hour following the induction of LTP, a period during which major steps in the initial consolidation of potentiation, and memory, take place.

Induction of LTP and the Encoding of Memory

LTP is Induced by Theta Activity

LTP can be seen as involving sequential stages of induction, expression, and stabilization. Induction is probably the best understood, and least controversial, phase in the sequence leading to stable potentiation. A high-frequency burst of afferent activity produces a profound depolarization of postsynaptic spines via activation of synaptic AMPA-type glutamate receptors and spatiotemporal summation of EPSPs. Depolarization of sufficient degree and duration unblocks voltage-sensitive *N*-methyl-D-aspartic acid (NMDA)-type glutamate receptors, which then pass calcium into the spine. Calcium, acting with other factors to be discussed, then sets in motion the steps that lead to LTP expression and consolidation.

A more precise description of the triggering events for LTP became available after the discovery that the induction process has a deep relationship with theta (5 Hz)-patterned neuronal activity that naturally occurs during learning and other behaviors. Specifically, delivery of a short train of high-frequency stimulation bursts (4 pulses per 30-ms burst) induces LTP when the bursts are separated by the 200-ms period of the theta wave (theta burst stimulation, TBS; see **Figure 1(a)**). Deviations from this pattern reduce the amount of potentiation. The peculiar efficiency of theta bursts has been traced to the feedforward GABAergic inhibition that accompanies excitatory (glutamatergic) transmission in the cortical telencephalon. Afferent fibers not only innervate principal cells (e.g., cortical and hippocampal pyramidal neurons) but, via collateral branches, contact a broad array of inhibitory, GABAergic interneurons with axonal projections to the same targets. Input activation, as expected from these

anatomical arrangements, generates a biphasic response consisting of an EPSP followed quickly by an inhibitory postsynaptic potential (IPSP) that shunts the excitatory currents. The latter event prevents a single theta burst from producing the depolarization needed to engage NMDA receptors. However, a second burst following at 200 ms results in the normal depolarizing drive but in much reduced inhibition and so leads to greater depolarization (**Figure 1(b)**) and a measurable influx of current through the NMDA receptors. This occurs because the interneuron terminals enter a refractory period, mediated by presynaptic autoreceptors, after being activated by the first burst, and this refractory state reaches its maximum at approximately 200 ms.

In all, the temporal parameters of IPSP suppression, along with other features, generate greater postsynaptic depolarization than that obtained with a continuous 100-Hz train (**Figure 2(b)**) and tune the theta burst pattern so as to maximize NMDA-receptor responses. These observations show how ubiquitous local network designs and a battery of physiological (and biophysical) properties act in concert over a 1-s period to initiate lasting changes to synapses. Beyond this, they make a strong case for the idea that LTP is a naturally occurring phenomenon – an argument that is further strengthened by studies showing that theta rhythm bursting occurs during learning.

Induction and Organizational Aspects of Learning

The above results provided some of the first insights derived from mechanisms of LTP into the cellular and neurochemical mechanisms of learning and memory. It has long been recognized that electrographic 4–8 Hz theta rhythms are intimately connected to learning, a relationship that is usually assumed to reflect a need for synchronizing brain contents that are about to be associated. This of course does not explain the particulars of the rhythm – its period, duration of bouts, etc. – or the import of the short bursts of neuronal firing that ride upon it. From studies of LTP we now see that all of these features are uniquely associated with the engagement of peculiar network properties and synaptic mechanisms that collectively produce time-locked calcium pulses in dendritic spines. The theta rhythm–LTP connection also helps explain the powerful role of temporal contiguity in learning. In the simplest sense, inputs arriving on successive peaks of the rhythm have a greater chance of being associated via LTP than those spaces apart by other intervals. Contiguity relationships based on subtler aspects of the induction process have also been found. One experiment entailed asynchronous theta burst stimulation of three afferents (#1, #2, and #3) to the same neuron with the drive from each, by itself, being too

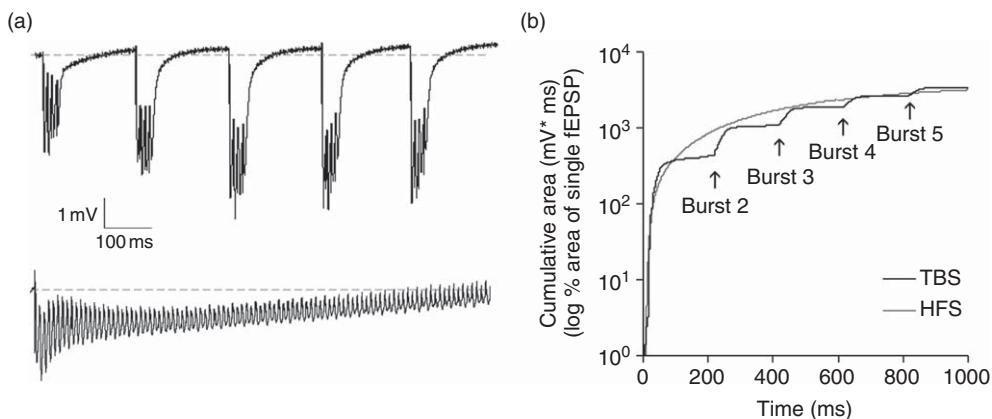


Figure 1 Theta burst stimulation is particularly effective for eliciting postsynaptic depolarization. Recordings of field excitatory postsynaptic potentials (fEPSPs) elicited by stimulation of Schaffer collateral-commissural afferents to field CA1 (adult rat hippocampal slices) illustrate differences in the magnitude of responses generated by the same period of stimulation applied as theta bursts (4-pulse 100-Hz bursts separated by 200 ms) or as a continuous 100-Hz high-frequency stimulation (HFS) train. Panel (a) shows representative responses to 1-s TBS (upper trace) and HFS (lower trace). With TBS the magnitude of the responses builds after the first burst, peaking at burst 3 or 4. In contrast, with HFS there is an initial facilitation (through 30–40 ms) followed by depression for the remainder of the train. (b) To assess the overall depolarization generated by the different forms of stimulation, the mean cumulative area under the response curve was plotted for four sets of responses to 1 s of TBS (black line) and HFS (gray line). Values were normalized to the area of a single test pulse that preceded the train and plotted as log values. Contributions of each theta burst are evident in the TBS curve (burst 2–5). As shown, despite the HFS train containing 5 times the number of pulses, the cumulative area, denoting the magnitude of the postsynaptic response, is nearly identical.

weak (i.e., engaging too few synapses) to trigger the depolarization needed to activate NMDA receptors and set the LTP-induction process in motion. However, serial activation of the inputs (#1>#2>#3) with bursts resulted in robust potentiation in the first member of the sequence (input #1) but not in the third member (input #3) when each episode of sequential activation was separated by the period of the theta wave. Thus, the order in which information arrives during theta activity dictates the strength for encoding the individual elements in the sequence.

Biologically realistic computer simulations confirm the suspicion that the order phenomenon just described results in a system that can learn to recognize complex cues according to the sequence of the elements within the cue (e.g., the words ‘raw’ and ‘war’); they also show that such a system has remarkable storage capacity. More broadly, the theta rhythm–LTP findings constitute the first steps toward the identification of synaptic learning rules that may determine how and why the cortical telencephalon organizes its representations of the world in the way that it does. This, of course, is not the end of the story. The vast networks in associational cortex, with their apparent lack of detailed topographic anatomy, are likely to represent a programmable system that allows the brain to escape from narrow synaptic rules. However, one suspects that even here, at the remote frontiers of neuroscience, LTP-induction mechanisms will influence the efficacy and organization of information storage.

Expression of LTP and Memory

Expression is Largely Postsynaptic

Theta burst stimulation is followed within 5–10 s by a marked increase in the size of EPSPs that is restricted to stimulated connections (i.e., it is synapse specific). This indicates that some form of potentiation is expressed almost immediately after the induction events, but it is unclear if this is supported by the same postsynaptic processes responsible for LTP. Indeed, the critically important question of when LTP expression first occurs remains a little-studied issue. A cleverly designed experiment by Gustafsson and colleagues led to the conclusion that the changes underlying LTP expression begin within seconds of induction and are fully realized 30 s later. Such extremely rapid expression would place a strong constraint on hypotheses about the nature of the modification that actually enhances synaptic currents. However, as discussed below, there are reasons to suspect that the synapse passes through multiple stages before a final stage of LTP expression is realized.

The discovery that NMDA receptors and increases in spine calcium content are required for the induction of LTP led to the early assumption that LTP expression also involves postsynaptic changes. Striking support for this came from studies showing that, within hippocampus, robust LTP is associated with changes in the magnitude of those components of the postsynaptic response mediated by the AMPA class, but not the NMDA class, glutamate receptors. In the absence of a set of unlikely assumptions, it is

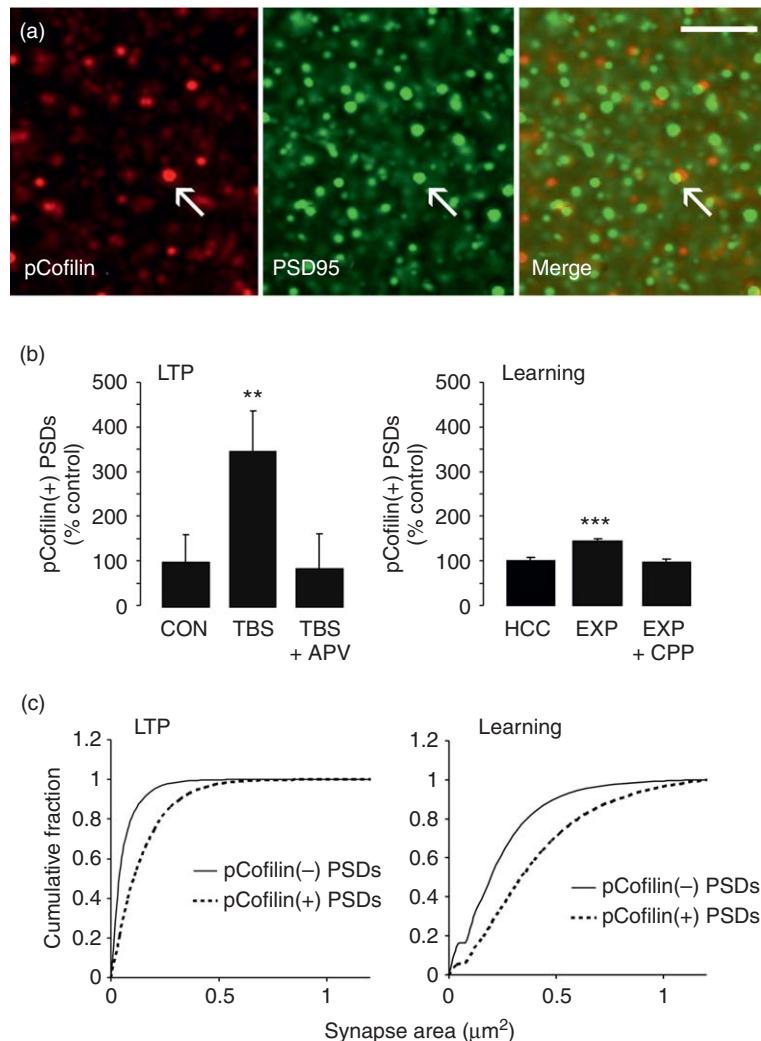


Figure 2 LTP and learning induce spine cytoskeletal remodeling and changes in synapse size. Immunofluorescent labeling for the actin regulatory protein cofilin and the postsynaptic density marker protein PSD95 was used to identify spines engaged in cytoskeletal remodeling and to measure the size of associated PSDs. Photomicrographs in (a) show double immunofluorescent localization of phosphorylated (p) cofilin and PSD95 in dendritic spines in hippocampal field CA1 of an acute slice: pCofilin labeling is reliably localized to a subset of spines with PSD95+ synapses (arrow indicates one double-labeled profile; iterative deconvolution images from CA1 str. radiatum; Scale = 5 μm). (b) The graph on the left shows that the induction of LTP by theta burst stimulation (TBS) produced a significant, three to fourfold increase in numbers of spines containing pCofilin [pCofilin(+)] PSDs ($**p < 0.01$; a two-tailed *t*-test) and that this effect was completely blocked by stimulation in the presence of the NMDA receptor antagonist APV. Similarly, unsupervised learning increased numbers of spines containing pCofilin (b, right); the graph shows counts of pCofilin+ spines (i.e., spine-like profiles associated with PSD95) in hippocampal CA1 str. radiatum in rats (1) maintained in their home cage (HCC), (2) engaged in 30 min unsupervised learning just before sacrifice (EXP), and (3) engaged in 30 min unsupervised learning under treatment with the NMDA receptor antagonist CPP (EXP+ CPP). The unsupervised learning group had 40% more pCofilin+ spines than did the home cage controls ($***p < 0.001$) and this effect of unsupervised learning was blocked by CPP (10 mg kg^{-1}). (c) To evaluate the effects of LTP, and learning, on synapse size, the areas of PSD95+ elements were measured for profiles immunolabeled in experiments illustrated in panel (b). Graphs in (c) show size-frequency distributions for PSDs associated with (dashed line) or not associated with (solid line) dense pCofilin; these distributions show that with both LTP (left) and learning (right) pCofilin-containing spines are associated with larger synapses.

difficult to see how a presynaptic increase in neurotransmitter release could act on just one subset of glutamate receptors co-localized at the synapse. Moreover, LTP is not accompanied by changes in paired-pulse facilitation of EPSPs, an effect that is sensitive to altered neurotransmitter release probabilities. Despite these points, the

above results opened one of the more intense controversies in recent neuroscience history: that is, the argument whether changes that underlie LTP are primarily pre- or postsynaptic. To address this issue, a number of laboratories applied whole cell recording techniques and methods of quantal analysis, originally developed to

study release at the neuromuscular junction, to hippocampal slices. Initial results indicated that the induction of LTP increases the probability of glutamate release and thus supported a presynaptic hypothesis for expression. However, questions soon appeared as to whether the essential conditions for quantal analysis had been satisfied in the slice studies and thus about the degree to which the results obtained actually challenged the postsynaptic hypothesis. When subsequent work on mechanisms of potentiation (see below) concurred with the conclusions from the studies on NMDA versus AMPA receptor changes, the controversy gradually subsided and a broad consensus emerged that expression is largely, and perhaps entirely, a postsynaptic phenomenon.

Active versus Structural Models of LTP Expression

What type of postsynaptic change underlies expression? One possibility considered was that synaptic potentiation arises from a change in spine resistance. However, tests of necessary predictions from this hypothesis proved negative. The alternative and favored hypothesis is that LTP expression involves another straightforward change that would lead to larger EPSPs; namely a modification involving postsynaptic AMPA receptors. Two versions of this idea have evolved over the past 5–10 years. The first, which will be referred to as the active model, posits that self-regenerating enzymatic activity acts on the AMPA receptors in such a way as to either increase their conductance or encourage their insertion into the synaptic membrane. The original version of the argument, involved calcium/calmodulin-dependent protein kinase II (CaMKII). Sacktor and colleagues subsequently advanced a second, and remarkably well-developed, active model based on protein kinase M-zeta (PKMzeta). Both of these enzymes are concentrated in the postsynaptic region, exhibit enduring activity, and influence AMPA receptor activities in ways that should increase the AMPA receptor component of the postsynaptic response. It is thus reasonable to propose that induction engages an enzyme that then (1) increases AMPA-receptor-mediated currents and (2) generates new copies of the active enzyme within the spine. The latter step ensures the maintenance of the potentiated state. Adding to the plausibility of this scenario are data indicating that activities of CaMKII and PKMzeta are increased with LTP induction and NMDA receptor stimulation. Questions naturally arise as to whether a self-regenerating system of this type could be sustained for the weeks and months over which LTP has been followed in chronic recording experiments. Positive data relating to a key prediction of the model – that inhibitors of the kinases will fully reverse established LTP without

affecting control synapses – have been reported, but additional work on this question would be helpful.

Structural models of expression hypothesize that induction increases the pool of synaptic AMPA receptors by changing the morphology of the postsynaptic region. This idea is grounded on two observations. First, early electron microscopic (EM) studies using hippocampal slices and acutely anesthetized rats obtained evidence that LTP is accompanied by changes in the postsynaptic density (PSD), the specialized anatomical region lying immediately beneath the synaptic contact zone. The mean effect of LTP on PSD sizes was small, but this is as expected given that the activated connections constituted a minority of the total synaptic population measured. These experiments also indicated that the PSD effects were associated with changes in the shape of dendritic spines. Collectively, the results led to the hypothesis that induction causes a rounding of spines and synapses and thus an increase in the area of the latter. Subsequent ultrastructural studies described other types of PSD and spine modifications, but in general terms supported the proposal that rapid structural changes are a concomitant of LTP. A second set of results pertinent to structural models came from immunogold/EM studies of the density of glutamate receptors at hippocampal synapses. Experiments of this type from different groups established that the number of AMPA (but not NMDA) receptors correlates positively with PSD size. Thus, LTP-related increases in PSD area, as found in some EM studies, would be expected to entail proportionate increases in synaptic AMPA receptor number and therefore to provide a satisfactory explanation for expression.

The advent of light microscopic techniques that allow for resolution and measurement of micron-level objects in the dense three-dimensional matrix of brain opened the way for new tests of the structural hypothesis for expression. Using these methods, convincing evidence has been obtained for the idea that the brief periods of high-frequency afferent activity used to induce LTP cause a rounding and enlargement of spines. Results of a recent, carefully executed experiment described a lasting 41% increase in spine volume following TBS along with a change in the shape of the volume–frequency distribution of the potentiated spines, an effect which is suggestive of a change in spine shape. Other work using immunocytochemical markers for potentiated synapses (see below, **Figure 2(c)**) found a 50–70% increase in PSD size following a conventional theta burst train that was again accompanied by a shift in the size–frequency distribution similar to that described for spines. Remarkably, the quantitative results for the PSD and spine studies are in good agreement with the magnitude of LTP and their congruence makes a powerful case for the hypothesis that expression of the potentiation effect is achieved by structural changes to spines and synapses.

Is there a syncretic model that incorporates both the active and structural arguments regarding LTP expression? Rapid filling of the expanded synapse with AMPA receptors presumably involves increased activity by the enzymes that promote movement of the receptors from lipid rafts in the perisynaptic membrane into the junc-tional zone. CaMKII and PKMzeta are well suited, both by their locations and known functions, to perform this role. The first step in exploring this idea would be to test if, within individual spines, activity of the two enzymes correlates with PSD size under resting conditions; positive results would constitute evidence that the machinery regulating receptor trafficking is linked to the factors controlling the area of synaptic apposition, and thus for an active/structural version of LTP expression.

LTP Expression and the Organization of Memory

Early work testing for evidence of LTP expression during behavior took advantage of several unusual features of the afferents to the olfactory cortex to substitute theta-pattern stimulation for sensory input to create electric odors, making it possible to measure EPSPs at precisely the same synapses that received inputs perceived by the animal to be a real-world cue. Learning of a two-odor discrimination, where one or both of the odors was electric, resulted in robust, synapse-specific, and long-lasting LTP. Importantly, failures to induce LTP with electric odors were invariably accompanied by failures to form memories. Recent experiments by Bear and colleagues obtained strong evidence that learning of a conditioned response enhanced synaptic responses in the hippocampus; critically, these effects occluded LTP, suggesting that they shared substrates with the potentiation effect, and involved only a portion of available synapses. The discovery that TBS increases the size of synapses made it possible to carry this line of work to the next level – to directly test, through visualization of individual synapses, whether the expression mechanisms for LTP are actually engaged by learning. The results were clear: spines in the hippocampus that were potentiated (i.e., contained a biochemical marker associated with the recent induction of LTP) by 30 min of unsupervised learning had synapses that were significantly larger than their neighbors that lacked the potentiation marker. These effects involved only a small percentage of the spine population, so they accord well with the expectation that the acquisition of substantial amounts of information (learning a complex environment) does not use up a significant fraction of the available synapses.

The parametric details of LTP expression, as was the case for induction, help explain how the biology of synaptic memory leads to one of the many possible internal representations of the external world. The approximately 50% increase in synapse size found in the imaging studies

aligns closely with the maximum amount of LTP obtained under standard conditions with naturalistic theta burst stimulation, a correspondence that seems unlikely and yet in line with the observation that synapse size scales linearly with the number of AMPA receptors (see above). What does it mean, in a computational sense, if synapses can change just this much but not more? Modeling research suggests that cortical networks incorporating LTP-based synaptic learning rules will inevitably form hierarchical categories in which learned cues are associated according to their similarity. In these models, the number of items needed to form a category is set by the magnitude of the smallest increment of LTP, while category breadth is determined by the maximum degree to which synaptic strength can be increased. According to these results, the serial learning of odors, to cite one well-studied example, results in the formation of classes (plants, animals) under which are subsumed subcategories (flowers, fruits), under which are specific cases (roses). Thus, the internal world generated by memory seems to be shaped by the ability of synapses to change size.

Consolidation

LTP Consolidation Depends on the Spine Actin Cytoskeleton

Nineteenth-century researchers were well aware that memory is unstable immediately after its encoding and then over the next 15 min becomes progressively more resistant to disruption. This reduced vulnerability to disruption is termed the consolidation of the memory trace. That LTP possesses a similar consolidation period was discovered in the early years of research on the potentiation effect, but the significance of this was not fully appreciated until several years later. Initial studies showed that LTP could be reversed by low-frequency stimulation applied within the first 5 min following LTP induction and that this erasure effect was selective to potentiated synapses (i.e., control, unpotentiated, pathways were unaffected). Later, chronic recording experiments confirmed that potentiation did not return following such erasure even after 24 h. Other studies showed that a number of manipulations (e.g., adenosine application and hypoxia) could selectively erase LTP when applied in the first minutes after induction but were without effect 10–20 min later. A sizeable number of experiments from different groups have confirmed these points.

How consolidation is achieved constitutes one of the major questions facing the field. It brings the plasticity effect face to face with one of the fundamental issues in memory research, and raises mechanistic questions that are likely related to a broad spectrum of neuropsychiatric

problems. Early attempts to explain consolidation posited that the structural modifications triggered by the induction process resulted in a lasting reorganization of the spine cytoskeleton, which then maintained the altered geometry of the synaptic junction. LTP in this reading would be a special case of the broad class of events whereby cells form specialized quasi-permanent connections with the extracellular matrix or other cells. An impressive wave of recent work suggests that this idea is largely correct. Intense stimulation of glutamate receptors in dissociated neurons or cultured hippocampal slices causes the rapid polymerization of actin within dendritic spines; evidence for activity-driven polymerization has also been reported for adult hippocampus, but in that instance it was not possible to clearly resolve individual spines. Subsequent work with novel technical modifications resolved the latter problem and showed that TBS causes a rapid (<2 min) increase in the number of spines containing high concentration of filamentous (F)-actin within the dendritic sublamina containing potentiated synapses. The effect had the same threshold (number of theta bursts) for induction as did LTP and was completely blocked, along with consolidation, by a toxin (latrunculin A) that prevents the addition of actin monomers to growing filaments.

While many lines of evidence indicate that TBS-induced F-actin assembly is essential for the production of stable potentiation, the effect by itself does not explain a defining feature of LTP consolidation: progressive development over a 15–30-min time frame. In other words, the protracted nature of consolidation points to stabilization processes that occur after TBS triggers actin polymerization (30 s to 2 min). One possibility is that the newly induced actin filaments are initially dynamic and then gradually transition into a more stable configuration. Work on growth cones shows that actin polymers engage in a treadmilling process wherein they add monomers at one end and release them at the other. Tests of whether this occurs in spines after LTP induction were made by applying latrunculin A at various times after TBS. As predicted, the toxin eliminated the several-fold increase in spines with dense concentrations of F-actin when infused at 2 min post-TBS but was without effect 10 min later. Its actions on LTP paralleled these results. These last findings provide a first candidate – F-actin stabilization – for the event that consolidates LTP.

Ongoing efforts to understand how a 1-s-long theta burst train, composed of five bursts of depolarization, can yield such dramatic changes to the spine cytoskeleton have already resulted in a novel model of synaptic function in which the junction contains adhesion and modulatory receptors along with the classical transmitter (glutamate) receptors. Integrin class adhesion receptors are known to potently influence the actin cytoskeleton across cell types. The $\beta 1$ -family integrins are

concentrated at glutamatergic, hippocampal synapses and three groups using different methods have shown that blocking their operation potently disrupts LTP consolidation. This confirms an early hypothesis that integrin engagement is a key step in producing stable LTP. The junctional zone also contains receptors for brain-derived neurotrophic factor (BDNF) and adenosine – the BDNF TrkB receptor promotes TBS-induced actin polymerization and LTP whereas the adenosine A1 receptor inhibits both processes. Paradoxically, then, theta bursts elicit the release of modulators that exert opposing effects on consolidation, an observation that will figure into a later discussion of how the synaptic chemistry of LTP not only encodes but shapes memory as well.

Do activities of glutamate receptors, integrins, and modulatory receptors coordinate the assembly and stabilization of actin networks within the confined space of the dendritic spine? The analysis of the small percentage of synapses involved in physiologically induced LTP in an adult brain was impossible prior to the very recent development of restorative deconvolution microscopic techniques for quantitative analysis of immunofluorescent labeling. These methods make it possible to ask if a subpopulation of synapses in the discrete dendritic layers targeted by TBS exhibit stimulation-induced increases in activated versions of proteins known from a vast literature on non-neuronal tissues to control actin filament assembly. Work using these methods revealed that, as predicted, TBS increases the number of synapses containing dense concentrations of phosphorylated p-21-activated kinase and cofilin, two proteins that act as intermediaries between integrins and the actin cytoskeleton. The cofilin result, as illustrated in **Figure 2**, is particularly diagnostic. The protein is constitutively active and functions to sever growing filaments; the suppression of this activity by phosphorylation, which is promoted by BDNF, is thus critical for actin-filament assembly and maintenance. These analytical techniques allow us to examine how much of the complex machinery regulating the actin networks in a diverse array of cell types is present in spines and engaged by LTP induction.

LTP Consolidation and Memory

Consolidation events described here are largely complete within 10–15 min following the induction of LTP. This time period aligns well with that first described by Ribot (see above) for the stabilization of memory. However, there is a large literature suggesting that consolidation involves multiple stages occurring over hours or even days. A long-standing question in memory research concerns the extent to which the proposed stages of consolidation are serial (where one causes the next) or parallel in the sense that different events with different latencies are activated by learning. The LTP work favors

the former idea. Specifically, studies by Vanderklish and Edelman show that the assembly of actin networks is necessary for activity-driven protein synthesis in the dendritic segments underlying spines. Sacktor independently reached this conclusion in his work on PKMzeta. Further discussion of these arguments, which would involve a survey of the vast literature on the possible roles of gene transcription and protein translation in memory and LTP, is beyond the scope of the present review.

Imaging memory consolidation. In addition to identifying actin-signaling pathways engaged by theta stimulation within spines, the quantitative immunofluorescence studies described above have provided a set of biochemical markers for recently potentiated synapses. This was a critical first step toward asking if LTP consolidation actually occurs in synapses during learning. Recent work established that unsupervised learning results in a modest, but statistically robust, increase in the number of hippocampal synapses associated with phosphorylated cofilin, one of the effects produced by TBS that is critical for generating new F-actin. This learning-induced increase was prevented by an NMDA receptor antagonist at concentrations that block both LTP and the production of long-term memory (**Figure 2(b)**). Collectively, these results constitute good evidence that memory consolidation involves the same mechanisms used to stabilize LTP. They also suggest a strategy for investigating one of the most important issues in all of behavioral neuroscience: Where in the cortical telencephalon are memories stored? The work with unsupervised learning focused on a single dendritic lamina in a subfield (CA1b) of mid-septotemporal hippocampus but, nonetheless, required significant sampling. Expanding such an analysis to include the entire hippocampus, but excluding the neocortex, using current technologies is a daunting prospect but may become realistic with further advances in computerized microscopy and automated identification and measurement of micron-sized structures.

The LTP work suggests the idea, rarely discussed in the behavioral literature, that the consolidation process affects the properties of memory. Parametric studies showed that 30-s trains of theta-frequency stimulation (i.e., single pulses, rather than bursts, delivered at 5 Hz) are sufficient to erase newly induced potentiation; this is informative because bouts of theta activity lasting this long are a routine feature of the hippocampus in freely moving animals. Possibly, then, the activity of ascending cholinergic projections and the behavior of the animal, two variables that regulate theta activity, in the post-acquisition period determine which aspects of the recently encoded information are erased or transferred into long-term memory. Results showing that the occurrence of extended periods of theta activity in behaving rats during the first few minutes after TBS eliminates LTP are in accord with this suggestion. In all, the consolidation effect

operating over several acquisition episodes may allow brain networks to actively construct a memory that highlights certain features of the learned material while omitting others.

LTP Rules Suggest Means for Enhancing Memory

Progress in explaining the induction, expression, and consolidation of LTP led to entirely new ideas about the failure of memory with aging and in neurodegenerative diseases and how it might be rescued. Closely related to this is a burgeoning body of work investigating neurobiologically grounded techniques for enhancing normal encoding. Beginning with induction, an early set of studies showed that antagonists of NMDA receptors thoroughly disrupt spatial learning, a result that first raised the idea that enhancing the triggering mechanisms for LTP might reduce the amount of training needed to acquire memory. It should be noted here that those same widely cited experiments also found that the antagonists left some forms of learning intact; the failure to recognize that LTP contributes to some but not all forms of memory added confusion to the subsequent extensive literature on the behavioral significance of the potentiation effect. In any event, the first attempts to exploit information about induction used an invented class of centrally active, allosteric modulators ('ampakines') that enhance the ionic currents passed by AMPA receptors. Since these currents provide the depolarization needed to unblock NMDA receptors, it was reasoned that they should reduce the activity threshold for inducing LTP and thereby facilitate the process. Both predictions were confirmed and ampakines were subsequently found to improve retention scores in a variety of learning paradigms in animals and humans. Moreover, these effects were obtained with ampakine dosages that produced little, if any, effect on baseline performance across multiple testing procedures.

The ampakine results lead to a conclusion with far-reaching implications: namely that the parameters for LTP induction and expression are less than optimal with regard to memory acquisition. This may be a very unlikely idea for some simply because learning is essential to survival and thus is presumably the target of strong adaptive pressures operating over tens of millions of years. In line with this suggestion, LTP-like effects have been recorded in representatives from multiple orders of mammals. If, as suggested by this, the phenomenon appeared with the earliest mammals, then it is at least 150 million years old. Note, however, that the tuning of LTP parameters would have occurred in environments that have only a passing resemblance to the settings in which memory tests are conducted – from this perspective

LTP settings that are less than ideal would not be unexpected. Acquisition is not the only learning-related variable affected by the LTP rules; as discussed, network models suggest that the categorization of information is intimately related to the induction threshold and expression ceiling for LTP. If this is correct, then experimental treatments acting on these variables so as to accelerate learning, or enhance the strength of memory, will likely affect the way in which information is organized in the cortex. Whether this would be to the benefit or detriment of cognitive operations can only be investigated using complex, real-world situations – tests of the point, which may become possible during clinical testing of LTP-related drugs, are likely to yield fascinating results.

Manipulation of consolidation offers some intriguing prospects for memory enhancement. In principle, such treatments would not affect acquisition but instead would improve the likelihood of translating the learned material into long-term storage. They could, in other words, be highly selective with regard to their impact on the organization of memory. Most of the work in this area has involved treatments directed at the synthesis of proteins thought to promote late phases of consolidation and so are beyond the scope of the present discussion. Factors that modulate activity-driven reorganization of the spine actin cytoskeleton offer obvious targets for enhancing the rapid phase of consolidation. Thus, several physiologically plausible manipulations are reported to increase the production of BDNF, which, as described, potently facilitates both TBS-induced actin polymerization and LTP consolidation. Surprisingly, there do not appear to be studies testing if, as predicted from the LTP work, elevation of brain BDNF levels improves retention scores in long-term memory paradigms. Another candidate suggested by recent experiments shows that agonists for estrogen receptor beta promote the formation of stable LTP and markedly improve performance in tests of long-term memory. Very little is known about the synaptic mechanisms responsible for these effects but estrogen

has a strong influence on actin dynamics in non-neuronal systems. In all, it appears that we are entering a period in which the growing body of knowledge about LTP will be used to design novel, mechanism-based strategies for enhancing memory.

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Thermo- and Mechanosensation via Transient Receptor Potential Ion Channels

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Glossary

Hyperalgesia – This word, literally, means an increased response to a painful stimulus. The phenomenon may involve a lowering of nociceptive threshold or an increase in the magnitude of pain evoked by suprathreshold stimuli. Hyperalgesia can occur at the site of tissue damage (primary hyperalgesia) and in the surrounding undamaged zones (secondary hyperalgesia). While primary hyperalgesia can result from changes in the sensitivity of nociceptors, the basis of secondary hyperalgesia remains unclear. The spread of hyperalgesia in the periphery may occur through the sensitization of nociceptors that project collateral branches, one of which innervates the site of injury.

Neuropathic pain – This type of pain may result from disorders of the peripheral nervous system or the central nervous system. Thus, it is divided into peripheral neuropathic pain, central neuropathic pain, or mixed neuropathic pain. Central neuropathic pain is found in spinal cord injury, multiple sclerosis, and some strokes. The common causes of painful peripheral neuropathies are diabetes, herpes zoster infection, human immunodeficiency virus (HIV)-related neuropathies, nutritional deficiencies, toxins, remote manifestations of malignancies, and genetic and immune-mediated disorders. Neuropathic pain is common in cancer as a: (1) direct result of cancer on peripheral nerves, (2) side effect of some chemotherapy drugs, and (3) result of radiation injury.

Potential receptor – A potential restricted to the receptive field that propagates electronically. It is generally produced by the opening of excitatory channels selective for cations. The movement of these ions brings the membrane potential toward the threshold for triggering an action potential. An example of this is mechanotransduction. A mechanical stimulus deforms the membrane, causing the activation of mechanosensitive channels. The ion flow through these channels depolarizes the membrane, activates voltage-gated Na^+ channels, and gives rise to an action potential.

Sensory neurons – Sensory information is conveyed from the periphery to the central nervous system via sensory neurons that have their cell bodies in dorsal root ganglia and in trigeminal ganglia. Somatosensory

modalities (touch, pain, and temperature) are subserved by separate populations of sensory neurons specialized in detecting innocuous and injurious stimuli. Sensory neurons roughly fall into three categories: (1) rapidly adapting mechanotransducers, which are neurons that respond to touch and non-noxious temperatures. They conduct action potentials rapidly and make a subset of nerve fibers referred to as ' $\text{A}\beta$ fibers'; (2) proprioceptive neurons, which are neurons that indicate the position of our muscles. They also conduct action potentials rapidly and comprise $\text{A}\beta$ nerve fibers; and (3) nociceptors, which are pain-sensing neurons that respond to painful mechanical or thermal stimulation. They also comprise the class of neurons that respond to chemical irritants (like capsaicin). They are lightly myelinated ($\text{A}\delta$) or unmyelinated (C-fibers), so they conduct action potentials more slowly than $\text{A}\beta$ fibers.

Transient receptor potential channels – TRP channels were named after the near-blind *Drosophila* mutant that displays transient potentials in response to continuous light. The identification of the TRP gene and recognition of its function as a Ca^{2+} -permeable cation channel led to the cloning of many channels that bear sequence and structural similarities to the *Drosophila* TRP. On the basis of sequence homology, the TRP family is divided into seven main subfamilies: the TRP canonical (TRPC) family, the TRP vanilloid (TRPV) family, the TRP melastatin (TRPM) family, the TRP polycystin (TRPP) family, the TRP Mucolipin (TRPML) family, the TRP ankyrin (TRPA) family, and the TRP NOMPC (TRPN) family. The characterization of TRP members has grown considerably during recent years, leading to a plethora of data on the roles of TRP channels in a variety of cellular functions, tissues, and species.

Vanilloids – They comprise a group of compounds including capsaicin, which is the primary pungent principle of hot pepper. It was first isolated by LT Thresh more than a century ago, who named this compound 'capsaicin,' and predicted that the structures of capsaicin and vanillin were closely related. Despite this early discovery, it was not until 1919 that the exact chemical structure of capsaicin was determined by EK Nelson. Today, capsaicin is used as a neuropharmacological tool to identify sensory neurons and their contribution to health and disease.

Introduction

Sensory neurons enable us to sense and perceive our environment, which is a condition for triggering appropriate behavioral responses. In vertebrates, these sensations are mediated by specialized sensory neurons, whose cell bodies are located in trigeminal ganglia (TGs) and dorsal root ganglia (DRGs). These neurons project long axons to the skin and deeper body structures where they detect chemical, thermal, and mechanical cues. Therefore, detecting and transducing several types of stimuli enable us to perceive touch, body movement, spatial orientation, as well as temperature. However, if the intensity of a particular stimulus is above a certain threshold, it can evoke the sensation of pain.

Sensory neurons are functionally heterogeneous: different subpopulations of neurons are specialized to detect different sensory modalities. The stimulus, whatever its nature, is always converted into an electrical signal, the receptor potential, which eventually leads to the generation of action potentials that propagate toward the central nervous system.

The transduction mechanisms underlying temperature sensation and mechanosensation have been the subject of intense study. Recently, several transient receptor potential (TRP) channels have been identified as candidate thermo- and mechanosensors. Channels from the TRP channel superfamily were first described in *Drosophila*, deriving their name from the mutant phenotype of a TRP channel in response to prolonged light stimulation. Thereafter, several subfamilies of TRP channels have been recognized

in both invertebrates and vertebrates and this led to the discovery of their roles in transducing mechanical, chemical, and thermal stimuli. To date, more than 70 TRP channel subunits have been identified in genomes of *Caenorhabditis elegans*, *Drosophila*, and humans, and have been classified into seven subfamilies: the TRP canonical (TRPC) family, the TRP vanilloid (TRPV) family, the TRP melastatin (TRPM) family, the TRP NOMPC (TRPN) family, the TRP ankyrin (TRPA) family, the TRP polycystin (TRPP) family, and the TRP Mucolipin (TRPML) family. Of these, the members of three families, that is, the TRPV channels, the TRPM channels, and the TRPA channels are of particular interest as thermo- and mechanosensors. This article summarizes findings regarding the role that TRP channels play in thermosensation and mechanosensation in mammals.

Thermotransducers in Mammals

The demonstration that certain TRP channels can be activated by increases or decreases in temperature, along with the recognition of their heterogeneous temperature sensitivities and expression pattern, has led investigators to evaluate these proteins as candidate thermosensors. Six highly temperature-sensitive TRP channels, whose genetic ablation alters thermally driven behaviors, have been implicated to mediate temperature sensation. These channels, expressed in sensory neurons or in the skin, are active at specific temperatures ranging from noxious cold to burning heat (**Table 1**).

Table 1 Thermal- and mechanosensitive mammalian TRP channels in sensory neurons and in the skin

Channel	Sensory stimulus	Agonists	Physiopathological role
TRPV1	T ≥ 43 °C	Capsaicin Vanilloid compounds Protons Anandamide Resiniferatoxin	Thermal pain sensation Thermal hyperalgesia Stretch-evoked sensation
TRPV2	T ≥ 52 °C Cell swelling	2-APB Carvacrol	Thermal pain sensation
TRPV3	T ≥ 31 °C	Thymol Eugenol 2-APB Camphor	Thermosensation Thermal preference
TRPV4	T ≥ 27 °C Cell swelling	4- α phorbol Eicosanoid	Osmotic sensation Thermal hyperalgesia
TRPM8	T ≤ 28 °C	Icilin Menthol Eucalyptol	Thermal preference Cold thermosensation
TRPA1	T ≤ 17 °C	Icilin Garlic (allicin) Cinnamaldehyde	Inflammatory pain Mechanosensation Thermosensation Cold-induced pain

Thermo-TRP Transducers to Noxious Heat

TRP vanilloid 1

TRP vanilloid 1 (TRPV1) is both a receptor for capsaicin, the ingredient of chili peppers, and related pungent vanilloid compounds, and a heat receptor, capable of depolarizing a subset of primary afferent neurons in response to noxious temperatures ($>43^{\circ}\text{C}$). Evidence for a contribution of TRPV1 to peripheral thermosensation has come from pharmacological, physiological, and genetic approaches. TRPV1 is expressed in small- to medium-diameter sensory neurons, consistent with the selectivity of vanilloid action on C- and A δ -fibers responsible for pain and warmth perception. In heterologous expression systems, TRPV1 can be activated not only by vanilloid compounds, but also by resiniferatoxin, extracellular protons, and arachidonic acid metabolites. Mice lacking TRPV1 have altered behavioral responses to capsaicin and resiniferatoxin. In cultures of DRG neurons, TRPV1 knock-out (KO) neurons exhibit a dramatic decrease in the occurrence of capsaicin-evoked cationic currents and do not respond to heating up to 50°C . Consistent with this, TRPV1 KO mice were found to exhibit prolonged latencies for tail withdrawal in assays of acute thermal nociception. However, analysis of thermosensation in these animals has produced a mixed picture. In cultured sensory neurons and skin–nerve preparations from TRPV1 KO mice, thermal sensitivity was defective, but not absent. Therefore, other heat transduction mechanisms, in addition to TRPV1, exist that can detect noxious temperatures (see below). TRPV1 channels are sensitized by inflammatory mediators such as low pH, bradykinin, nerve growth factor, and prostaglandins. TRPV1 sensitization by inflammatory mediators typically produces decreased threshold or latencies of withdrawal from thermal stimuli. In TRPV1 KO animals, the signs of thermal hyperalgesia following inflammation are markedly reduced, demonstrating that TRPV1 contributes to the transduction of painful heat stimuli, particularly in the context of inflammation.

TRP vanilloid 2

The residual responsiveness to heat stimuli in mice lacking TRPV1 argues for the existence of an additional heat transduction mechanism. TRP vanilloid 2 (TRPV2), which shares 50% amino acid identity to TRPV1, is a candidate. TRPV2 is expressed in a subpopulation of medium- to large-diameter sensory neurons that give rise to A δ /B fibers. TRPV2 shows responsiveness to high temperatures ($>52^{\circ}\text{C}$, $\sim 10^{\circ}\text{C}$ higher than that of TRPV1), but it is unresponsive to typical activators of TRPV1, including low pH and capsaicin. TRPV2 may be the thermotransducer mediating type I heat response in A δ nociceptors because these afferent fibers are unresponsive to intradermal capsaicin administration.

TRPV2 has been shown to heteromultimerize with TRPV1. This raises the possibility that TRPV2 may contribute to thermodetection within a temperature range lower than that required to activate homomeric TRPV2 channels.

Thermo-TRP Transducers to Non-Noxious Heat

TRP vanilloid 3 and TRP vanilloid 4

TRP vanilloid 3 (TRPV3) and TRP vanilloid 4 (TRPV4) share 40–50% homology with TRPV1. TRPV3 can be activated by temperatures above 33°C , and by camphor, which causes the sensation of warmth when applied to human skin, and irritant extracts from oregano, thyme, and clove. TRPV4, although activated by warm temperatures (25 – 34°C), was first described to be activated by hypotonic stimuli, metabolites of arachidonic acid, and by phorbol esters. TRPV4 currents show marked desensitization upon repetitive thermal challenge, in contrast to TRPV3 currents that exhibit an increase in amplitude in response to repetitive heating stimulation. TRPV3 and TRPV4 are expressed at low levels in sensory neurons, but are strongly expressed within skin keratinocytes. The expression of TRPV3 and TRPV4 in skin epidermis led to the hypothesis that keratinocytes are involved in the detection of warmth stimuli. In line with this, TRPV3 KO mice have a diminished sense for warm temperatures, in that they show an increased latency in the hot-plate and tail-immersion assays at relatively high temperatures. TRPV4 KO mice also show defect in acute thermally evoked withdrawal responses in the tail-immersion assay. Compared to wild-type (WT) animals, TRPV4 KO mice show a preference for warmer innocuous temperatures, suggesting that the channel is important for dictating the precise range of preferred temperatures within the innocuous range.

Collectively, these studies demonstrate impairment of thermotaxis behavior in TRPV3 and TRPV4 KO mice. In addition, they suggest that keratinocytes, through release of paracrine factors, such as ATP, opioid peptides, and cytokines, among others, sensitize the sensory nerve endings. Evidence for the role of keratinocytes in peripheral thermosensation remains to be established *in vivo*.

Thermo-TRP Transducers to Noxious Cold

TRP ankyrin 1

The TRP ankyrin 1 (TRPA1) channel exhibits strong expression within sensory ganglion neurons and is present in a subset of small- to medium-diameter DRG neurons that expressed TRPV1. TRPA1 is unique in that it possesses a relatively long N-terminus domain with numerous ankyrin-repeat motifs. It is insensitive to capsaicin, but is activated by a variety of pungent chemical ligands, including isothiocyanates such as those found in

mustard oil, wasabi, and garlic. Intracellular calcium can directly activate TRPA1. In addition, it has been suggested that TRPA1 may act as a sensor of painfully cold temperatures. Support for a role of TRPA1 in transducing noxious cold has come from the following observations: first, TRPA1 is activated by temperatures of about 17 °C; second, antisense oligonucleotides directed against TRPA1 have been reported to reduce behavioral hyperresponsiveness to cold after peripheral inflammation, suggesting that TRPA1 may sense cold in these disease states; and third, TRPA1 KO mice have been reported to exhibit blunted behavioral responses to a cold metal surface and to acetone-mediated cooling. However, these conclusions have been challenged. In contrast to these findings, certain papers showed that TRPA1 KO mice have a normal sensitivity to cold temperatures, while DRG neurons from these animals are still sensitive to noxious cold. Thus, the involvement of TRPA1 in transducing noxious cold certainly awaits further experiments.

Thermo-TRP Transducers to Non-Noxious Cold

TRP melastatin 8

TRP melastatin 8 (TRPM8) is a member of the TRP melastatin subfamily of TRP channels expressed in a subset of small DRG neurons distinct from those that express TRPV1. The TRPM8 channel is activated by temperatures below 26 °C, consistent with a role in innocuous cooling perception. Cooling-mimetic chemicals, including menthol and eucalyptol, activate TRPM8 and shift to warmer temperatures the threshold for TRPM8 activation. Icillin, which is structurally not related to menthol or eucalyptol, activates TRPM8 and produces a sensation of cooling in humans. Additional support for the participation of TRPM8 in physiological cold transduction comes from the observation that TRPM8 KO mice show reduced sensitivity to non-noxious cold

stimuli, whereas their behavioral responses to noxious cold temperatures are only slightly altered.

Mechanotransducers in Mammals

Although all animals employ mechanical sensations to apprehend their external and internal environments, the molecular transduction mechanisms involved in the detection of mechanical stimuli remain obscure. The ability of mechanoreceptive sensory neurons to detect mechanical information relies on the presence of mechanosensitive (MS) channels that rapidly transform external mechanical forces into electrical signals. In the few past years, genetic approaches coupled to functional studies have provided insights into the basic mechanisms by which the senses of touch and pain are transduced in mammals. TRP channels have again emerged as candidate mechanosensors ([Table 1](#)).

Properties of Native MS Channels in Sensory Neurons

Recordings of mechano-gated currents under voltage clamp were first made by JD Levine and colleagues in 1999. MS currents evoked in sensory neurons have a relatively short latency, which argues against activation of a second-messenger cascade and favors direct activation of a mechanically activated channel. In response to sustained mechanical stimulation, MS currents decline or adapt, through closure of the transduction channels. Based on these adapting kinetics, three main classes of MS currents have been identified and loosely classified as either rapidly adapting (RA), intermediately adapting (IA), or slowly adapting (SA) ([Figure 1](#)). An MS current that does not adapt within the testing range has been occasionally observed in sensory neurons. This nonadapting MS current exhibits the particularity to peak during

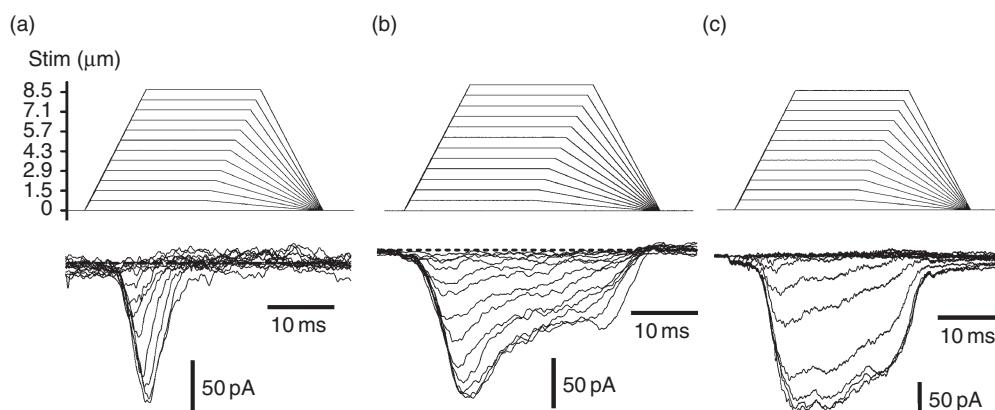


Figure 1 Families of mechanosensitive current traces evoked by a series of mechanical steps in 0.7- μm increments. Mechanosensitive currents showing (a) rapid-, (b) intermediate-, and (c) slow-adapting kinetics recorded in sensory neurons. Mechanical ramp stimulations (top traces) were applied at a holding potential of -60 mV ; the probe velocity was $850\text{ }\mu\text{m s}^{-1}$.

the stationary part of the mechanical stimulus. The majority of large-diameter sensory neurons express RA MS currents, whereas small- to medium-diameter sensory neurons preferentially display SA/nonadapting MS currents. The differences in properties of MS currents among sensory neuron subpopulations *in vitro* are consistent with the *in vivo* physiological properties of low-threshold mechanoreceptors (LTMs) and nociceptors.

RA and SA MS currents in sensory neurons exhibit reversal potentials at about 0 mV and are carried by channels nonselective for cations and impermeable to anions. The similarity in the ionic selectivity of these MS currents among different populations of sensory neurons suggests that closely related ion channel subunits mediate these currents. The ability of RA MS currents to pass large organic molecules, such as TEA, choline, and Tris, strongly favors the view that the pore of the underlying MS channels is relatively large.

While MS currents show the ability to be carried by Ca^{2+} and, to a lesser extent, by Mg^{2+} , both Ca^{2+} and Mg^{2+} at physiological concentrations produce a partial block of MS currents. Although the mechanism of the block is still unknown, it may be due to a Ca^{2+} -binding site within the pore causing reduced permeation to Na^+ .

Pharmacological studies of MS channels have been dominated by the use of unselective blockers such as metal cations. Not surprisingly, gadolinium (Gd^{3+}), a widely used blocker of various cationic conductances, blocked all MS currents in sensory neurons. Amiloride, another nonselective drug that blocks many epithelial Na^+ channels and TRPP-like channels, inhibited most MS currents at relatively high concentrations. These properties are shared by many mechano-gated cationic channels in other systems, including spider mechanoreceptor neurons, *Xenopus* oocytes, and mammalian hair cells. Recently, it has been shown that a conopeptide analog termed 'NMB-1' (for noxious mechanosensation blocker 1) preferentially blocks SA MS currents in sensory neurons. Indeed, NMB-1 has an approximate 30-fold selectivity for MS channels mediating SA currents over those carrying RA currents. Biotynylated NMB-1 principally binds to peripherin-containing sensory neurons, which are typically considered as nociceptors. Consistently, animals show reduced mechanically evoked behavioral responses to high-intensity mechanical stimuli in the presence of NMB-1. Collectively, these data reinforce the view that SA and RA MS currents are expressed in functionally distinct sensory neuron populations and are carried by different molecular entities.

Non-TRP Mechanotransducers

Acid-sensing ion channels

Acid-sensing ion channels (ASICs) belong to an H^+ -gated subgroup of the DEG/ENaC channel family of cation

channels. Four ASIC genes have been cloned and encode seven channel subunits. Although all ASIC channel subunits are expressed in the peripheral nervous system, only ASIC2a, b and ASIC3 are specifically expressed in mechanosensory neurons. They are expressed in DRGs and transported from the cell bodies to specialized mechanosensory structures, such as Meissner corpuscles and Merkel cells, as well as to penicillate and lanceolate nerve endings surrounding hair follicles. Both channel subunits are also expressed in unmyelinated free nerve endings of the skin, suggesting a role in the transduction of mechanonociceptive stimuli.

Recombinant ASIC channels failed to demonstrate any mechanosensitivity. Furthermore, the biophysical properties and pharmacology of these heterologously expressed ASIC channels differ from MS cation channels recorded in native sensory neurons, suggesting that ASIC subunits may not contribute to the pore-forming subunit of the mechanosensory apparatus. In line with this, no differences in the current amplitude or kinetics of MS currents were seen in ASIC2/ASIC3-null mutant mice.

The role of ASIC channels has been also investigated in behavioral studies using mice with targeted deletion of ASIC channel genes. ASIC2 KO mice exhibit a decreased sensitivity of both RA and SA LTMs. By contrast, ASIC3 KO mice show an increase in sensitivity of RA LTMs, no change in SA LTMs, and reduced responses of myelinated high-threshold mechanoreceptors (HTMs) to noxious stimuli. Although these data argue for the involvement of ASIC subunits in mechanotransduction, the definite demonstration that ASIC subunits carry MS currents in mammalian sensory neurons awaits further experiments.

Stomatin

In *C. elegans*, MEC-2 encodes an integral membrane protein with a stomatin homology domain involved in touch receptor function. MEC-2 exhibits a central sequence of 247 amino acids that possess 64% homology with the mammalian protein stomatin. The use of mutant mice lacking stomatin-like protein 3 ($Slp3^{-/-}$) provided evidence to suggest that SLP3 is an important determinant of skin mechanoreceptor functions. About 36% of sensory neurons recorded *in vitro* show no responses to mechanical stimuli in $Slp3^{-/-}$ mice, compared with less than 5% in WT sensory neurons. Because the proportion of cells that normally display RA and SA MS currents decreased conjointly, it can be hypothesized that SLP3 is necessary for both types of underlying MS channels. At the behavioral level, the loss of SLP3 impairs tactile discrimination capability and touch-evoked pain following neuropathic injury. Therefore, SLP3 appears as an essential element of the mechanosensory apparatus in mammalian mechanoreceptors. Although its precise function is still unknown, SLP3 may be envisioned as a

putative linker between MS channel subunits and the underlying microtubules, as proposed for its *C. elegans* homolog MEC-2.

TRP Mechanotransducers

TRPV channels

TRPV channels are best known for sensing heat and mediating neurogenic inflammation; however, some have also been implicated in mechanosensation. TRPV1, which is activated by capsaicin and heat, is also required for normal stretch-evoked reflexes in the bladder and for osmosensation in circumventricular neurons. However, it cannot directly sense and respond to mechanical stimuli and *Trpv1*-null mutant mice exhibit normal acute mechanical sensitivity and mechanical hyperalgesic responses following tissue inflammation.

Some evidence has implicated TRPV2 in aortic myocyte responses to membrane stretch and hypotonic stimulation and in the constriction of blood vessels under increasing pressure. Although TRPV2 is preferentially expressed in large-diameter somatosensory neurons, its role in mechanotransduction remains to be tested.

TRPV4 has been proposed to act as an osmosensor because, in addition to warm temperature and acidic pH, it is activated by cell swelling through an indirect mechanism requiring fatty acid metabolites. In that sense, TRPV4 cannot be considered as a real mechanosensor and there must be an upstream element to fulfill this function. It is a crucial determinant in shaping the response of nociceptive neurons to osmotic stress. Disrupting *Trpv4* expression in mice has only modest effects on acute mechanosensory thresholds, but strongly reduces sensitivity to noxious mechanical stimuli. In addition, TRPV4 has been implicated in mechanical hypersensitivity during inflammation.

TRPC1 channels

Known as a store- and receptor-operated channel, the canonical TRP channel TRPC1 has been recently proposed to form the stretch-activated cation channel constitutively expressed in *Xenopus* oocytes. TRPC1 antisense reduces the endogenous stretch-activated current in oocytes, whereas overexpression of TRPC1 increases the current. Moreover, TRPC1 expression in Chinese hamster ovary, clone K1 (CHO-K1) cells results in a fivefold increase in MS cation current density. Against the presumed role of TRPC1 in mechanotransduction, however, is the observation that this channel is widely expressed in mammalian cells, most being devoid of mechanosensory functions. Perhaps, the MS signaling properties of TRPC1 rely on its interaction with other MS TRP subunits. Evidence has appeared to suggest that TRPC1 can interact with the putative MS TRPP2, a channel involved

in the mechanical detection of laminar fluid flow in renal epithelial cells. Whether TRPC1/TRPP2 heteromers form functional channels that sense mechanical stimuli remains to be tested.

TRPA1 channels

No mechanoreceptor potential C (NomPc), a member of the TRPN cation channel subfamily in *Drosophila*, together with its homologs in *C. elegans* and vertebrates, has been consistently implicated to play critical roles in mechanotransduction. A unique feature of these TRPN-related channels is their large N-terminal domains harboring numerous ankyrin repeats. This prompted the suggestion that these N-terminal domains may serve as tension transmission structures to the pore-forming region.

TRPN channels are not present in the genome of reptiles, birds, and mammals. The only mammalian TRP subunit with an extended domain of ankyrin repeats is TRPA1. This subunit was suggested to form the main mechanotransducing channel of the inner ear, but this proposal was not corroborated by knock-out strategies since *Trpa1*^{-/-} mice showed normal auditory responses and hair-cell transduction currents. Recently, the *C. elegans* ortholog of mouse TRPA1 has been shown to be expressed in mechanosensory neurons and contributes to neural responses of these cells to touch. In addition, mechanical pressure can activate *C. elegans* TRPA1 heterologously expressed in mammalian cells. These data demonstrate for the first time that *C. elegans* TRPA1 encodes an ion channel that can be activated in response to mechanical pressure.

The expression of TRPA1 in small-diameter neurons of DRGs and TGs in mammals led to the suggestion of its possible implication in mechanical pain sensation. Consistent with TRPA1's expression pattern in sensory neurons, mice lacking TRPA1 are deficient in the detection of acute high-threshold mechanical stimuli applied to the extremities. However, contrary to these findings, the Julius group did not report deficit in responses to noxious mechanical stimuli in *Trpa1*^{-/-} mice. Kwan and co-workers in 2006 went on to further demonstrate a role of TRPA1 in mechanical hyperalgesia by showing that the mechanical pain threshold after bradykinin-induced inflammation is significantly higher in *Trpa1*^{-/-} mice compared to WT. Taken together, these data provide substantial evidence suggesting that TRPA1 mediates responses to high-threshold mechanical stimuli in nociceptive neurons. However, further investigations are clearly needed to determine whether TRPA1 is a real mechanotransducing channel and to clarify the existing discrepancies regarding its role in mammalian mechanosensation.

Conclusions

Emerging features of the TRP channels include their physiological roles in sensory functions. Some TRP channels, especially TRPM and TRPV, are equipped to detect thermal changes from noxious cold to noxious heat. TRPM8 and TRPV3/TRPV4 encode cool and warm, respectively, and TRPV1 and TRPV2 sense noxious heat. TRPV4 and TRPV1 respond to thermal, chemical, and mechanical stimuli as well. Others, including TRPA1, are probably involved in mechanosensation and cold transduction. Typically, TRP channels are activated by a plethora of chemical and physical stimuli. Such gating promiscuity allows TRP channels to play a key role in a wide range of sensory processes, including osmoregulation, thermal, and chemical signaling. Importantly, some of these TRP channels are involved in life-threatening human diseases such as mucolipidosis IV, polycystic kidney disease, hypomagnesemia, glomerulosclerosis, and many others. TRPV1 has been linked to chronic pain, neuropathies, bladder disorders, irritable bowel syndrome, and gastroesophageal reflux disease, among others.

While the discovery of thermosensitive TRP candidates has greatly enhanced our understanding of transduction mechanisms of thermal stimuli, findings in selective KO animals indicate that additional, yet unknown, transduction mechanisms contribute to thermal sensation. In addition, some important channels involved in touch and painful mechanical stimuli have not been unequivocally identified, and the exact role of TRP channels in mechanotransduction remains to be investigated in depth. Future studies will undoubtedly be focused on gaining insight into thermal and mechanical activation and the role of TRP channels in disease pathophysiology.

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See also: Drug Sensitization and Drug Abuse; From Sensation to Perception; Neurotransmitters and Neuromodulators Regulating Sleep and Wakefulness; Pain and Addiction; Thermoregulation.

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Hormonal Contributions to Arousal and Motivation

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Glossary

Appetitive behavior – A word with many meanings, including the variable behavior impelled by internal deficits or any behavior that increases the probability of satisfaction of a need.

Arousal – It determines an organism's responsiveness to sensory stimuli, level of voluntary motor activity, and emotional reactivity.

Consummatory behavior – Any stereotyped behavior pattern supposed to end a sequence of behavior or causes the fulfillment of a need.

Lordosis – Female mating posture consisting of immobility with back arched downward, extended hindlegs, and the tail deflected to the side. In many mammals, this behavior pattern is absolutely dependent on estrogens.

Motivation – A concept referring to the ease by which a behavior is activated as well as the specific direction of behavior and its persistence.

Hormones link the brain with the rest of the body and help to render behavior concordant with the state of the body and with the environment. One of the ways they accomplish this is through changes in specific motivational states such as sexual desire, hunger, or fear. However, they also work through changes in the underlying states of arousal, global central nervous system (CNS) functions that provide for the activation of behavior. This article deals both with specific motivational forces and with generalized CNS arousal.

Motivation

The ability of sex hormones to increase the performance of sexual behaviors, when all other aspects of the experiment are held constant, is proof of the ability of these sex hormones to increase sexual motivation. That is, in the quantitative relations between environmental stimuli and biologically important responses, changes in motivation account for changes in behavioral response when the environment is held constant. For both experimental animals and human behavior, motivational states are subdivided into their specific aspects (e.g., sex, hunger,

thirst, and fear) and their general aspects (accounting for the activation of any behavior). These general aspects usually go under the name 'arousal,' which in turn can be subdivided into specific forms (leading to alertness, attention, etc.) and general forms (including but not limited to the sleep/wake cycle).

The general features of hormonal actions on motivation have been summarized in the form of several principles:

1. Hormones can either increase or decrease motivational states.
2. A single hormone can have many effects.
3. Combinations of hormones may be required for motivational change.
4. Hormonal metabolites may be the motivationally active compounds.
5. There may be optimal hormone concentrations; too much or too little can be damaging.
6. In molecular terms, both membrane-initiated and genomic actions of hormones may be necessary and may synergize with each other.
7. Hormonal effects on motivation may depend on the environmental context.
8. Hormonal mechanisms have been largely conserved from the animal brain into the human brain.

Arousal

It is clear that sex hormones heighten CNS arousal. In female four-footed animals, estrogens cause greatly increased muscular tension and increased locomotion. In male animals, androgenic hormones cause the male to fight for territory and mates, to explore to seek out females, and then to mount them.

Sex hormones are certainly not the only types of hormones that can influence CNS arousal. Consider thyroid hormones, including the chemical form in which the thyroid secretes the hormone, thyroxine (T4), and the chemical form most active at thyroid hormone receptors, triiodothyronine (T3). High levels of these hormones in the human produce nervous, jittery, hyper-aroused individuals. Abnormally low levels produce a sluggish, underaroused patient, a form of disease called myxedema.

The most primitive, elementary form of arousal is called ‘generalized arousal,’ and has been given the following operational definition: a more aroused animal or human being

1. is more responsive to sensory stimuli in all modalities.
2. emits more voluntary motor activity, and
3. is more reactive, emotionally.

Ascending neuroanatomical systems supporting generalized CNS arousal are well known, and include noradrenergic, dopaminergic, histaminergic, serotonergic, and cholinergic systems, augmented by the actions of orexin/hypocretin. All of these systems are influenced by hormones.

A Specific Hormone-Influenced Motivated Behavior, and How Hormone-Influenced Generalized Arousal Influences It

The most complete analysis of a motivated behavior is the estrogen-driven mating behavior of female four-footed mammals. It is called lordosis behavior and constitutes a dorsiflexion of the vertebral column – a sway-backed posture by which the female permits fertilization. Successful analysis of this behavior shows how steroid hormone effects on nerve cells can direct natural, instinctive behaviors.

First, discovering exact cellular targets for steroid hormones in the brain. A system of hypothalamic and limbic forebrain neurons with sex hormone receptors, discovered in rodents, was later found to be present in species ranging from fish through to primates. This hormone-sensitive system is apparently a general feature of the vertebrate brain. *In situ* hybridization studies to distinguish gene expression for estrogen receptor alpha (ER- α) from estrogen receptor beta (ER- β) revealed remarkably different distributions for the two and showed the estrogenic-feedback sensitivity of ER- α gene expression. We have visualized these ER- α -producing cells using modern transgenic techniques: in the ventromedial nucleus (VMN) of the hypothalamus, not only ER- α but also ER- β mRNA can be downregulated by estradiol administration. Use of an adeno-associated viral (AAV) vector encoding a small interfering RNA (siRNA) directed against the mRNA for ER- α allowed us to microinject it into the VMN with the result that proceptive and lordosis behaviors were abolished.

Second, working out the neural circuitry for hormone-dependent female reproductive behavior, the first behavior circuit elucidated for any mammal. Electrical activity in neurons at the top of this circuit, the VMN of the hypothalamus predicts a chemical compound’s ability to influence lordosis. Third, involves the demonstration of several genes that are turned on by estrogens in the forebrain.

Fourth, showing that these gene products facilitate reproductive behavior. For example, the induction of one of them – the gene for the progesterone receptor – showed that the hormone estrogen could activate another transcription factor important, in turn, for behavioral control.

Taken together, these four sets of studies have proved for the first time how specific chemicals acting in specific parts of the brain could determine individual behavioral responses.

How do generalized arousal forces influence hormone-responsive hypothalamic neurons to increase sexual arousal and sexual behaviors? For the female, we have four mechanisms, discovered by biophysical techniques, that work in parallel: histamine and norepinephrine, generalized arousal transmitters, increase the electrical excitability of neurons in the hypothalamic cell group that drive estrogen-dependent sexual behavior. The other two routes are inhibitory. μ -Opioid peptides and prostaglandin D reduce generalized arousal and reduce female sexual behavior.

Lessons

A series of surprising lessons in gene–behavior relations has emerged from a long series of studies using knockouts of ER- α and ER- β genes. (1) The effect of a given gene on a given type of behavior depends on where in the brain and exactly when that gene is expressed. (2) The effect of the ER- α gene on aggressive behavior in males is the opposite of that in females. (3) The effects of ER genes on aggressive and locomotor behaviors can depend upon the age at which the behavioral assay is conducted. (4) The effect of a specific gene on aggressive behavior can depend on the type of aggression tested and the nature of the opponent.

Finally, comparisons of the causal influences of the genes encoding ER- α and ER- β on the reproductive and aggressive behaviors of female and male mice led to the principle that, for the mammalian CNS, we have moved beyond the classical ‘one gene–one enzyme’ concept of George Beadle and Edward Tatum derived from the classical, Nobel-recognized work with *Neurospora*. Instead, for mammalian CNS and behavior we have ‘patterns’ of gene expression governing ‘patterns’ of behavior.

The ‘Appetitive versus Consummatory’ Distinction in Motivated Behaviors

Progress in understanding the hormonal, neuroanatomical, biophysical, and molecular underpinnings of hormone-regulated motivation has been so remarkable that we now can turn back to certain classical intellectual problems in the field and look at them with a new view.

Perhaps the most important is the distinction between appetitive behaviors (ABs) and consummatory behaviors (CBs). Clearly, the CNS arousal systems described above serve both. Because generalized arousal is essential for the activation of any behavior, it is easy to see, for example, in the case of male mating behavior, that a high level of CNS arousal is required for the male to search for and find a female; but then, of course, it is also required to support mounting, erection, and ejaculation.

One of the present authors (DP) subscribes to the AB/CB distinction for three reasons. (1) From a common sense point of view it is intellectually, intuitively appealing. (2) It was useful in the work of the classical ethologists such as Konrad Lorenz and Niko Tinbergen for the description of natural behaviors in the home environments of the animals. (3) From a neuroscientific point of view, it may provide a terminology to help separate out different stages in the production of a motivated behavior and analyze those stages individually. For example, it is clear that preoptic neurons regulate both AB and CB for male sexuality. In some animals, more rostral preoptic region cell groups affect AB, and more caudal, CB. It is a sign of high accomplishment in this field that scientists can turn to this type of question with considerable cellular and molecular data that bear on the issue. Some previous authors have criticized the distinction (1) because they find it difficult to identify a clear boundary between AB and CB; and (2) because in a long chain of behaviors an individual response might be the CB of a preceding behavior and the AB of a subsequent behavior.

The other author (AA) does not think the ‘appetitive/consummatory’ distinction is useful. The reasoning behind this rejection is straightforward, but it needs to be put into a context in order to be comprehensible. Basically, we need to accept that behavior is a sequence of motor patterns. These motor patterns are frequently classified in categories based on prominent and/or stereotyped actions. Motor patterns characteristic of food ingestion can be grouped under the label eating, while motor patterns typical of copulation can be grouped under the label sexual behavior, for example. However, before executing the stereotyped movements associated with food consumption or copulation, an animal needs to detect and approach food or a mate. The motor patterns displayed while approaching food or a mate are highly variable. In fact, they are determined by the context. Furthermore, identical motor patterns may be employed for approaching different kinds of stimuli. Thus, while the motor patterns displayed during actual commerce with a stimulus like food or a mate are highly specific for that particular stimulus, motor patterns leading to approach are independent of the stimulus activating them.

It might intuitively appear convenient to label the variable approach behaviors appetitive and the ensuing stereotyped behaviors consummatory. However, the

common use of the terms appetitive and consummatory rarely coincides with this distinction. In the case of female rat sexual behavior, some stereotyped patterns like ear wiggling and hop-darting, behaviors specifically activated by proximate stimuli from the male, are frequently considered appetitive, while another stereotyped motor pattern, lordosis, equally activated by proximate stimuli provided by the male, is considered consummatory. The logic behind that distinction is impenetrable unless we accept that the term consummatory means fulfillment. Lordosis is a motor pattern making vaginal penetration possible, thereby eventually allowing the male to deposit sperm which would represent the fulfillment of the biological purpose of copulation. However, a female rat will display lordosis in response to a male’s mount during the entire period of proestrus, essentially irrespective of how many ejaculations she might have received. Thus, the purported fulfillment associated with lordosis resides in the eyes of the beholder and not in the female rat’s brain.

Furthermore, the fact that the female continues to display lordosis as long as her brain is exposed to adequate amounts of ovarian hormones excludes another use of the term consummatory, namely acts that bring a behavior sequence to an end. The display of one lordosis does not reduce the likelihood for the display of a second or third, and so on. Likewise, the attainment of one ejaculation in a male rat does not reduce the likelihood of attaining a second or third in rapid succession. The inevitable conclusion is that it is no exaggeration to maintain that the concept of consummatory behavior is arbitrary, in the same way as appetitive behavior.

A most illustrative example of this arbitrariness would be an animal walking from a very hot place to a place with a temperature close to the thermoneutral zone. Whether the behavior of walking is appetitive or consummatory is impossible to determine. It is appetitive because it brings the subject into contact with a desirable end-state, and it is consummatory because it fulfills the biological purpose of behavioral thermoregulation (and, coincidentally, ends the behavioral sequence). How we decide to label the walking behavior becomes a matter of personal inclination, which is an undesirable situation in science.

Terms with many simultaneous meanings are always a source of confusion, and they probably obscure the understanding of basic behavioral processes. Consequently, if we wish to persist in the habit of subdividing the continuous flow of behavior into categories, this division should at least not be based on the empirically void distinction between appetitive and consummatory. It could be based upon the distinction between arbitrary approach behaviors – independent of the stimulus being approached – and specific stereotyped behaviors activated by the stimulus, as already suggested. Such a distinction coincides with current knowledge of brain function. There are several examples of experimental manipulations affecting the stereotyped

behaviors activated by a specific stimulus while leaving the variable approach behaviors to that same stimulus intact. Although both kinds of behaviors ultimately depend on adequate motivation and general arousal, approach is controlled by distant stimuli while the stereotyped behaviors are activated when the stimulus source is in close proximity.

It is even likely that the variable approach behaviors are controlled by stimulus modalities different from those controlling the stereotyped motor patterns displayed when in direct contact with the stimulus. In the female rat, for example, olfaction is essential for approach to a mate, while tactile stimuli control lordosis. No wonder, then, that the central nervous circuits controlling approach are partly different from those controlling lordosis. However, when a behavior is influenced by hormones, both the variable approach behaviors and the stimulus-specific stereotyped behaviors seem to be affected by the same hormone, and it is possible that hormone action at a single brain site controls both.

See also: Genes and Behavior: Animal Models; Motivation.

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Incentive Motivation and Incentive Salience

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Glossary

Hedonic impact ('liking') – Hedonic impact or 'liking' corresponds to activation of brain mechanisms that generate the pleasure of a reward. Hedonic generating mechanisms are sometimes called 'hedonic hotspots,' and include cubic-millimeter sites in the nucleus accumbens and ventral pallidum where opioid and endocannabinoid signals can amplify 'liking' for sweet tastes and related sensory pleasures.

Incentive salience ('wanting') – Incentive salience is a form of motivation, sometimes called 'wanting,' that is generated by brain mesolimbic systems involving dopamine. Incentive salience is actively attributed to brain representations of reward stimuli and their associated cues. Attribution makes those stimuli and cues become attention grabbing and perceived as attractive or 'wanted,' and able to elicit behavioral approach and pursuit. Under some circumstances, incentive salience can occur without conscious desire, resulting in unconscious 'wanting.'

Incentive sensitization – A brain-based theory of addiction, which posits that drugs produce long-term sensitization changes in brain mesolimbic dopamine-related systems that mediate 'wanting' or incentive salience for rewards. Sensitization produces increases in 'wanting' to take drugs, without necessarily increasing 'liking' for the same drugs. Sensitization may last years after drug use is stopped. Sensitized 'wanting' may thus cause relapse back into drug taking, even for drugs that will not cause much pleasure, and even if the addict no longer suffers from withdrawal.

Many types of evidence combined against reduction concepts, but perhaps the single most important line of evidence against drive reduction concepts, came in the 1960s from studies of brain stimulation reward and related studies of motivated behavior elicited by "free" brain stimulation. A single electrode in the lateral hypothalamus could both elicit motivated behavior (if just turned on freely) and have reward or punishment effects (if given contingent on the animal's response). Many behavioral neuroscientists of the time believed in drive reduction theory. So, at first, they expected to find that brain sites where stimulation would reduce eating (presumably by reducing drive) would also be the sites where stimulation was rewarding (again, presumably by reducing drive). Conversely, they believed the opposite would be true too. They expected to find that punishing electrodes would sometimes activate drives such as hunger.

Surprisingly to most behavioral neuroscientists of the era, the theory was not true, and many of the predictions based on it turned out to be wrong for explaining natural motivations such as hunger, thirst, or sex. In most cases, the opposite results were found instead. The brain sites where the stimulation caused eating behavior were almost always the same sites where stimulation was rewarding. Eating electrodes were not punishing electrodes. Instead, the eating electrodes were reward electrodes. Stimulation-induced reward and stimulation-induced hunger drive appeared identical or, at least, had identical causes in the activation of the same electrode. This meant that the reward of the lateral hypothalamic electrode could not be due to drive reduction from the same electrode. Instead, the reward electrode increased the motivation to eat; it did not reduce that drive. In other words, the reward must be understood as a motivational phenomenon of its own independent from drive reduction, involving its own active brain mechanisms.

Today, the active brain mechanisms of incentive motivation for reward are the topic of much research in affective neuroscience. Incentive motivation concepts rose as drive concepts fell beginning in the 1960s. One of the most useful incentive concepts is the Bolles-Bindra-Toates theory of incentive motivation. The theory posits that individuals want and like the cues that predict pleasant rewards (pavlovian conditioned stimuli), much as they ant and like the rewards themselves (food, drink, sex, drugs, etc.; pavlovian unconditioned stimuli).

This incentive motivation concept was the psychological forerunner of the incentive salience concept. The

Incentive Motivation Concepts: From the Ashes of Drive Reduction

Up until the 1960s, drive reduction reigned as the chief concept for explaining motivation. It posited that brain mechanisms of motivation acted chiefly to reduce aversive drives. However, to a large extent today drive reduction concepts have been largely replaced by incentive motivation concepts for natural motivations such as hunger, thirst, and sex. In several ways, incentive motivation explains more of the data from more experiments than drive reduction.

neurobiological forerunners of incentive salience were hypotheses that brain mesolimbic dopamine systems mediated the pleasure of rewards and the appetitive motivation to obtain them. The incentive salience concept essentially adopted the Bolles–Bindra–Toates formulation for how incentive motivation worked but split it into separate ‘liking’ and ‘wanting’ concepts, stripped the pleasure ‘liking’ role from dopamine, and assigned dopamine to a specific ‘wanting’ role.

Incentive Salience as a ‘Wanting’ Module

Incentive salience is a ‘wanting’ module: it is a particular subcomponent of what is ordinarily meant by the word, wanting. Incentive salience has evolved to add a visceral ‘oomph’ to mental desires. This is part of what makes ‘wanting’ a unique module and quite different from wanting (no quotation marks) in the usual sense of the word as a conscious, cognitive desire. That is why the author and his colleagues have put quotation marks around ‘wanting’ when writing about incentive salience. Ordinarily, incentive salience ‘wanting’ is congruent with other forms of desire such as cognitive wanting, and is also congruent with the hedonic pleasure or ‘liking’ of the outcome. But dissociations can occur among all of these utility forms, and when they do incentive salience is revealed as a distinct module of desire.

Comparison to Ordinary Meaning of the Word Wanting

How does ordinary wanting in the usual sense of desires differ from ‘wanting’? Ordinary cognitive desires involve explicit thoughts of the target or reward. In cognitive desires, or wanting in the ordinary sense without quotation marks, you know what you want, or at least think you do, you expect to like the wanted target, and you may have some idea of how you intend to get it. Such desires are guided by explicit memories of how nice the target was in the past, or if never before experienced then at least on imagination of what it would be like to experience.

By contrast, none of this cognition needs to be part of incentive salience ‘wants.’ Incentive salience is a percept-bound type of ‘wanting,’ which typically occurs as relatively brief peaks upon encountering a reward or a physical reminder of the reward (a cue). Incentive salience does not require a clear cognition of what is wanted, and does not even need to be consciously experienced as a feeling of wanting, at least in some cases (though when it is brought into consciousness, it can considerably intensify feelings of desire). Perhaps a reason for the difference is that incentive salience is mediated chiefly by mesolimbic dopamine-related brain mechanisms which are largely

subcortical, whereas cognitive forms of desire are more dependent on higher cortex-based brain systems. Incentive salience may have evolved using ‘early’ brain systems as a distinct ‘wanting’ module to pursue particular innate incentives. Possibly, it gave an elementary form of goal directedness, which could guide behavior in the right direction in advance of experiencing the goals. Later in evolution ‘wanting’ may have become harnessed to serve ‘liking’ and learned features of reward, so that most incentive salience in our lives today is probably attributed to learned reward cues.

Incentive salience as a module is only one type of wanting. It is not the one we are most aware of in daily life, because our awareness or consciousness of desire focuses on our cognitive representations of potential rewards. Cognitive representations of reward can be different from more basic incentive salience (as when you cognitively believe a reward will be bad for you, but you still ‘want’ it). However, incentive salience is important in daily life, needed to color conscious desires with motivational power, to make them compelling spurs to action. It may be a crucial component of our most intense and visceral desires, and especially important in the pathological intensity of some addictions and compulsive desires.

Incentive salience can be viewed as a motivational transform of a brain signal corresponding to the perceived object of desire or its mental. When attributed to a stimulus representation, incentive salience transforms the mere sensory shape, smell, or sound into an attractive and attention-riveting incentive. Once attributed, the incentive percept becomes difficult to avoid noticing, the eyes naturally move toward the incentive, it captures the gaze and becomes motivationally attractive, and the rest of the body may well follow to obtain it.

Signature Psychological Features of ‘Wanting’

How can incentive salience be recognized? There are several distinguishing psychological features that help it be recognized even in animal experiments as well as in human daily life. Incentive salience gives a ‘motivational magnet’ property to stimuli it is attributed to, and makes those stimuli attractive, ‘wanted,’ and potently able to elicit approach. Incentive salience also triggers momentary peaks of intense motivation to obtain a cued reward. Such features (reward cues becoming motivational magnets, cues as objects of desire, peaks of cue-triggered ‘wanting’ for the actual reward) allow us to recognize incentive salience in behavioral neuroscience experiments with animals as well as in people.

In many relevant neuroscience experiments, the brains of rats or mice are manipulated in painless ways that alter the operation of underlying substrates for ‘wanting.’ For

example, microinjection of tiny quantities (a two-thousandth of a milliliter) of amphetamine solution is directed into a targeted brain structure, such as the nucleus accumbens, or into its major target, the ventral pallidum. The drug stimulates dopamine release by neurons in the target brain site.

When activated by a brain microinjection, the chief features of incentive salience that become enhanced are cue-triggered ‘wanting’ for rewards, the potency of reward cues as motivational magnet, and their potency as conditioned reinforcers. These features are described below.

Cue-Triggered ‘Wanting’: Temporary Peaks of Desire

One feature of incentive salience is to endow reward-related cues (in experiments these are pavlovian conditioned stimuli or CSs) with ability to trigger powerful peaks of ‘wanting’ for their own associated reward. For example, the scent of food may suddenly make you ravenous as lunchtime approaches even if you were not feeling particularly hungry moments before that cue occurred. The tinkling sound of an email arriving in your computer inbox may trigger a sudden urge to check the message. In all such cases, cue-triggered ‘wanting’ occurs as a temporary peak, bound to a particular encounter with a cue or to a vivid mental image of the reward. Peaks of cue-triggered ‘wanting’ are sudden, intense, temporary, reversible, and repeatable.

Behavioral neuroscience experiments have dramatically amplified cue-triggered ‘wanting’ by stimulating brain mesolimbic dopamine systems. The most specific way to do that is by placing a tiny droplet of amphetamine drug in the nucleus accumbens. Amphetamine causes the ends of dopamine-containing neurons to release extra amounts of dopamine onto their target neurons. The psychological result is typically to increase the incentive salience that cues trigger for their reward without altering other components of desire or reward. In such experiments, a rat’s brain would have been constantly flooded with dopamine after an amphetamine microinjection, yet its ‘wanting’ came and went with the coming and going of the physical cue for sugar. Incentive salience thus reflects a synergy between brain states of mesolimbic activation and particular events happening in the world. Both must be present to trigger the ‘want.’ These constraints of operation help show the psychological boundaries of the ‘wanting’ module and reveal it as just one among several psychological mechanisms of desire – albeit a powerful one when present.

Cues as Motivational Magnets: Good Enough to Eat

A second feature of incentive salience is that its attribution to a reward-related stimulus may make that stimulus ‘wanted,’ even if the stimulus is just the cue. The pavlovian

CS stimulus becomes an attractive ‘motivational magnet’ itself, in addition to triggering ‘wanting’ for its hedonic reward, although the CS cue is only a learned predictor for the reward that has little to no intrinsic value of its own. In a sense, a cue can even become ‘good enough to eat.’ For example, incentive salience may make a rat try to ‘consume’ a metal object whose presence predicts sugar, with nibbles and sniffs of the metal cue that are similar to movements used in eating actual sugar. Cues for other rewards become attractive in their own ways. Crack cocaine addicts, for instance, have been reported to compulsively ‘chase ghosts’ when they have no cocaine, that is, to scrabble around on the floor after tiny whitish specks under the table, even if they know the specks are more likely to be sugar. They are chasing a visual cue for cocaine: white specks. Such motivational magnet features of attractive cues are made much more potent by activation of mesolimbic systems, such as the amygdala or nucleus accumbens. Motivational magnet features of reward cues may account for a host of phenomena in which individuals become pulled toward reward cues acting as beacons to guide brain motivation systems.

Conditioned Reinforcers: Working to Obtain the Cue

A related mark of a motivational magnet cue is that individuals may ‘want’ to possess that cue, just as they ‘want’ its hedonic reward. Animals in an activated brain state will work harder to obtain a CS or cue that is attributed with incentive salience, just as they would work to obtain the actual reward. This is sometimes called conditioned reinforcement.

Unconscious Aspects of Incentive Salience ‘Wants’

Another way in which incentive salience diverges from intentionality, as well as from the ordinary sense of explicit desire that one is aware of, is that it need not always be conscious. Examples of unconscious core ‘wanting’ have been demonstrated people ranging from drug addicts to ordinary college students.

Brain Bases of Incentive Salience

Incentive salience is generated chiefly by subcortical brain circuits, especially the mesocorticolimbic dopamine system. Other neurotransmitters important to incentive salience include opioids (natural brain neurochemicals similar to poppy-derived opiate drugs such as opium, heroin, or morphine) which can also cause ‘liking’ as well as ‘wanting’ when delivered into cubic-millimeter

hedonic hot spots in nucleus accumbens or ventral pallidum, glutamate from the brain cortex and from amygdala, thalamus and hippocampus, and gamma aminobutyric acid (GABA) from many sources. We have often used dopamine and opioid activations in mesolimbic structures to turn on incentive salience in our laboratory. The dopamine neurons project from the midbrain where their neuronal cell bodies are sending axon fibers upwards to nucleus accumbens, other parts of striatum, amygdala, and prefrontal neocortex. Opioid enkephalin neurons exist within these structures themselves, and other opioid neurons enter as beta-endorphin neurons from the arcuate nucleus of the hypothalamus. Amygdala and cortex neurons project down to nucleus accumbens, forming a common interchange there. Nucleus accumbens in turn projects downwards to ventral pallidum and eventually up again into prefrontal cortex, and also again back down to midbrain, where the process can start over, forming recursive loops for reward-related signals.

Manipulations of dopamine neurotransmission have proven especially useful in manipulating and isolating incentive salience to reveal its psychological features. Elevations in dopamine, more than other neurotransmitters in this system, appear to selectively activate ‘wanting’ as a psychological function. Manipulations that selectively boost brain mesolimbic dopamine signals tend to specifically increase ‘wanting,’ without necessarily increasing other reward aspects such as ‘liking’ or hedonic pleasure, or other motivation aspects such as cognitive desires. In addition to activating dopamine systems by direct drug administration, it is also possible to make them permanently hyper-reactive by inducing what is called neural sensitization via a repeated series of binges with addictive drugs. Neural sensitization of mesolimbic systems by drugs causes a similarly selective but enduring enhancement of cue-triggered ‘wanting.’ Thus, it is a useful shorthand to think of brain dopamine systems as powerfully controlling ‘wanting’ (though other neural stages of the mesocorticolimbic circuit are involved too).

Addiction and Incentive Sensitization

Human drug addiction may be a special illustration of intense “wanting” that results from permanent sensitization of mesocorticolimbic. Sensitized ‘wanting’ may rise to quite irrational levels. That is, the intensity of cue-triggered ‘wanting’ to take drugs for brain-sensitized addicts could outstrip their ‘liking’ even for pleasant drugs, outstrip their expectation of how much they will like the drugs, and outlast any feelings of withdrawal if they stop. Brain-sensitized addicts may be unable to give a reason for their drug taking in such a case. Indeed, there is no reason; there is only a cause for why they ‘want’ so much. Sensitization is a second reason besides withdrawal

escape for why addicts are so prone to relapse. Sensitization also lasts much longer, and may possibly be permanent, and so it may especially contribute to relapse in ‘recovered’ addicts who have already succeeded through detoxification therapy. Even though a sensitized addict may no longer experience strong withdrawal symptoms, they will be vulnerable to intense peaks of cue-triggered ‘wanting’ to take drugs again.

Sensitization of mesolimbic systems arises as a permanent change that addictive drugs can produce in the brains of susceptible individuals. Sensitization increases the neurochemical responsiveness of these neurons and can even change their physical shapes. Individual susceptibility is determined by genetic factors, hormonal factors, previous drug experiences, and previous experiences with major stresses in life. Sensitization is also influenced by drug dose and the speed with which it reaches the brain, and is facilitated most when the drugs are taken in binges. Neural sensitization of mesolimbic dopamine systems means that the brain system becomes hyper-reactive to drugs. The system is not constantly hyperactive in a stable fashion, but it can be put temporarily into a hyperactive state by reaction to the drug again or to related cues: it is hyper-reactive to particular stimuli. Sensitization of mesolimbic systems may create compulsive levels of ‘wanting’ for drugs or other addictive incentives. A sensitized brain responds with extra incentive salience to reward cues just as a brain that has been drugged with amphetamine does – even if the sensitized brain has no drug on board at that moment. A sensitized addict’s brain, on encountering the right drug cue, would irrationally “want” the cued reward at that moment because of excessive incentive salience – even if the person cognitively expected not to like it very much and eventually did not like it much in the end. Crucially, sensitization may last years after an individual stops taking any drugs.

‘Liking’: Hedonic Hot Spots for Generating Pleasure

Different from ‘wanting’ is ‘liking’ or the core process of hedonic pleasure. A much more restricted brain circuit appears to mediate hedonic ‘liking’ than incentive ‘wanting.’ The generation of pleasure ‘liking’ is more restricted neurochemically: opioid stimulation but not dopamine stimulation (whereas ‘wanting’ is enhanced by both). ‘Liking’ is also more restricted anatomically: enhanced by opioid ‘hot spots,’ which are about a cubic millimeter in the brains of rats and probably about a cubic centimeter in human brains, but not by the rest of the same limbic structure that contains the hot spot (even if the entire structure can enhance ‘wanting’). ‘Liking’ generation is also more restricted as a brain circuit, requiring unanimous activation of multiple hot spots simultaneously

(whereas ‘wanting’ can be enhanced by a single hot spot). In short, enhancement of pleasure ‘liking’ is fragile, and brain pleasure systems are relatively recalcitrant to activation compared to ‘wanting’ systems. Consequently, our limbic mechanisms may consign us more often to states of desire than of pleasure.

Conclusion

‘Wanting’ a reward is distinct from ‘liking’ the same reward. The two can sometimes diverge, and notably, ‘wanting’ can become unjustifiably high in certain circumstances. The identity of incentive salience as a distinct module within desire creates conditions under which in some cases, such as addiction, incentive salience ‘wanting’ to persist in the face of a fully sincere cognitive intention to do the opposite.

See also: Acute Dependence; Alcoholism; Animal Models of Behavior: Alcohol Addiction; Animal Models of Sexual Function; Basal Ganglia; Brain Imaging and Addiction; Brain Stimulation and Addiction; Cellular Plasticity in Cocaine and Alcohol Addiction; Cognition: Attention and Impulsivity; Cognition: Learning and Memory: Pavlovian; Compulsive Buying; Conscious and the Unconscious; Control of Food Intake; Drug Addiction; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Drug Priming; Drug Sensitization and Drug Abuse; Drug Withdrawal – Motivational View; Emotion-Cognition Interactions; Emotions; Ethanol and Nicotine Interactions; Feeding; Genes and Behavior: Animal Models; Hallucinogens; Hormonal Contributions to Arousal and Motivation; Impulsive–Compulsive Sexual Behavior; Molecular Neurobiology of Addiction; Mouse Genetic Approaches to Psychiatric Disorders; Motivation; Motor Function and Motivation; Neural Systems of Motivation; Neurobiology of Opioid Addiction;

Neurophysiology of Drug Reward; Nicotine; Novelty; Obesity and Binge Eating Disorder; Pain and Addiction; Parkinson’s Disease; Pathological Gambling; Pleasure; Problematic Internet Use; Psychostimulants; Rewarding Brain Stimulation; Stress and Drug Craving; Stress and Reward; Subjective Experience and the Expression of Emotion in Man; Taste Perception and Behavior in Rodents and Flies; Transition to Addiction; Value of Animal Models for Predicting CNS Therapeutic Action; Vulnerability Factors in Addiction Disorders; Δ9-THC.

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Motor Function and Motivation

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Glossary

Effort – The conscious exertion of power; work done to achieve a particular end.

Energy – The capacity of acting or being active; vigorous exertion of power: i.e., effort.

Motivation – The set of processes through which organisms regulate the probability, proximity and availability of stimuli; motivation has both directional and activational aspects.

Work – The sustained physical or mental effort to overcome obstacles and achieve an objective or result.

Thus, when discussing motivation it is useful, even necessary, to understand the relation between motivational processes and other important concepts such as homeostasis, allostasis, emotion, cognition, learning, reinforcement, and sensation. For this article, the primary focus is upon the fundamental relation between motivational and motor functions.

Psychological constructs persist in the literature because they are useful; the main utility of the construct of motivation is that it provides a convenient summary and organizational structure for observable features of behavior. It is often said that behavior is directed in relation to particular stimuli, many of which can be referred to as goals. More broadly, the behavior of organisms is directed toward or away from particular stimuli, as well as activities that involve interacting with those stimuli. Organisms seek some stimulus conditions (i.e., food, water, and sex) and avoid others (i.e., pain, stress, and discomfort), both actively and passively. Another important feature of motivated behavior is that it typically takes place in phases. The terminal stage of motivated behavior, which reflects the direct interaction with the goal stimulus, is commonly referred to as the consummatory phase. The word consummatory, which often is associated with Craig, does not refer to consumption, but instead to consummation, a word of French/Latin origin; to consummate means to complete or to finish. In view of the fact that motivational stimuli usually are available at some physical or psychological distance from the organism, the only way to gain access to these stimuli is to engage in a pattern of behavior that brings them closer, or makes their occurrence more probable. This phase of motivated behavior often is referred to as appetitive, preparatory, or instrumental. The word instrumental was suggested for this specific purpose because it is equally applicable for both approach and avoidance behavior, and also because the term already is widely used in psychology (i.e., instrumental behavior or conditioning).

In addition to directional aspects of motivation, another fundamental feature of motivated behavior is that it has activational aspects. Because organisms are usually separated from motivationally relevant stimuli by a long distance, or by various obstacles or work-related response costs, engaging in instrumental behavior to obtain access to these stimuli typically involves work. Whether it is foraging in the wild or running in a maze, whether it is a rat pressing a lever in an operant chamber, or a human walking several blocks to reach a soda

Motivation is a term that is widely employed in psychology and neuroscience, which has been discussed in many different contexts and defined in several different ways. Like many psychological concepts, the discussion of motivation had its origins in philosophy. For example, in describing motivational causation, the German philosopher Schopenhauer considered how organisms must be in a position to “choose, seize, and even seek out the means of satisfaction.” Since the emergence of modern psychological sciences, motivation has been a core area of interest. Early psychologists such as Wundt and James included motivation as a subject in their texts. Although Skinner did not place much emphasis on motivation, viewing the concept as largely unnecessary, neobehaviorists such as Hull and Spence readily applied motivational concepts such as drive and incentive. In his book *Motivation and Emotion*, P.T. Young defined motivation as “the process of arousing actions, sustaining the activity in progress, and regulating the pattern of activity.” According to a more recent definition, motivation is “the set of processes through which organisms regulate the probability, proximity and availability of stimuli.” Broadly speaking, the modern psychological construct of motivation refers to the behaviorally relevant processes that enable organisms to regulate both their external and internal environment. Although we can define motivation in terms that make it distinct from other concepts or constructs, it should be recognized that, in fully discussing either the behavioral characteristics or neural basis of motivation, one must also consider related functions. The brain does not have little dotted lines or box-and-arrow diagrams that neatly separate core psychological functions into discrete, nonoverlapping neural systems.

machine, an animal must allocate resources toward stimulus-seeking behavior. Motivated behavior is therefore characterized by considerable effort, that is, speed, vigor, persistence, and high levels of work output. Although the exertion of this effort can at times be relatively brief (e.g., a predator pouncing on its prey), under many circumstances it must be sustained over long periods of time (combing over large areas while foraging, maintaining operant behavior across long sessions). These effort-related capabilities are highly adaptive, because in the natural environment survival value can depend upon the extent to which an organism can overcome work-related response costs. For these reasons, the notion that behavioral activation is a fundamental aspect of motivation has been a persistent feature of the literature in psychology and animal behavior for several decades. Early studies of the neural basis of emotion and motivation highlighted the importance of arousal and energy mobilization. Hull and Spence used the concepts of drive and incentive to emphasize the energizing effects of motivational conditions on measures of instrumental behavior, such as run speed in a maze. Cofer and Apley suggested that there was an anticipation–invigoration mechanism that could be activated by conditioned stimuli, and which functioned to invigorate instrumental behavior. Scheduled noncontingent presentation of primary motivational stimuli such as food can induce various activities, including drinking, licking, locomotion, and wheel running. Several researchers have studied the impact of work requirements on the performance of an instrumental task, which ultimately helped to lay the groundwork for the development of economic models of operant behavior. Ethologists also have employed similar concepts. Animals foraging in the wild sometimes need to exert considerable energy in order to obtain access to food, water, or nesting material, and optimal foraging theory was proposed to account for the fact that the amount of effort or time expended to obtain these stimuli was an important determinant of choice behavior. In humans, pathological aspects of behavioral activation processes also have considerable significance for clinical psychologists and psychiatrists. Fatigue, anergia, and psychomotor slowing are common symptoms of depression, and can also be present in a number of other psychiatric or neurological disorders.

As noted above, motivational processes do not stand alone, but instead should ultimately be viewed in the overall context of related psychological functions. For example, instrumental behavior is not only influenced by the motivational properties of reinforcing stimuli. Learning obviously is extremely important as well. Animals learn to engage in specific instrumental responses that are associated with particular reinforcing or punishing outcomes; effectively, organisms must learn which responses lead to which stimuli. Furthermore,

behavioral activation often is instigated by conditioned stimuli that predict motivational outcomes. In various ways, motivational functions are intertwined with cognitive, emotional, and other functions. A thorough review of all these diverse features of motivation is beyond the scope of this article. Instead, the primary focus is on the relation between motivational and motor functions. Of course, in some basic sense, every behavioral activity is fundamentally related to motor control. Consummatory activities such as food intake involve organized patterns of motor activity, such as food handling, chewing, and swallowing. However, it is particularly evident that activational aspects of motivation directly reflect features of motor output. Indeed, there is a considerable degree of conceptual overlap between motor control and activational aspects of motivation, as well as functional overlap in terms of the behavioral parameters measured and the neural systems involved.

Illustrative examples of the relation between activational aspects of motivation and motor control processes can be seen in numerous behavioral tasks. Food deprivation can make a rat run faster in a maze. Is the fundamental process in operation under these conditions motivational, motoric, or some combination of both? Locomotor activity clearly involves actions of the neural systems that regulate movement. Nevertheless, locomotor activity also is very sensitive to the impact of motivational conditions. Enhanced locomotor activity in rats can be induced by novelty or food deprivation. Furthermore, scheduled presentation of small food pellets to a food-deprived rat can generate excessive wheel running, or levels of locomotor activity comparable to those seen after administration of amphetamine. In addition, if an organism is presented with a work-related challenge during instrumental performance, it often responds to that challenge by exerting greater effort. When a rat that has been trained to lever press on a continuous or fixed ratio, 1 reinforcement schedule (1 lever press yields 1 food pellet) is then shifted to a schedule with a higher ratio requirement (e.g., fixed ratio 5; 5 lever presses yield 1 food pellet), this increased work requirement creates a substantial upward pressure on response rates. Indeed, if over multiple sessions the ratio requirement were to continue to get increase, up to levels such as fixed ratio 60 or more, the rat would eventually display a radical shift in its response speed, emitting thousands of lever presses per half hour, with the vast majority of responses occurring at local rates considerably faster than once every 500 ms. Similarly, if a rat trained to run in a maze for food reinforcement is suddenly confronted with a barrier that blocks the maze arm, it quickly adjusts its behavior and leaps over the barrier to obtain the food. If, over subsequent sessions, the barrier gets larger and larger, the rat continues to adjust to the challenge and climb the barrier. It is as though portions of the neural systems that regulate

motor output operate at the behest of those neural systems that direct behavior toward or away from particular stimuli.

The examples listed above illustrate precisely why scholars in this field have found it to be useful and important to emphasize the activational components of motivation. This is why Young defined motivation in terms of arousing actions and sustaining activity, and why Cofer thought it so necessary to posit that there was a mechanism allowing for the ability of conditioned stimuli to invigorate instrumental behavior. Conceptually, the examples listed above highlight the necessity of recognizing that there is a basic overlap between motor control and motivational processes. Of course, the terms motor control and motivation do not have exactly the same meaning. Moreover, one can easily find points of nonoverlap. For example, parkinsonian tremor is clearly more in the realm of movement control, while initiation of osmotic thirst by activation of osmoreceptive cells is more related to directional aspects of motivation. Nevertheless, it is also evident that there is a fundamental overlap as well. For that reason, it is illuminating to consider that the English words motivation and movement both are ultimately derived from the Latin word *move*, to move (i.e., *moti* is the past participle of *move*).

Over the last several decades, behavioral neuroscientists have intensively studied the brain mechanisms thought to be involved in various aspects of motivation. Considerable research and scholarship in this area has come to place emphasis on the role of nucleus accumbens as a point of functional overlap between brains systems involved in aspects of motivation and those involved in motor control. In a seminal paper aptly entitled "From motivation to action: functional interface between limbic system and the motor system," Mogenson and colleagues identified the nucleus accumbens as a point of functional interaction between limbic brain areas involved in motivation, emotion, and cognition and basal ganglia regions involved in generating behavioral output. In particular, nucleus accumbens dopamine (DA) has been strongly implicated in activational aspects of motivation and effort-related processes. As described above, scheduled presentation of food pellets to food deprived animals can induce high levels of various activities, including wheel running, drinking, and locomotion. Evidence indicates that these environmental conditions are associated with substantial increases in accumbens DA release, and also demonstrates that low doses of DA antagonists and accumbens DA depletions can block these schedule-induced activities. In contrast, interference with nucleus accumbens DA transmission, even at a level that can impair spontaneous or schedule-induced locomotor activity, generally has no effect on food consumption, little effect upon food-reinforced fixed ratio 1 performance, and minimal actions upon the discrimination of reinforcement magnitude in maze choice tasks. It has also

been reported repeatedly that the effects of accumbens DA depletions on various operant procedures are substantially different from those of pre-feeding and appetite suppressant drugs. Taken together, this evidence of fundamental aspects of food motivation being preserved after interference with accumbens DA transmission has been used to support the hypothesis that nucleus accumbens DA depletion or antagonism can impair activational aspects of motivation while leaving directional aspects largely intact.

Furthermore, the effects of nucleus accumbens DA depletions on food-reinforced operant behavior depend greatly upon the requirements of the instrumental response. Accumbens DA depletions have little effect on total response output with rats responding on a fixed ratio 1 schedule; their main impact is to produce an initial slowing of responding that does not closely resemble extinction. Similarly, nucleus accumbens DA depletions exert little influence upon performance of variable interval schedules, such as variable interval 30, 60, or 120 s. Overall, the primary pattern of effects that has been observed is that nucleus accumbens DA depletions affect food-reinforced operant responding in a manner that is directly related to the ratio requirement of the schedule. Although fixed ratio 1 performance shows little sensitivity to the effects of accumbens DA depletions, schedules with higher ratio requirements (fixed ratios of 16 and 64 in one study, or up to 200 to 300 in another) are substantially impaired by interference with accumbens DA transmission. Essentially, nucleus accumbens DA depletions produce two effects on food-reinforced ratio performance: they reduce responding on schedules that have moderately high ratio requirements, and they dramatically suppress responding on schedules with very high ratio requirements, a phenomenon known as ratio strain. In other words, nucleus accumbens DA depletions suppress the response enhancing effects of moderate size ratio requirements, and enhance the response suppressing effects of large ratios.

There are several noteworthy features of the effects of accumbens DA depletions on ratio lever pressing performance. First of all, the reduction of overall responding that occurs when there are low-to-moderate size ratio requirements is directly related to the baseline rate of responding; the higher the response rates, the greater the diminution of responding. Second, the reduction in responding appears to have two major components: there is a slight decrease in the local rate of responding as reflected by alterations in the interresponse times, and a very substantial increase in the number and duration of pauses in responding. Thus, lever pressing becomes more fragmented or disengaged. Another feature of the effects of accumbens DA depletions on ratio performance is that the overall pattern of effects of DA depletion is completely different from the overall pattern of effects observed

after pre-feeding to reduce food motivation. Finally, the ratio strain that develops with very high ratios (i.e., fixed ratio 200–300) appears to be relatively independent of the rate of responding, and more dependent upon the sheer size of the ratios.

In addition to being involved in the exertion of effort in response to work-related obstacles or challenges, nucleus accumbens DA is also involved in effort-related choice behavior. An animal in a complex environment can select from alternative sources of food and other motivational stimuli. There are diverse paths for obtaining each of these stimuli, and each distinct trajectory can entail different response requirements related to time, work, and other parameters. Several behavioral paradigms have been developed that assess how animals allocate resources based upon analyses of reinforcement value and response cost. In some studies, a concurrent lever pressing chow feeding procedure is employed. With this task, animals can choose between lever pressing for a more preferred food reward (e.g., high carbohydrate operant pellets) versus approaching and consuming a less preferred food, such as common laboratory chow. Performance on this task is affected by the ratio requirements on the lever pressing component, thus demonstrating some degree of sensitivity to the work load placed on the operant schedule. Importantly, with rats responding on a fixed ratio 5 schedule for the preferred reward, untreated animals generally get most of their food from lever pressing. Several studies have shown that accumbens DA depletions or antagonism shift the choice behavior of these animals, decreasing lever pressing and substantially increasing chow intake. This pattern of effects is not produced by prefeeding or appetite suppressant drugs. Another commonly used procedure is a T-maze task; the two arms of the maze can have different reinforcement densities (e.g., 4 vs. 2 food pellets, or 4 vs. 0), and under some conditions a 44 cm barrier can be placed in the arm with the higher reward density to present a work-related challenge to the animals. When there is no barrier present in the arm with the high reinforcement density, untreated rats generally prefer that arm, and neither haloperidol nor accumbens DA depletions affect preference of the arm with the higher reward density. In contrast, DA antagonism and accumbens DA depletions dramatically alter choice behavior when the high density arm (four pellets) has the barrier in place, and the arm without the barrier contains an alternative food source (two pellets); rats with impaired DA transmission show decreased choice of the high density arm (i.e., reduced barrier climbing), and increased choice of the low density arm without the barrier. Researchers also have developed lever pressing and T-maze versions of effort discounting tasks, and the results all point in the same direction, that is, interference with DA transmission alters effort-related choice behavior across a wide variety of tasks. These findings indicate that manipulations that affect nucleus

accumbens DA transmission set constraints on effort-related choice behavior, biasing animals toward low cost alternatives.

The effects of nucleus accumbens DA depletions on exertion of effort and effort-related choice behavior in food-motivated tasks do not appear to be dependent upon a general reduction of appetite for food, or an incapacity to respond, or the induction of severe motor impairments. Nucleus accumbens DA depletions alter response output across different ratio schedules in a manner that does not closely resemble the effects of prefeeding. Similarly, the effects of accumbens DA antagonism and DA depletion on effort-related response allocation in the concurrent lever pressing/chow feeding procedure do not mimic the actions of prefeeding or appetite suppressant drugs. Rats with nucleus accumbens DA depletions show normal levels of food intake, and also show normal patterns of reaching, grasping, and food handling. Furthermore, injections of DA antagonists into nucleus accumbens core or shell subregions, which alter choice behavior in the concurrent lever pressing chow feeding task, do not affect the form of lever pressing by increasing lever press duration. The shift from lever pressing to chow intake observed in the concurrent fixed ratio 5/chow feeding choice task can be observed in animals with mild DA depletions that do not show impaired lever pressing on the fixed ratio 5 schedule, indicating that these DA depleted rats are capable of responding, and are therefore choosing to select lab chow over lever pressing, when both are concurrently available. Similarly, the ratio strain induced by accumbens DA depletions is not just occurring because of a ceiling on response rates, DA depleted rats responding on a fixed ratio 64 schedule actually press less than DA depleted rats responding on a fixed ratio 16. In a variant of the T-maze choice task in which the barrier arm contained four pellets, but the other arm contained no pellets (i.e., the only way to get food was to climb the barrier), rats with accumbens DA depletions still chose the high density arm, climbed the barrier, and consumed the pellets. Taken together, this pattern of results does not appear to resemble the characteristics of either a reduction in directional aspects of food motivation (i.e., appetite), or a severe impairment in motor execution. Granted, there are brain areas at which dopaminergic manipulations can produce such effects; for example, hypothalamic DA is involved in appetite, and ventrolateral neostriatal DA depletions can produce motor impairments in reaching, grasping, forepaw usage during feeding, feeding rate, and lever press duration. Nevertheless, nucleus accumbens DA depletions produce more subtle, context-dependent effects that are at the very cusp of motivation and motor control functions, but not solely in either realm.

Of course, nucleus accumbens DA does not participate in behavioral activation and effort-related processes in isolation. Rather, it is a nodal point imbedded into a larger

forebrain circuitry that includes other neurotransmitters and brain regions. Basolateral amygdala and prefrontal/anterior cingulate cortex also participate in effort-related choice behavior. Within the accumbens, evidence indicates that DA interacts with the neuromodulator adenosine to regulate behavioral activity. Stimulation of adenosine A_{2A} receptors in nucleus accumbens can mimic the effects of DA depletions, and blockade of A_{2A} receptors can reverse the effects of DA antagonism. The gamma aminobutyric acid (GABAergic) ventral striatopallidal pathway, the major output pathway from nucleus accumbens to ventral pallidum, also participates in behavioral activation and effort-related functions. Within the last few years, researchers have begun to sketch the outline of the brain circuitry involved in motivational aspects of motivation and related decision-making processes.

Research in this area has shed considerable light on the neural mechanisms underlying motivational aspects of motivation for natural reinforcers. However, it also has considerable clinical significance. Exertion of effort is not only important for natural motivation; excessively focused exertion of effort is a hallmark of drug abuse and addiction. Drug addicts show a radically altered preference structure for motivational stimuli, with drug selection taking a distorted position atop the hierarchy of motivational stimuli. Addicts can show markedly reduced behavioral activation in response to natural reinforcers, and can display psychomotor slowing during drug withdrawal, while also being able to allocate a disproportionate and extreme amount of behavioral (and financial) resources into drug seeking. This exaggerated effort invested in drug seeking in addicts may involve DA, though it is also possible that it is relatively DA independent, relying upon the recruitment of other components of the circuitry. Nevertheless, these patterns of behavior suggest that the addiction process is characterized by a dramatic restructuring of both directional and motivational aspects of motivation. Another clinical manifestation of the role of behavioral activation is related to psychomotor dysfunctions in depression, mania, and other disorders. Energy-related symptoms such as anergia, fatigue, and psychomotor slowing are common symptoms of depression. Clinical research has shown that these symptoms are strongly related to outcome measures in depressed people. In terms of the neural systems involved, evidence indicates that there is a striking similarity between the brain areas implicated in psychomotor dysfunction in depression and the circuitry that is involved in regulating behavioral activation in animals. To a certain extent, psychomotor slowing in depression shares some characteristics with parkinsonism, which is consistent with the use of antiparkinsonian drugs for the treatment of anergia or fatigue. In stark contrast to depression, mania is accompanied by dramatically heightened levels of behavioral activation. People in the manic phase of bipolar disorder often work

copiously, and have an exaggerated sense of self-efficacy, capability, and power.

Several decades of behavioral neuroscience research has shown us that the conceptual structure inherited from psychology is useful, even necessary, for understanding brain function, but also that it can be somewhat limiting. In addition to considering the neural systems that perform the operations related to core psychological functions, we also must recognize that the borders between these functions are not always distinct, that they do not map neatly onto brain systems, and that there can be considerable overlap and integration of function. For example, a particular behavioral function can be sensory/motor, or cognitive/affective. So it is with the relation between motor function and motivation. Clearly, motivational aspects of motivation represent an area of overlap between motivational and motor processes, and the neural systems involved in behavioral activation, such as nucleus accumbens DA, display the characteristics of involvement in this type of functional integration. Continued research on behavioral activation and effort-related processes will help elucidate how brain mechanisms participate in the regulation of both natural and pathological features of motivation, and also how the selection of paths toward specific motivational stimuli can ultimately be translated into action.

See also: Depression; Drug Addiction; Incentive Motivation and Incentive Salience; Motivation; Neural Systems of Motivation.

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Novelty

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Glossary

- Cloninger's novelty seeking** – One of three dimensions measured by the Tridimensional Personality Questionnaire (TPQ); the other two dimensions are harm avoidance and reward dependence.
- Habituation** – Nonassociative loss of responsiveness in a neural or psychological process over time when a constant stimulus is applied.
- Mesocorticolimbic dopamine reward system** – Neural system that projects from ventral tegmental area (VTA) to nucleus accumbens (NAcc), medial prefrontal cortex (mPFC), and associated limbic structures subserving the rewarding effect of novelty, drugs of abuse, and other reinforcers.
- Novelty detection** – Identification of a new or unknown stimulus based on past experience.
- Novelty-induced place preference** – Free-choice preference displayed for a novel context over a familiar context.
- Novelty seeking** – Approach to or preference for a new stimulus relative to a familiar stimulus.
- Sensory adaptation** – Loss of responsiveness in a sensory transducer over time when a constant stimulus is applied.
- Zuckerman's sensation seeking** – Personality trait measured by the Zuckerman–Kuhlman Personality Questionnaire (ZKPQ) that is characterized by a need for novel, complex, and ambiguous stimuli and a willingness to take a risk for obtaining these stimuli.

Background

From a historical perspective, one of the most interesting set of psychological experiments conducted during the 1950s involved the use of extreme sensory deprivation in human subjects. Volunteer subjects were instructed to lie on a bed in a sound-attenuated cubicle and sensory information was restricted by having the subjects wear goggles and gloves. After several hours, the prolonged sensory deprivation had a debilitating effect on normal functions, often producing hallucinatory experiences and unpleasant emotional feelings. Immediately upon termination of the sensory deprivation period, significant deficits in performance on various cognitive tasks were also noted.

However, not all individuals suffered deleterious effects, and there were large individual differences in the reaction to the sensory deprivation.

These early sensory deprivation experiments were important because they helped to usher in the modern view that organisms are not merely passive sensory neural systems, but rather that they are built to require a virtually continuous stream of sensory experience. Repetitive sensory stimulation is not sufficient to maintain the organism, but instead there is a need for varied experience. When faced with a lack of sensory input or a lack of varied input, the organism must become active to seek out the needed stimulation. Thus, novelty engages not merely sensory detection properties, but also incentive motivational neural systems. Both sensory and motivational neural systems are tuned exquisitely to process novel information because attending to and approaching new stimuli leads to potential sources of food, water, and sexual partners, while providing information about potential sources of danger.

A corollary idea is that individuals differ in the degree to which they need varied experience, with some individuals having a high need for novelty and others having a low need. This can be demonstrated by presenting a novel visual stimulus to two different individuals. One individual may habituate rapidly to the stimulus and thus turn away relatively quickly, whereas another individual may habituate slowly and thus attend to the stimulus for a longer period of time. Even though both individuals are attracted initially to the stimulus (novelty detection), the first individual who turns away more quickly to find other sources of stimulation may have a higher need for novelty (novelty seeking). Such individual differences may have important clinical implications for the expression of risk-related behaviors, as discussed below.

Before proceeding, it is important to point out that the term novelty is difficult to define precisely because any discrete stimulus or situation is experienced in the context of other stimuli or situations. In an absolute sense, a novel stimulus is one that has never been experienced before. However, a never-experienced stimulus may be relatively more novel when viewed within a familiar context, as opposed to being viewed in a novel context. Conversely, a previously experienced stimulus may be novel when it occurs unexpectedly in a different context. Hence, novelty is not an absolute attribute of any stimulus or situation, but rather it reflects the relative difference compared to a background.

Mechanisms of Novelty Seeking

Adaptation and Habituation

A general principle of neuroscience is that sensory and integrative systems are tuned to attend to changes in environmental events, as opposed to repetitive monotonous events. In the sensory transduction process, the visual system provides a good example of this general principle. Continuous stimulation of a specific retinal region of the eye with light will fatigue the photoreceptive transduction that occurs at the level of the rods and cones. Such fatigue leads to a temporary loss of visual acuity because the photo pigments of the sensory cells become depleted, a process that is known as sensory adaptation. This adaptive process is partially offset by the saccadic or jerky movements of the eyes, which varies slightly the location of a visual stimulus on the retina.

Within the peripheral and the central nervous systems, constant repetitive stimulation also leads to a loss of function. This process is called habituation, and it represents a primitive form of learning that is important in the context of understanding novelty detection. For example, an auditory stimulus (tone) that is presented for the first time may elicit orienting responses. However, when the same stimulus is presented repeatedly at regular intervals, these orienting responses weaken and may dissipate completely. However, with the passage of time, some of the weakened response may return, a process referred to as spontaneous recovery. Behaviorally, this process is illustrated by placing a rat into the same stimulus environment on repeated occasions. When a rat is first placed into a novel context, it is highly active due to exploratory behavior and this exploratory behavior diminishes across the exposure sessions. Following a rest period, there is some return of the exploratory behavior, although it is somewhat weakened relative to the first exposure. Without this habituation process, an organism would be unable to detect a novel stimulus because the background in which they occur would also be novel.

Motivational Aspects

Not only does novelty engage attention systems, it elicits active exploratory behaviors that engage incentive motivational neural systems. This phenomenon is illustrated in rats by spontaneous alternation in a T-maze. In this test, even when both arms of a T-maze are reinforced with food, rats tend to alternate their choice between the two arms, a behavior that is thought to reflect novelty preference. Rats will also interact more with a novel object than a familiar object and will choose a novel environmental context over a familiar context, suggesting further that novelty engenders positive incentive motivation.

Perhaps the most cogent evidence that novelty has positive incentive value comes from operant conditioning experiments in which novelty is used as a reinforcer. Rhesus monkeys will solve complex discrimination tasks when novel visual and auditory stimuli are presented contingent on correct lever-press responding. Rats will also exert effort (lever pressing) to earn novel visual and auditory stimuli. In contrast to other types of reinforcers such as food, water, or sex, novelty does not necessarily reduce a drive state (hunger, thirst, or sexual drive), and there is no consummatory response that leads to satiation. In this regard, novelty is somewhat analogous to the phenomena of brain stimulation reward, a motivated behavior that can be maintained for prolonged periods with little diminution in intensity.

Development

Novelty seeking represents an important functional aspect of the nervous system across the life span. Prelingual human infants are attracted to complex visual patterns. This preference can be demonstrated by exposing infants to a pattern on a computer screen repeatedly, followed by presentation of a novel stimulus. Using an eye-tracking system, it has been shown that infants spend more time viewing novel stimuli than familiar stimuli. If a discrete novel stimulus is presented as a feature against a background of familiar stimuli, infants are readily able to detect the stimulus, a phenomenon that is sometimes referred to as the pop-out effect.

Adolescence is a developmental period that is characterized by an especially heightened expression of novelty seeking. This is a period in which mammals become fully capable of moving away from parental protection into an environment where potential mate selection will be available. In many mammals, including humans, activity levels and risk taking increase at the same time as the onset of puberty. While this is a period of heightened risk, it should be emphasized that this is generally an adaptive strategy for species survival. Moreover, novel stimulation plays a critical role in promoting normal development, as well as maintaining an optimal level of arousal for information processing.

Neural Mechanisms

While there are undoubtedly multiple brain regions and neurotransmitters involved in novelty seeking (see **Table 1**), one critical part of the neural circuitry is the mesocorticolimbic dopamine (DA) system. Many studies have shown that exposure to novelty (either forced exposure or free-choice exposure) activates the mesocorticolimbic DA system. Similar to the effects of many drugs of abuse, rats exposed to novel environmental stimuli display an increase in locomotor activity. Rats also

Table 1 Some of the various brain systems activated following exposure to novelty

<i>Brain region(s)</i>	<i>Neural system</i>
Ventral tegmental area	Dopamine
Nucleus accumbens	
Locus ceruleus	Norepinephrine
Neocortex	
Hippocampus	
Raphe nucleus	Serotonin
Amygdala	
Neocortex	Acetylcholine
Hippocampus	
Brainstem	Glutamate
Neocortex	
Hippocampus	
Brainstem	GABA
Neocortex	
Hippocampus	
Hypothalamus	Corticotropin-releasing factor

show a conditioned place preference when novel objects are paired with one compartment of a place preference apparatus. When entering a novel compartment from a familiar compartment, there is a transient and rapid surge in DA activity in the nucleus accumbens (NAcc) recorded by *in vivo* voltammetry. This rapid increase in voltammetric signal is not obtained when the rat re-enters the now familiar compartment, and thus it is not an artifact of locomotor activity or a general stimulus change. Further, injection of the neurotoxin 6-hydroxydopamine (6-OHDA) into the mesocorticolimbic DA system disrupts the increase in locomotion and rearing normally elicited by novel stimuli. Novelty-induced place preference is also blocked by DA antagonist drugs (both D1 and D2 antagonists) and by lesioning the NAcc. Even though the 6-OHDA-induced blockade of novelty-induced place preference is reversed after several weeks, a deficit in exploratory behavior persists.

In recent reports using electrophysiological techniques in nonhuman primates, novelty signal detection has been observed in the ventral tegmental area (VTA) of behaving animals. VTA neurons show tonic impulse activity, but respond with short-latency, short-duration bursts of activity to novel stimuli, unpredictable positive rewards, and conditioned stimuli that reliably predict positive rewards. VTA neuron burst firing is thought to occur when the stimulus input is sufficiently novel to warrant a change in behavior. Similarly, in human subjects, anticipation of novelty reward activates the ventral striatum and this activation is correlated positively with scores on the novelty-seeking subscale of Cloninger's Tridimensional Personality Questionnaire.

Monoamines other than DA also play a role in novelty detection and seeking. For example, accumulating evidence indicates that norepinephrine activity in the

hippocampus is also involved in novelty signal detection. Although no direct neural connections have been found between the VTA and the hippocampus, most hippocampal targets send efferent projections to the VTA. For example, the hippocampus sends efferents to the medial prefrontal cortex (mPFC), which sends excitatory input stimuli to VTA. Similarly to the novelty-induced increase in mesocorticolimbic DA, exposure to novel environmental stimuli increases the concentration of extracellular norepinephrine in the frontal cortex assessed by *in vivo* microdialysis, which presumably reflects an increased response of neurons in the locus ceruleus. In any case, these results indicate that the VTA acquires information about stimulus novelty through circuitry involving the prefrontal cortex and hippocampus, sites for memory storage, and novelty detection.

Serotonin and glutamate systems have also been implicated in novelty seeking. The 5-HT_{1A} agonist 8-OHDPAT produces a dose-dependent decrease in novel object exploration in rats, whereas the 5-HT_{1A} antagonist WAY-100635 produces an increase in novel object exploration. Importantly, these serotonin-mediated effects are obtained without any change in locomotor activity, thus demonstrating that the effects are specific for approach to novelty rather than general exploratory behavior. Similarly, a role for glutamate has been illustrated in rats conditioned to approach an environmental context paired previously with a novel object. The noncompetitive *N*-methyl-D-aspartic acid (NMDA) antagonist MK-801 given during the conditioning phase blocks this effect, thus implicating a role of NMDA receptors in learning produced by stimulus novelty.

Individual Differences in Novelty Seeking

Genetic Influences

There is considerable evidence that novelty seeking is, at least in part, under genetic control. For example, different inbred strains of mice display a different level of locomotor activity in a novel apparatus, as well as a different rate of contact with novel objects. Along with these behavioral differences, strain differences have been observed in a number of DA neurochemical markers, including the density of DA striatal D₂ receptors, metabolism of DA in the NAcc, and drug-stimulated DA release.

Recently, Akil and colleagues at the University of Michigan have initiated a selective breeding study in rats based on the novelty-seeking phenotype. Based on the level of locomotor activity in an inescapable novel environment, selectively bred high responders show decreased anxiety-like behaviors compared with low responders. In addition to alterations in DA systems, phenotype differences between high and low responders are observed in mRNA signal for serotonin receptor

transcripts in the olfactory tubercle, dentate gyrus, dorsal hippocampus, and thalamo-cortical projection areas, implicating a contribution of 5-HT systems to novelty seeking.

In humans, both twin-pair and family history approaches have revealed a genetic influence on the expression of novelty seeking. While there is some variability in published results across different population samples, genetic factors alone generally account for about 50% or more of the variance in novelty-seeking scores measured by either Zuckerman's sensation seeking scale or Cloninger's novelty seeking scale. Some evidence also suggests that the degree of inheritance may be greater in males than in females.

Novelty seeking was reported initially in 1996 to be associated with a polymorphism of the DA D₄ receptor (DRD4) gene. Subsequent reports using independent populations have either refuted or confirmed a role of the DRD4 gene in novelty seeking, thus generating considerable debate in this area. In another line of work, high novelty seekers have been shown to have low monoamine oxidase (MAO) activity in blood platelets. Since MAO is the major enzyme that metabolizes monoamine neurotransmitters such as DA, these results suggest that the activity of monoamine brain systems, and DA in particular, may differ between high and low novelty seekers. However, this conclusion is speculative presently, since platelet MAO activity is not a reliable index of MAO activity in the brain.

Environmental Influences

While individual differences in novelty seeking are under some genetic control, they are also influenced by environmental factors. One important environmental factor that alters novelty seeking is the amount of novel sensory experience that an individual encounters during development. One paradigm that has been used widely is the environmental enrichment paradigm. In this paradigm, rats are housed for several weeks in either an enriched condition (EC) with novel objects and social cohorts or an isolated condition (IC) without objects or cohorts; rats can also be raised in a social condition (SC) in which cohorts are available, but no novel objects. A host of studies has shown that EC rats display a wide range of neurobehavioral changes relative to IC rats. In general, EC rats find novelty to be less stressful than IC rats, and they will approach novel stimuli more rapidly than IC rats. For example, while EC rats display less activity than IC rats in an inescapable novel environment, in a free-choice situation, they enter a novel compartment and manipulate novel objects more than IC counterparts do. While they approach novelty more readily, EC rats also habituate to novel stimuli more rapidly than IC rats. EC rats are less motivated than IC rats to make an operant response for a

weak auditory or visual reinforcer, suggesting that the incentive value of novelty is reduced by enrichment.

The enrichment-induced alterations in novelty seeking may be mediated by various neural substrates. Studies have shown that environmental enrichment produces profound neuroanatomical and neurochemical changes in the neocortex, particularly in the visual cortex, which likely explains the enhanced visual acuity by EC rats. Environmental enrichment increases dendritic arborization, as well as increasing neocortical weight and thickness, primarily due to an increased density of glial and capillary endothelial cells. These enrichment-induced anatomical changes are initiated by the expression of various gene-encoded transcription factors, some of which are involved in inducing neurotrophic factors. Similar to environmental enrichment, repeated exposure to stimulant drugs such as amphetamine and cocaine increases dendritic branching in NAcc and mPFC.

As regards specific neurotransmitter systems, relatively little is known about the effect of environmental enrichment on subcortical neurotransmitter systems, and on NAcc activity in particular. In one study, no difference in DA D1 or D2 receptor levels in NAcc (core, shell, and rostral subregions) was found between EC and IC rats. However, it has been shown that EC rats have greater glucose utilization in NAcc following exposure to a novel environment relative to IC rats, but it is not known if this increase in metabolic activity reflects a specific activation of DA neurons. In cortex, environmental enrichment increases cholinesterase activity and increases levels of norepinephrine and DA. In mPFC, environmental enrichment decreases the density of DA D1 receptors and the number of cell-surface DA transporter proteins, indicating that the repeated exposure to novelty during development downregulates both pre- and postsynaptic proteins involved in DA transmission. It remains to be determined if this change in mPFC underlies the enrichment-induced alterations in novelty seeking.

Risk-Related Novelty Seeking

Drug Abuse

Laboratory animal studies

Similar to novelty-seeking behavior, there is ample evidence that drug-abuse-related behaviors also involve ascending mesocorticolimbic DA projections. While a full review of that literature is beyond the scope of the current article, Piazza and colleagues at the University of Bordeaux have shown that individual differences in locomotor activity within an inescapable novel environment predict stimulant self-administration, with high-responder rats self-administering more cocaine and amphetamine than low-responder rats. Compared with low responders, high responders also exhibit greater

neuronal firing in VTA, greater levels of extracellular DA in NAcc following exposure to novelty or stimulant drugs, greater velocity of DA uptake in NAcc, and greater mRNA levels for tyrosine hydroxylase and DA D1 receptor proteins. In addition, high responders have fewer DA D2 receptors in NAcc, suggesting a decreased number of release-regulating autoreceptors or a compensatory downregulation of postsynaptic receptors in response to increased presynaptic DA release. Despite the wide use of this model, it is not clear to what extent it reflects individual differences in novelty exploration (novelty seeking), as opposed to individual differences in stress reactivity within the inescapable novel environment. Stress reactivity likely plays some role, since high and low responders differ in levels of the stress hormone corticosterone.

Other rat models have examined free-choice approach to a novel context or novel objects as a more direct measure of novelty seeking. Individual differences in novelty place preference have been shown to predict amphetamine reward, and this relation is probably not due to individual differences in stress hormones because free-choice novelty does not elevate levels of corticosterone. Instead, approach to novelty likely activates directly the reward-relevant mesocorticolimbic DA circuitry.

Evidence from inbred rat strains also supports the importance of genetics in the control of novelty-seeking and drug-seeking behaviors. The Lewis (LEW) and Fischer (F344) rat strains have received considerable attention. Relative to F344 rats, LEW rats display increased locomotor activity in an inescapable novel environment and increased sensitivity to the locomotor-stimulant effect of methamphetamine. LEW rats also display greater intake of various drugs of abuse and more rapid acquisition of intravenous cocaine self-administration. The greater drug intake in LEW relative to F344 rats likely reflects an enhanced sensitivity to the reinforcing effect, as LEW rats also show greater cocaine conditioned place preference. At the neurochemical level, although DA receptor levels are similar between LEW and F344 rats, LEW rats display greater tyrosine hydroxylase activity in VTA and greater cocaine- and methamphetamine-induced DA release in NAcc.

More recently, selective breeding of rats that are either high or low on activity in an inescapable novel environment indicates that high responders self-administer more cocaine than low responders do. Interestingly, while both male and females show these heritable differences, high-responder females show the most dramatic increase in acquisition of cocaine self-administration, suggesting that female novelty seekers may be especially vulnerable to stimulant abuse. However, this conclusion is tempered somewhat by other work showing that mice selectively bred for differences in exploratory head dipping do not consume differential amounts of ethanol or methamphetamine.

As for environmental influences, just as environmental enrichment alters mesocorticolimbic DA neurocircuitry, it also alters the neurobehavioral effects of drugs of abuse such as amphetamine. EC rats are more sensitive than IC rats to the locomotor stimulant effect of acute amphetamine and are more sensitive to amphetamine conditioned place preference. These behavioral differences are accompanied by an enrichment-induced increase in amphetamine-stimulated DA release and metabolism in NAcc. With repeated amphetamine injections, however, EC rats show an attenuation of locomotor sensitization relative to IC rats, as well as a reduction in the discriminative stimulus effects of amphetamine. EC rats also exhibit a reduction in amphetamine self-administration across repeated sessions. Thus, although some of the initial effects of amphetamine may be enhanced, environmental enrichment reduces the psychostimulant effects of repeated amphetamine treatment.

Human studies

In humans, numerous studies indicate that individual differences in novelty or sensation seeking, measured by either the Cloninger or Zuckerman questionnaires, reliably predict drug use and abuse, with high novelty seekers being at increased risk. Recent meta-analytic reviews have found small-to-moderate effect sizes between novelty seeking scores and alcohol use, with the effect size tending to be greater among studies that include higher percentages of male subjects. Individual differences in response to stimulant drugs are also related to novelty seeking. For example, in controlled human laboratory studies, high novelty seekers show greater amphetamine-induced subjective stimulation and elevated mood compared to low novelty seekers. High novelty seekers have been shown to be more sensitive than low novelty seekers to the rewarding effects of drugs of abuse as measured by self-administration. The relation between novelty seeking and positive response to amphetamine in healthy volunteers is consistent with the idea that common neural mechanisms underlie both measures, and that such mechanisms may be in part responsible for the increased vulnerability to psychostimulant use and abuse among high novelty seekers.

It is generally assumed that novelty seeking is an antecedent trait that increases risk for abuse; however, much of this work has involved cross-sectional analyses, which does not allow for determining the exact relation between novelty seeking and drug use. While longitudinal studies have confirmed that novelty seeking early in life does predict later drug use, there is also evidence that drug use can increase novelty seeking. This latter finding suggests that drug use may predict later novelty seeking due to a third variable (e.g., common deviant peer influence) or due to a direct effect of the drug on brain function. In any case, it appears that there is a reciprocal relationship between novelty seeking and drug use.

Other Risk-Related Behaviors

In addition to increased drug-abuse vulnerability, individual differences in novelty seeking predict other negative health outcomes in humans. Novelty or sensation seeking is associated with aggressive and criminal antisocial behaviors. High novelty seekers are also more likely to engage in sexual risk taking, including having multiple sex partners, causing or becoming pregnant, and having unwanted sex while drunk. Further, high novelty seekers are more likely to gamble, drive fast, and suffer from physical injuries. In survey studies, high novelty seekers report a greater likelihood than low novelty seekers to not wear seatbelts, beat other drivers at the getaway, weave through traffic, enjoy passing other vehicles, and driving at high speeds. Extreme and dangerous sports, such as skydiving, hang gliding, motorcycle racing, ocean kayaking, rock climbing, and scuba diving, have also been correlated with high novelty seeking. Finally, novelty seeking has also been associated with various eating disorders; in particular, individuals with bulimia nervosa or anorexia nervosa characterized by binge eating and purging have higher novelty seeking scores than controls.

In conclusion, research in the field indicates that novelty seeking is a phenotypic behavior that is highly adaptive and conserved within the species. It serves to direct attention toward unexpected events that may have important biobehavioral relevance for survival and, thus, novelty has an affective positive incentive value. However, unbridled novelty seeking can be maladaptive, consistent with the evidence linking it to risk-related negative health outcomes. Thus, the most adaptive individual may be one who not only has the ability to attend to and approach novelty, but also has that ability to counterbalance that propensity with appropriate behavioral inhibitory control when needed.

Acknowledgments

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See also: Acute Dependence; Alcoholism; Animal Tests for Anxiety; Attention and Speed of Information Processing; Behavioral Development and Socialization; Brain Evolution in Vertebrates; Cellular Plasticity in Cocaine and Alcohol Addiction; Cognition: Attention and Impulsivity; Developmental Neurogenesis; Drug Addiction; Drug Sensitization and Drug Abuse; Emotion-Cognition Interactions; Environmental Influences on Adult Neurogenesis; Genes and Behavior: Animal Models; Habituation; Incentive Motivation and Incentive Salience; Molecular Neurobiology of Addiction; Motivation; Neural Basis of Recognition Memory in Nonhuman Primates; Neural Systems of Motivation; Neurogenesis and Memory; Orientation and Navigation; Psychostimulants; Stress and Drug Craving; Vulnerability Factors in Addiction Disorders.

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Sexual Motivation

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Glossary

Incentive sexual stimuli – Primary or conditioned stimuli that stimulate sexual arousal and desire. Conditioned sexual incentives appear to require both opioid and dopaminergic activation for their salience, whereas primary (unconditioned) sexual incentives do not appear to require dopamine. **Sexual arousal** – It refers to increased activation of the autonomic nervous system that prepares the body for sexual responding. This includes increased parasympathetic actions that control genital blood flow and increased sympathetic actions that increase heart rate and breathing rate. It is typically divided into physiological arousal (reflecting blood flow into or out of tissues) and subjective arousal (reflecting a conscious awareness of one's physiological state). Key neurochemical activators include noradrenaline and acetylcholine both in the brain and the periphery to modulate the arousability of the organism, and dopamine, oxytocin, and vasopressin in the hypothalamus and pituitary to direct autonomic outflow.

Sexual desire – It refers to wanting or craving sexual activity, along with behaviors aimed at acquiring sex partners or sexual reward, including operant responses for conditioned sexual incentives. It is synonymous with sexual excitation and most obviously reflective of sexual motivation. It is altered by hormonal actions and the effect of priming stimuli on neural systems for incentive motivation in general and sexual responding in particular, but also by the degree of sexual inhibition present at any given time. Sexual desire is typically viewed as conscious, although it can be expressed as an ability to be distracted by primary or conditioned sexual incentive stimuli, or as an inability to be distracted away from such stimuli. Key neurochemical activators include central actions of dopamine, melanocortins, and oxytocin, to modulate incentive sexual responding and partner preference.

Sexual inhibition – It refers to a dampening of sexual arousal and desire. This occurs in varying degrees as a function of sexual reward and satiety, as occurs during sexual refractoriness or exhaustion, or as a function of stress, fear, or punishment, as might occur in situations in which having sex would be dangerous for the

organism. Key neurochemical activators of the inhibitory state appear to be central opioid transmission that gives rise to a sexual reward state or that is activated by stress or fear, and central serotonin transmission that gives rise to a state of satiety that inhibits both incentive sexual responses and genital blood flow.

Sexual reward – It refers to the pleasure derived from sexual gratification. This occurs most typically as a function of the reward state induced by orgasm, but may also include the intimacy, bonding, and control inherent in sexual activity with one or more partners. A key neurochemical activator appears to be central opioid transmission, most notably in the medial preoptic area of the hypothalamus and ventral tegmental area of the midbrain.

Sexual satiety – A state of refractoriness in which sexual responding is inhibited and in which further sexual stimulation may be aversive or painful.

What Is Sexual Motivation?

Definitions

Sexual motivation can be defined as the energizing force that generates one's level of sexual interest at any given time. It drives our sexual fantasies, compels us to seek out, attend to, and determine the quality of sexual incentives, regulates our levels of sexual arousal and desire, and enables us to masturbate, copulate, or engage in other forms of sex play. It seems like a straightforward concept, yet it runs into trouble at several important levels. As a concept of something internal, it is often circular, being inferred from the strength of a behavior or content of a fantasy. Of course, people can be highly motivated sexually but suffer from an arousal disorder, such as erectile dysfunction. The concept also runs into problems of definition when it is presented as a fuzzy dichotomy, like "physiological versus subjective arousal," or when it is used to define a set of internal motivational processes that are somehow distinct from the outward expression of sexual performance (as if any sexual performance is not also sexually motivated). Defining the goal of sexual behavior – a necessity if we are to understand what the

motivation is driving the organism to do – is often tricky because it ranges from reward to reproduction.

The value of a concept like sexual motivation lies in its ability to provide a framework, or ‘heuristic,’ within which sexual behaviors and their neural correlates can be more easily organized and understood. It should be able to handle animal and human sexual behaviors at different levels of analysis, and despite their outward dissimilarities. General models of sexual behavior are almost always conceptual in nature, and try to distill the behavioral components down to core variables that can be applied across species or situations.

Common Components of Sexual Behavior

All organisms that engage in sexual behavior share a common set of principles and endpoints that define the behavior. First, they must be able to respond to hormonal and neurochemical changes that signal sexual arousal and desire. This ability underlies a moment-to-moment level of sexual arousability and contributes to the internal state that is commonly referred to as sex drive. Organisms must be able to identify external stimuli that predict where potential sex partners can be found, to seek out, solicit, court, or otherwise work to obtain sex partners, distinguish external cues and behavioral patterns of potential sex partners from those that are not sexually receptive, and to pursue sexual partners once sexual contact has been made. Neural mechanisms exist that allow the stimulation received during sexual contact to be perceived as rewarding. Such reward alters subsequent behavior, for example, by contributing to the formation of preferences for salient stimuli associated with positive sexual reinforcement, and also leads to a state of sexual satiety in which inhibitory neural systems are activated.

For all animals, sexual behavior occurs as a sequence or cascade of behavioral events. Researchers have recognized the heuristic value of separating sexual behavior into respective appetitive and consummatory phases. Appetitive behaviors bring an animal from distal to proximal and into contact with goal objects or incentives. Consummatory behaviors are performed once an animal is in direct contact with the incentive (i.e., to consummate the goal). Consummatory behaviors tend to be species specific, sexually differentiated, and stereotyped, whereas appetitive behaviors are more flexible. Indeed, survival often depends on an animal’s ability to learn a variety of strategies to obtain goals in different appetitive circumstances. Heuristically, it is useful to conceive of appetitive and consummatory behaviors as two overlapping Venn diagrams (**Figure 1**) in which the behavioral stream moves from appetitive to consummatory incentive sequences. The intersection of the diagrams defines pre-copulatory behaviors made once animals come into contact with potential sex partners. The diagrams are

overlapping, rather than discontinuous, because the division between the two phases is rarely fixed. Some responses, such as solicitation, can be placed into both phases, especially if sexual interaction comes in bouts. Solicitation would, therefore, be defined as a precopulatory behavior that acts as a transition from appetitive to consummatory.

It is also useful to describe sexual motivation in terms of components of sexual behavior displayed across species, including sexual arousal, desire, reward, and inhibition. In turn, the neural substrates that control those aspects of sexual function can be studied in terms of how they are altered by hormones, sensory stimulation, conditioned cues, drugs, and lesions or stimulation of different brain regions.

Sexual Arousal

Sexual arousal in the humans and other animals can be defined as increased autonomic activation that prepares the body for sexual activity. This includes both parasympathetic blood flow that keeps blood in genital tissues such as the clitoris, labia, vaginal epithelium, and penis, and sympathetic blood flow from the heart to striated and smooth muscle that participate in sexual responses. Sexual arousal also includes a central component that increases neural tone or preparedness to respond to sexual incentives. Both peripheral and central arousal may be detected as part of the perception of subjective sexual arousal, and both clearly lead to changes in responsiveness in genital tissues and control certain copulatory responses, such as the latency to orgasm or ejaculation.

Both in males and females, removal of the gonads or conditions in which gonadal output is depressed or inhibited (rendering the individual hypogonadal, as happens to women after menopause) lead to decreases in sexual arousal. Penile, clitoral, and vaginal tissues shrink in such conditions and blood flow to those tissues is compromised. Those effects can be counteracted by hormone-replacement therapy (e.g., exogenous administration of androgens in males and females, and/or androgens and estrogens in females). Hypothalamic and, possibly, limbic structures are key targets for steroid hormones in the stimulation of autonomic outflow and in the processing of sexually arousing cues that lead to autonomic outflow. Many treatments that enhance genital blood flow in men and women enhance penile erection and vaginal vasocongestion in rats and rabbits (e.g., phosphodiesterase (PDE)-5 inhibitors such as sildenafil, dopamine (DA) agonists such as apomorphine, melanocortin agonists such as melanotan-II (MT-II) and its active metabolite bremelanotide, prostaglandin E₁, oxytocin, α_2 -adrenergic receptor antagonists like yohimbine, idazoxan, or imiloxan, vasodilators that act through nitric oxide substrates, such as nitroglycerine, sodium nitroprusside, and linsidomine).

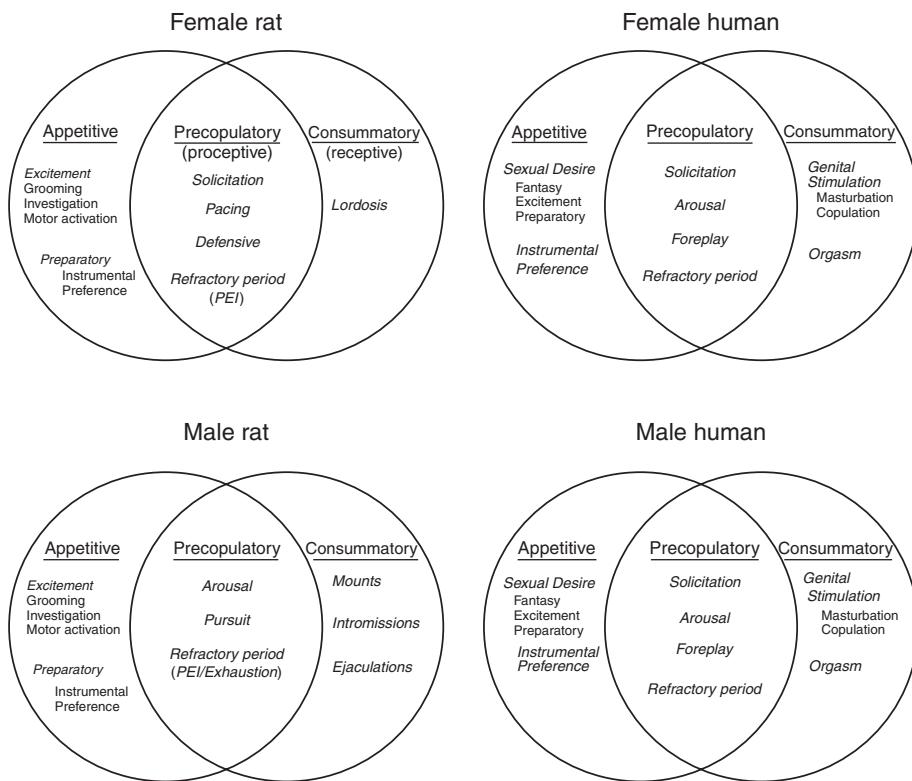


Figure 1 Incentive sequences for human and rat sexual behavior. The behavioral stream moves from left to right, through appetitive, precopulatory, and consummatory phases of behavior. This conforms to the movement of animals from distal to proximal to interactive with respect to the sexual incentive. Three types of appetitive responding reflect relative degrees of learning and necessity. ‘Preparatory’ behaviors are learned responses that animals must make in order to acquire the incentive (e.g., operant behaviors, pursuit). ‘Anticipatory’ behaviors are learned responses that occur in anticipation of an incentive, but are not necessary to obtain it (e.g., conditioned psychomotor stimulation that characterizes behavioral excitement). Unlearned appetitive responses also exist that are innate (e.g., unconditioned anogenital investigation). These aspects of behavior also occur once copulatory contact has been made, especially if copulation occurs in bouts (as it does in rats). Adapted with permission from Pfau JG (1999) Revisiting the concept of sexual motivation. *Annual Review of Sex Research* 10: 120–157

Selective activation of DA receptors in the medial preoptic area (mPOA) of the anterior hypothalamus alters autonomic arousal, with selective agonist actions on D1 receptors facilitating erections and inhibiting seminal emissions in male rats, and selective actions on D2 receptors doing the opposite. This suggests that the erectogenic actions of the DA agonist apomorphine in rats occur through its binding to D1 receptors in this or other brain regions, such as the paraventricular nucleus of the hypothalamus (PVN) – another region that controls erection through the release of oxytocin. A similar mechanism may exist in the control of female vaginal blood flow, as melanocortin agonists increase vaginal blood flow in women and stimulate DA release selectively in the mPOA in both male and female rats.

Sexual Desire and Reward

Perhaps the most obvious examples of sexual motivation are found in behaviors that denote sexual desire. Clinicians and motivational theorists alike view sexual

desire as distinct from sexual arousal both in animals and the humans, a distinction that generally reflects blood flow to the genitals and erectile tissues versus a psychological sexual interest in which individuals want or crave sex. When an individual expresses sexual desire, attention and behavior focus on obtaining some form of positive sexual reinforcement. This can occur alone in fantasies or together with others in goal-directed social and sexual behaviors. Thus, in addition to people stating colloquially that they feel horny (with or without corresponding arousal), desire encompasses the work people will perform to obtain sexual rewards, the excitement displayed in anticipation of such rewards, and the strength of the incentive value ascribed to a particular sexual stimulus. It must be noted that sexual desire and sexual reward go hand-in-hand, and the strength of desire is usually directed by the anticipation of reward. Many behaviors that reflect the reward state, for example, conditioned place or partner preferences, are typically tested using measures of sexual desire (e.g., movements into the

preferred place, solicitations of the partner associated with greater sexual reward, and choice of partner for ejaculation).

Similar to the humans, animals increase their motor output in anticipation of copulation and work for the opportunity to copulate or to obtain primary or secondary (conditioned) sexual rewards associated with copulation. Animals will also choose among two or more sexual incentives based on the strength of the incentive cues and the animal's own internal drive state. What characterizes these behaviors is that they occur before copulation: Solicitation, courtship, operant responses, conditioned locomotion in anticipation of sex, time spent near a particular sexual incentive, or choices made between two or more incentives can all be considered analogies of anticipatory sexual desire. Desire can also be inferred from certain appetitive behaviors that occur during copulation. Those aspects of sexual behavior are altered in a relatively selective fashion by certain drugs that are known to alter desire in the humans.

It is generally agreed that sexual reward in the humans reflects the pleasure obtained during or after genital stimulation (e.g., the pleasure inherent in genital stimulation during copulation and/or orgasm, and intimacy during the delicious afterglow). In male rats, the reward state induced by ejaculation supports the development of conditioned place and partner preferences. However, the sexual reward state also depends on the level of sexual arousal experienced prior to ejaculation. Both conditioned place and partner preferences in male rats can be abolished by treatment with the opioid antagonist drug naloxone during conditioning, indicating that the release of endogenous opioids during copulation and/or ejaculation are a critical feature of the reward state. Female rats also form conditioned place and partner preferences if they are allowed to control or pace the initiation and rate of copulation. This can be accomplished in open fields and bilevel chambers (in which the female can enforce her preferred copulatory interval by running from the male, forcing the male to chase her), or in pacing chambers bisected by a barrier with holes that allow only the female to cross into and out of the male's side. Similar to males, treatment with the opioid antagonist naloxone during conditioning abolishes the development of both conditioned place and partner preferences. Moreover, female rats given their primary sexual experiences under the influence of naloxone display significantly fewer solicitations and other sexual interactions with males when taken off the drug, indicating that the lack of opioid-induced reward during their formative sexual experiences lead to a decrease in measures of sexual desire. It thus appears that opioid turnover during copulation, particularly in hypothalamic and/or limbic regions of the brain, constitutes a critical feature of sexual reward.

Instrumental Responding for Primary or Conditioned Sexual Rewards

Sexual rewards may come in the form of primary reinforcers (e.g., orgasm in the humans; ejaculation in male rats or the ability to regulate or 'pace' copulation in female rats), or secondary reinforcers, such as stimuli associated with sexual gratification (e.g., certain facial features, clothes, or smells in the humans; certain odors or place cues in rats). All animals work to obtain such rewards. In male rats, those behaviors have included performance in obstruction boxes, straight-alley running, maze learning, crossing of electrified grids, nose-pokes through a wire-mesh screen that prevents contact with a sexually receptive female, digging through sand, bar-pressing for a sex partner or for second-order cues such as light associated with the arrival of a sex partner. Indeed, to gain access to receptive females, male guinea pigs will learn to run an alley, male pigeons will learn to peck keys, and male stickleback fish will learn to swim through rings.

Some appetitive instrumental sexual responses are expressed without much prior experience (e.g., nose-pokes, digging, obstruction-box performance, crossing electrified grids), whereas others require some degree of training (maze learning or bar pressing). Those behaviors can be reduced following castration, indicating that gonadal steroid actions in the brain are necessary for their development and/or maintenance, or following lesions of certain steroid-concentrating brain regions, for example, basolateral amygdala. They can also be reduced after a devaluation of the sexual reward (e.g., switching from receptive female to no female, an extinction procedure), or following infusions of DA antagonists to the nucleus accumbens (NAcc). DA antagonists administered systemically to male rats reduce running speed in a runway that leads to a goal box containing a sexually receptive female, and microinjections to the mPOA disrupt maze learning for sexual reinforcement in the male rats. It is likely that different DA terminal regions play a role in different types of unconditioned and conditioned sexual activity. Female rats will bar-press for access to gonadally intact, sexually active males, and access to intromissions from a male that were made contingent on poking a lever with the nose increased the incidents of nose-poking in sexually receptive female rats. Contingent access to male bedding or emulsified preputial gland secretions did not support increases in behavior, indicating that the copulatory stimulation was rewarding. As noted above, paradigms which allow females to pace the rate of copulation result in sexual reward. Any imposition of an operant prior to copulatory stimulation, therefore, allows females to pace copulation at their own preferred rate. Although copulation in general increases DA release in the mPOA, NAcc, and striatum of male and female rats, such operant pacing of copulation increases DA release more in the striatum relative to

unpaced copulation. Consistent with this, lesions of the striatum reduce the ability of females to pace the copulatory interactions, whereas lesions of the NAcc result in females that avoid sexual contact.

Desire and Reward Inferred from Copulatory Behaviors

The strength of certain copulatory behaviors can be taken as measures of sexual motivation. In female rats, for example, such behaviors include solicitations, pacing, and the magnitude of lordosis. In male rats, such behaviors include the latency to mount, the number of intromissions before ejaculation, and the total number of ejaculations the male can achieve in a circumscribed test. Lesions of the mPOA in female rats abolish solicitations and other precopulatory indices of sexual motivation. Lesions of the mPOA in male rats abolish copulatory behavior altogether, although they leave bar-pressing for second-order sexual incentives intact. Systemic treatment with the DA agonist apomorphine increases solicitations and reduces pacing in females, a pattern of behavior indicative of a greater degree of sexual desire and time spent copulating with males. In males, apomorphine stimulates mounts, intromissions, and ejaculations, although at high doses this excitatory effect is overpowered by the psychomotor stimulating effects of the drug. Conversely, systemic treatment of female rats with DA antagonists, such as haloperidol, dramatically reduces solicitations, increases pacing, and makes females unable to disengage from lordosis once they have engaged in the posture. In males, DA antagonists increase the mount and intromission latencies, and reduce the number of ejaculations that males are capable of attaining. As mentioned above, DA release is increased in the mPOA, NAcc, and striatum of both female and male rats during copulation, and in males, DA release falls immediately after each ejaculation, and rises again prior to the initiation of copulation. The role of DA in the NAcc appears to play a role in attention toward sexual incentives, especially during appetitive phases of sexual behavior, whereas the role of DA in the striatum appears to enhance motor output, but especially if an operant is imposed. DA in the mPOA appears to modulate appetitive responses, perhaps by activating efferent connections to the ventral tegmental area (VTA) to drive sexual-incentive motivation.

Other neurotransmitter systems are also important for sexual desire. For example, melanocortin agonists, such as MT-II and bremelanotide, increase solicitations selectively in female rats. Both agonists induce this effect after microinfusions to the lateral ventricles or directly to the mPOA, but not the ventromedial nucleus of the hypothalamus (VMH). Interestingly, systemic administration of bremelanotide increases DA release selectively in the mPOA, and blockade of D1 receptors

in the mPOA can reverse the increase in solicitations induced by systemic bremelanotide treatment. This suggests that melanocortin receptors in the mPOA potentiate sexual desire in females by promoting DA release and subsequent binding to D1 receptors. Recent clinical trials with bremelanotide have confirmed that the drug administered intranasally stimulates sexual desire both in pre- and postmenopausal women with hypoactive sexual desire disorder. The VMH plays a key role in regulating lordosis by estrogens, perhaps through a disinhibitory process by which glutamate interneurons in the VMH (whose activation inhibits both appetitive and consummatory measures of female sexual behavior) are inhibited by the estrogenic stimulation of γ -aminobutyric acid (GABA) and/or other inhibitory transmitters directly onto glutamate neurons. In contrast, GABA transmission in the mPOA inhibits female sexual behavior, whereas nitric oxide-potentiated glutamate release in the mPOA stimulates DA release presynaptically to enhance male sexual behavior. This complex neurochemical interplay in hypothalamic regions appears to involve both excitation and disinhibition in the activation of sexual desire. In turn, hypothalamic outputs combine with more general motivational systems in limbic regions of the striatum, amygdala, and piriform cortex (and likely elsewhere) to focus attention on primary and conditioned-incentive sexual stimuli in the external environment and direct motor patterns that enable animals to seek out and act upon those incentives ([Figure 2](#)).

Sexual Inhibition

All behaviors have a beginning, middle, and end. The end typically comes with the attainment of reward large enough to activate mechanisms of satiety. In any motivational system, reward is a dynamic function with an inverted U-shaped relationship to ongoing behavior: Low rewards do not generally sustain behavior, moderate-to-ideal rewards do, and high rewards induce the inhibitory feedback that characterizes satiety. With regard to sexual behavior, rewards that sustain sexual arousal and desire might be considered low-to-moderate, whereas high rewards like orgasm might be those that induce a period of sexual refractoriness. Although sexual satiety decreases sexual responding in the short term, the reward associated with it in male and female rats is necessary for the conditioning of sexual preferences in the long term.

The notion of separate (but interactive) neural systems for behavioral excitation and inhibition goes back to the work of early neurophysiologists like Sechenov, Sherrington, and Pavlov, and more modern psychologists like Jeffrey Gray. It has important implications for motivation, in general, because it posits that behavior can commence either due to direct excitation or through a process of disinhibition.

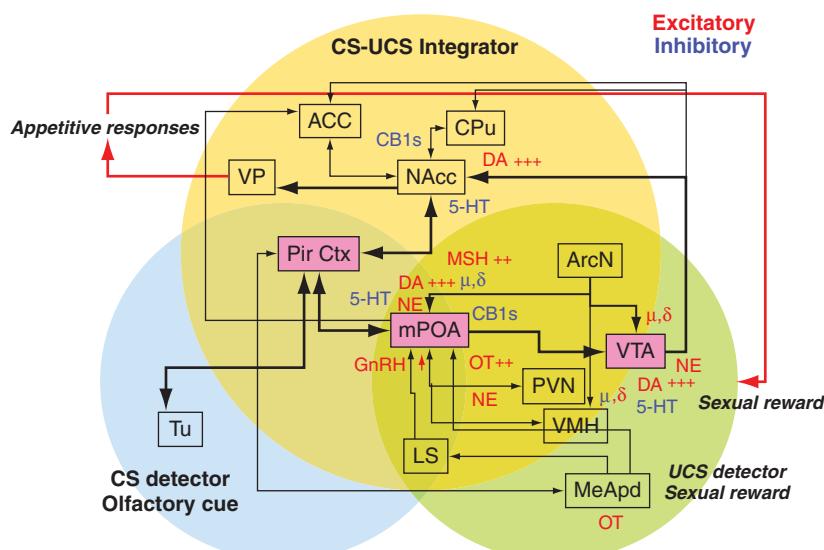


Figure 2 Neural systems which are critical for the display of conditioned olfactory preferences in the rat. Appetitive behaviors made toward conditioned stimuli lead to sexual reward that is processed by three interactive systems. Two systems process olfactory stimuli and sexual reward relatively independently, whereas a third – mesolimbic DA system – acts to integrate both the conditioned olfactory cue and its rewarding sexual outcome. Three common regions – the piriform cortex, mPOA, and VTA – are activated in male and female rats by conditioned olfactory stimuli. Opioid actions in the VTA potentiate mesolimbic DA activation, whereas opioid actions in the mPOA inhibit sexual arousal and desire. Neurotransmitter systems or their receptors in red are excitatory for sexual motivation whereas those in blue are inhibitory. Note that opioids can be excitatory in the VTA, inhibitory in the mPOA, or either in the VMH (depending on the receptor type). Abbreviations: ACC = anterior cingulate cortex; ArcN = arcuate nucleus of the hypothalamus; CB1 = cannabinoid Type 1 receptor; CPU = caudate-putamen (striatum); DA = dopamine; δ = delta opioid receptors; GnRH = gonadotropin-releasing hormone; LS = lateral septum; MeApd = posterior-dorsal nucleus of the medial amygdala; mPOA = medial preoptic area; MSH = melanocortin-stimulating hormone; μ = mu opioid receptors; NAcc = nucleus accumbens; NE = noradrenaline; OT = oxytocin; PirCtx = piriform cortex; PVN = paraventricular nucleus of the hypothalamus; Tu = olfactory tubercle; VMH = ventromedial nucleus of the hypothalamus; VP = ventral pallidum; VTA = ventral tegmental area; 5-HT = serotonin.

A Dual Control Model of sexual function has been advanced by researchers that posits excitatory and inhibitory mechanisms in the brain that interact to produce a level of sexual responsiveness in all individuals during all phases of the sexual response cycle. This model stresses the adaptive nature of both excitatory and inhibitory processes. For example, the adaptive nature of sexual excitement would drive individuals to seek out sexual partners for reproductive or reward purposes. The adaptive nature of sexual inhibition would guard against situations that threaten the individual, including chronically stressful life events. However, too little activation of the excitatory mechanisms, as might occur in hypogonadal individuals, or too much activation of the inhibitory mechanisms, would be expected to lead to decreased sexual motivation. The study of sexual inhibition is also critical if we are to understand how certain events or prosexual drugs (e.g., alcohol, cocaine) induce sexual disinhibition.

Sexual inhibition is most likely to occur in the presence of an obvious threat or a conditioned cultural proscription against the activity (complete with the possibility of getting caught). However, this raises some interesting issues with regard to the arousing nature of

inhibition. As mentioned above, central excitation and inhibition both activate the autonomic nervous system. A small degree of stress or threat (i.e., something naughty or even painful) can be arousing, especially for individuals with low levels of arousability. Translated to a sexual situation, such arousal could be transferred directed into sexual activity, perhaps to the point where, in some individuals, it becomes a necessary antecedent. Anger, fear, or even short-lived terror, can be preludes to sex because they stir passion or arousal. The stimuli that evoke excitation and inhibition may be different for different individuals and what inhibits one person may actually excite another.

Punishment and Stress

Although punishment with shock can suppress a variety of appetitive and consummatory behaviors in rats, such punishment has never been reported to induce sexual inhibition in males. In fact, shock, short-term pain (e.g., tail-pinch), or neutral stimuli paired with them, actually stimulate mounting in sexually sluggish or inactive male rats, and reduce the number of intromissions required for

ejaculation in sexually active males. As mentioned above, both male and female rats will readily cross electrified grids to gain access to sexually receptive partners, and arousal is a necessary antecedent to sexual activity and sexual reward in both male and female rats. However, more reliable methods of punishment exist. Rodents live in a predominantly olfactory world, and pairing estrous odors with gastrointestinal distress (induced by contingent injections of lithium chloride that make animals sick) induces a conditioned odor-aversion in male rats and hamsters that translates to avoidance of female vaginal secretions, increased mount and intromission latencies, decreased proportion of males that ejaculate, or avoidance of copulation altogether. This effect occurs if conditioning took place when the males were juveniles, and conditioned males utter distress vocalizations in the presence of vaginal secretions. Male rats can also learn to avoid copulation with sexually receptive females scented with odors that predict gastrointestinal distress, while copulating normally with unscented females.

When are animals in the wild exposed to circumstances in which they must inhibit their sexual responses? Dominance hierarchies are important in controlling copulation in the wild, and, in some primate species, subordinate males will not attempt to copulate with females in the presence of dominant males. Males of many species also inhibit their copulatory advances in the presence of sexually nonreceptive females. This is in marked contrast to laboratory settings in which male rats will attempt to copulate with nonreceptive females placed into chambers in which the males have copulated previously with sexually receptive females. However, training sexually active male rats to differentiate between sexually receptive and nonreceptive females on alternating test trials leads quickly to a substantial reduction in the proportion of males that attempt to copulate with nonreceptive females. The aversive stimuli provided by the nonreceptive females include a thwarting of attempted copulations due to the female's lack of receptivity, along with aggressive and defensive behaviors (e.g., boxing, biting, kicking) when the male attempts to mount. By virtue of the females being nonreceptive, these behaviors are paired with a lack of estrous odors. Males learn quickly to differentiate the presence of estrous odors with preceptive and receptive behaviors, and the lack of estrous odors with thwarted sexual advances and female aggression (which can be severe). In the wild, such conditioning may occur normally during adolescence. As juveniles, male and female rats mount almost anything, including one another. As the expression of this behavior transitions into adult forms, males likely attempt to mount adult females that are not in heat. Of course, those females teach the males not to try it again. Using this type of conditioned inhibition, researchers have shown that low-to-moderate doses of alcohol can release sexual behavior

from inhibition and compel male rats to attempt copulation with nonreceptive females. This finding is of interest for several reasons, most notably because alcohol intoxication figures in high-risk human sexual activity. A neutral odor paired with sexually nonreceptive females can also inhibit male sexual advances toward a sexually receptive female bearing the odor.

Refractoriness and Sexual Satiety

If male rats are allowed to copulate to sexual exhaustion, they display reduced responsiveness to female solicitations and many will not copulate to ejaculation for 24–48 h. This inhibition is due to neurochemical events that underlie sexual satiety, and can be partly or fully reversed with the α_2 -adrenergic receptor antagonist yohimbine (which increases noradrenergic tone), the 5-HT-1a agonist 8-OH-DPAT (which decreases serotonin release), and the opioid-receptor antagonists naloxone or naltrexone. This suggests that increased release of serotonin and endogenous opioids, and decreased release of noradrenaline, somewhere in the brain underlie the inhibitory state of sexual satiety. As with men, sexual inhibition in women can occur as a result of stress, lack of intimacy, sexual nonreward, or in the refractory state after orgasm that denotes satiety. Sexual inhibition in female rats has been explored rudimentarily in paradigms of estrus termination (in which sexual behavior is inhibited by a large amount of vaginocervical stimulation prior to testing), after subthreshold administration of ovarian hormones to ovariectomized rats (which stimulates a low level of lordosis but no appetitive behaviors), and following sexual nonreward (induced as noted above by naloxone treatment during the females' early sexual experience). Appetitive sexual behaviors like solicitation are the most sensitive to those treatments. As the intensity of the inhibition progresses, females display increases in pacing and defensive behaviors and a decrease in high-intensity lordosis.

Integration

Motivation imbues all aspects of sexual behavior. In all animals, sexual arousal, desire, reward, and inhibition are integrated along neural mechanisms of sexual excitation and inhibition. These mechanisms are set up by steroid hormone actions in the hypothalamus (notably the mPOA) and limbic system (notably the NAcc, amygdala, and cells in the VTA that give rise to the mesolimbic DA system) and altered conditionally (and continually) by interaction with sexual incentive stimuli that lead to sexual reward. Hormonal actions stimulate the synthesis of proteins that act as transmitter receptors, synthesizing enzymes, uptake mechanisms, etc., that create a different 'state' of neurotransmitter actions in hypothalamic,

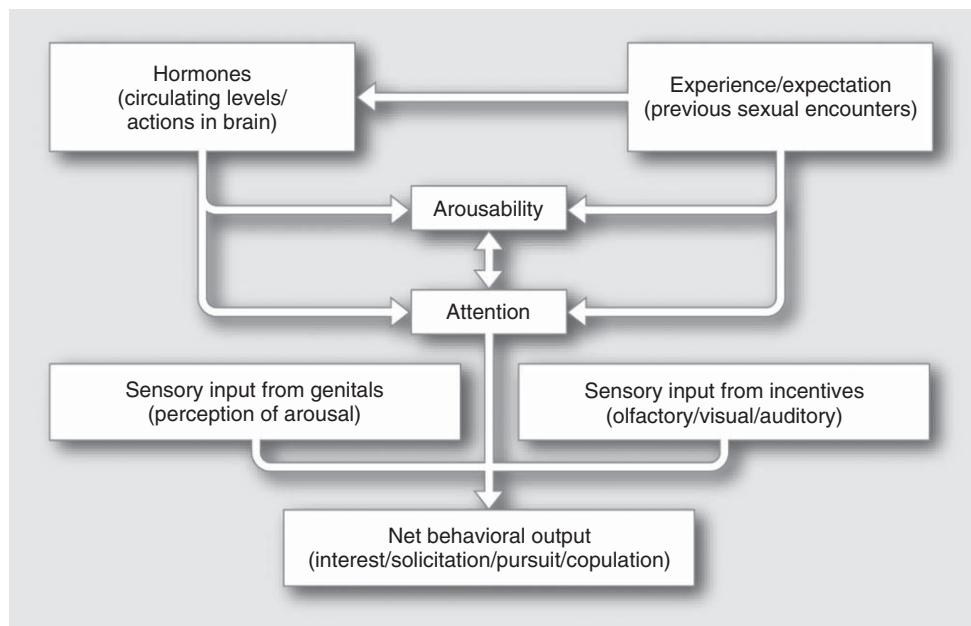


Figure 3 Hypothetical relationship between experience, hormonal activation, arousability, attention, and stimulus processing from genital sensations and external incentives on net sexual responding at any given time. Note that excitatory and inhibitory feedback can occur anywhere in this flowchart to strengthen or reduce responding. Such feedback provides moment-to-moment modulation of sexual motivation. Adapted with permission from Pfau JG and Scepkowski LA (2005) The biological basis for libido. *Current Sexual Health Reports* 2: 95–100.

limbic, and cortical sites to support sexual responding. The brain's excitatory and inhibitory systems for sexual behavior are likely in constant interaction. The excitatory system involves the coordinated activation of noradrenergic pathways for central and autonomic arousal, and mesolimbic, nigrostriatal, and hypothalamic DA pathways for attention toward external incentive stimuli and the activation of specific sexual responses. These systems are themselves modulated by certain neuropeptides, notably melanocortins, which activate DA release in the mPOA selectively and conditionally in response to sexual incentive stimuli, and may modulate oxytocin which selectively and conditionally activates genital blood flow. The inhibitory system involves the coordinated activation of opioid pathways that support sexual reward states and serotonergic pathways that induce feelings of satiety after orgasm or ejaculation. However, those systems may also be activated in response to stressors or environments in which sexual responding is dangerous or otherwise not in the organism's best interest. Some transmitter pathways feed-forward to sensitize mechanisms of bonding (e.g., oxytocin, vasopressin), activate reproduction and steroid hormone synthesis (e.g., gonadotropin-releasing hormone), and to sensitize mesolimbic and other DA pathways so that conditioned sexual incentives can prime the excitatory system into action. The sum of those influences (Figure 3) generates a net 'sexual motivation' that is either at a hypothetical zero, in the case where excitatory and inhibitory influences are equal, or is

enhanced or inhibited depending on the dominant influence at the time.

See also: Control of Food Intake; Hormonal Contributions to Arousal and Motivation; Incentive Motivation and Incentive Salience; Infant Bonding and Attachment; Sex Hormones, Mood, and Cognition.

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Organization of Human Locomotion: Proprioception and Quadrupedal Coordination

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Glossary

Central pattern generators (CPGs) – Neuronal circuits located in the thoraco-lumbar spinal cord that generate the locomotor pattern. This pattern is inborn, is shaped by proprioceptive input, and is under the control of cortical and brainstem centers.

Electromyography (EMG) – A device used to record muscle activity by needle or surface electrodes. It reflects the activity of motoneurons within the spinal cord.

Monosynaptic stretch reflex – This reflex is the simplest reflex system. Muscle spindles within a muscle monitor changes in muscle length. The afferent discharge of the muscle spindles is mediated by fast-conducting afferents to the motoneurons of the muscle of origin (i.e., one synapse). The excitatory input leads to a discharge of the motoneurons. This again leads to a contraction of the muscle which becomes stretched. The delay between the stretch of the muscle and its contraction is therefore within a minimal range (e.g., latency for the lower leg muscle is around 40 ms). The monosynaptic stretch reflex plays a role in the neurological examination in order to recognize, for example, a spastic paresis.

Proprioception – Afferent information from the limbs (muscles, skin, and tendons) to the spinal cord. This information is used by spinal neuronal circuits to shape the locomotor pattern according to the actual requirements.

Propriospinal neurons – Neuronal circuits within the spinal cord. These circuits activate and control movements of the different limbs, for example, the coordination of upper and lower limbs during locomotion.

Clinical Relevance

The study of movement control has relevance to our general understanding of brain function; however, it also has implications for specific fields, such as neurology, cognitive neuroscience, rehabilitation medicine, and robotics. Our understanding of movement disorders and their appropriate treatment depends on knowledge of the

neuronal mechanisms that underlie normal functional movements. Movement disorders are the focus of one of the most rapidly expanding fields in medicine, leading to increasing costs of treatment and rehabilitation. There is increasing evidence that movement disorders, such as spasticity and Parkinson's disease, involve the defective use of afferent input in combination with secondary compensatory processes. This article focuses on the role of proprioception and a quadrupedal organization of human locomotion, which can serve as a paradigm for functional movements.

In a general sense, locomotion is representative of movement control. It is a subconsciously performed, everyday movement that is highly reproducible and is adapted automatically to existing conditions, such as ground irregularities, within a large safety margin. Furthermore, knowledge about the neuronal control of human locomotion is of wide interest for clinical reasons. Characteristic disorders of locomotion are often the first sign of a central or peripheral lesion of the motor system.

Neuronal Control of Locomotion

It is generally accepted that locomotion in mammals depends on neuronal circuits (networks of interneurons) in the spinal cord (the central pattern generator (CPG)) that can act in the absence of any afferent input. Afferent information influences the central (spinal) pattern and, conversely, the CPG selects appropriate afferent information according to external requirements. In addition, proprioceptive information provides the basis for a conscious representation of our body in space, which becomes severely disturbed in deafferented individuals. Both the spinal locomotor center (CPG) and the reflexes that mediate afferent input to the spinal cord are under the control of the brainstem. In addition, there is a phase-linked corticospinal control of locomotion in humans. Voluntary commands have to interact with the spinal locomotor generator to change, for example, the direction of gait or to avoid an obstacle. Any disturbance of this finely coordinated interaction between afferent inputs and pattern generation after a central lesion, such as stroke or spinal cord injury, leads to a movement disorder.

Irrespective of the conditions under which stance and gait are investigated, the neuronal pattern that is evoked

during a particular task is always directed to hold the body's center of mass over the base of support. All control mechanisms must therefore be considered and discussed in this respect. One consequence is that the selection of afferent input by central mechanisms must correspond to the requirement for body stabilization. Neuronal signals of muscle stretch or length are insufficient for the control of bipedal posture. Only a combination of afferent inputs can provide the information that is needed to control the body's equilibrium during locomotion.

The control of locomotion involves the use of afferent information from a variety of sources in the visual, vestibular, and proprioceptive systems. As a rule, spinal reflex pathways and descending pathways converge on common spinal interneurons to integrate these inputs. Furthermore, the amount of proprioceptive feedback from the legs during various locomotor activities determines the influence of vestibulospinal input on the stabilization of body movement. Conversely, somatosensory loss increases vestibulospinal sensitivity.

Reflex Mechanisms

The adaptation of the locomotor pattern to external demands is achieved by proprioceptive input that continuously modulates the programmed pattern during locomotion as per the information from peripheral sensors. Proprioceptors include receptors of the locomotor system that are located in the muscles and tendons, as well as other mechanoreceptors in the joints and the skin. The impulses of these receptors – which signal, for example, muscle stretch or tension – are conveyed to the spinal cord by afferent nerve fibers of different diameters, and

hence of different excitability and conduction velocity. On the basis of these differences, the input from various types of receptors can be separated into fiber types of group I to group IV (Table 1). For example, impulses from sensors of dynamic muscle length (spindles) are mediated by group Ia afferents, whereas those representing static muscle length are carried by group II fibers, and information about tension developed at the tendons (measured by Golgi organs) is transmitted by group Ib fibers to the spinal cord. Only the group Ia fibers have direct excitatory connections to the motoneurons of the same muscle – they are part of the monosynaptic stretch reflex, which has a characteristic short latency (~40 ms). The other afferent fibers converge on spinal interneurons that project in a more complex way to the motoneurons of leg muscles. Consequently, their afferent input usually leads to responses in synergistic muscle groups with a longer latency (starting at 70–80 ms). This pathway represents the polysynaptic, or long-latency, reflex mechanism.

For methodological reasons, there has been a bias toward the simplest reflex system, the monosynaptic stretch reflex, in experimental studies of the afferent sources that contribute to the regulation of human gait. Most studies have focused on the contribution of type Ia afferents using the H-reflex technique (which measures the monosynaptic reflex response of the muscle to low-intensity electrical stimulation of group Ia afferents). However, as discussed below, the contribution of this reflex system to the regulation of locomotion is limited. The significance of, for example, type Ib, II, or III afferents to locomotor movements has been underestimated for many years.

In fact, there is multisensory afferent input during locomotion both in the cat and in humans. This proprioceptive input arises from muscles, skin, joints, and

Table 1 Prorioceptive reflexes suggested to be involved in locomotion

Appropriate stimulus	Receptor pathway	Afferent connection	Reflex	Suggested function
Dynamic muscle stretch (small amplitude)	Muscle spindles (nuclear bag)	Group I	Monosynaptic stretch reflex	Compensation for ground irregularities; running/hopping (?)
Muscle stretch (large amplitude; static)	Muscle spindles (nuclear chain)	Group II (III)	Polysynaptic spinal reflex	Compensation for perturbations of gait
Change in body's center of mass	Golgi tendon organs	Group Ib	Polysynaptic; convergence spinal interneurons	Control of body's center of mass
Joint-movement position	Muscles around joint; mechano-sensors of joint capsule	Group I,II	Polysynaptic; convergence spinal interneurons	Influence on locomotor pattern (hip); local compensation (other leg joints)
Skin deformation	Mechanosensors of skin	Group II (III)	Polysynaptic; convergence spinal interneurons	Adaptation to actual ground conditions
Noxious stimulus; pressure	Free endings; Pacini corpuscles	Group III, IV	Spinal interneurons (CPG); flexor reflex	Withdrawal reflex

CPG, central pattern generator.

tendons. One of the primary functions of proprioceptors is to detect unexpected events and to initiate rapid compensatory electromyographic (EMG) responses. Recently, it has become clear that proprioceptors have further roles in the regulation of motor output during unperturbed movements. This is the case, for example, during the push-off phase of locomotion, running and hopping, and the control of the unperturbed step cycle (cf. **Figure 1**).

Monosynaptic Reflexes

The monosynaptic reflex represents the most extensively investigated proprioceptive reflex system. Monosynaptic reflex responses can be recognized clearly by the characteristic short-onset latency of the EMG responses, which alter muscle stretch in different motor conditions.

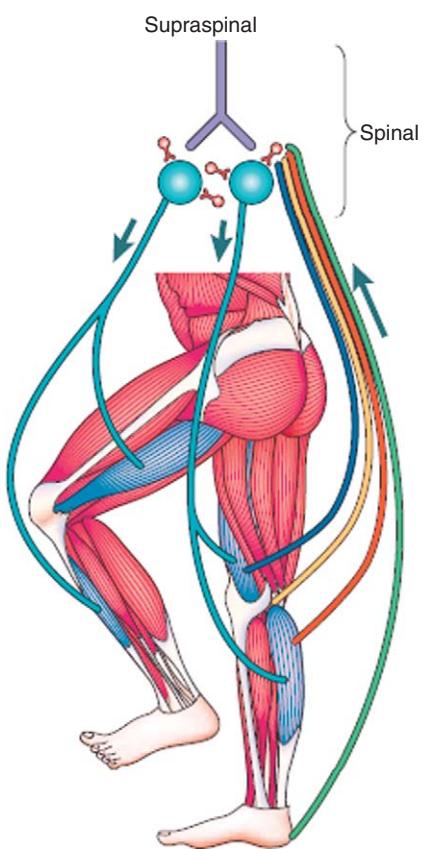


Figure 1 Schematic drawing of the neuronal mechanisms involved in human gait. Physiological condition. Leg muscles become activated by programmed pattern that is generated in spinal neuronal circuits (turquoise pathway). This pattern is modulated by multisensory afferent input, which adapts the pattern to meet existing requirements. Both the pattern and the reflex mechanisms are under supraspinal control. In addition, there is differential neuronal control of leg extensor and flexor muscles. Whereas extensors are mainly activated by proprioceptive feedback, the flexors are under central control. From Dietz V (2002) Proprioception and locomotor disorders. *Nature Reviews Neuroscience* 3: 781–790.

The potential significance to locomotion of group Ia afferent input lies in the fact that its gain can be modulated by presynaptic inhibition (i.e., type Ia excitatory input to the motoneurons can be attenuated, e.g., by supraspinal influences) and by changes in muscle-spindle sensitivity. This sensitivity is controlled by the fusimotor system, which can vary the strength of activation of intrafusal muscle fibers in muscle spindles by γ -motoneurons.

During locomotion, the threshold and amplitude of the soleus H-reflex is modulated over the entire step cycle. The functional implications of this modulation of group Ia afferent input during locomotion are suggested to be threefold: First, facilitation of the gastrocnemius/soleus stretch reflex at the end of the stance phase contributes to compensation for ground irregularities and assists during the push-off phase. Second, the depression of type Ia inputs to leg extensor motoneurons during the swing phase prevents the occurrence of the extensor stretch reflex during ankle dorsiflexion. Finally, type Ia afferents are proposed to have an important role in the continuous online control of joint movements.

Nevertheless, the functional significance of monosynaptic stretch reflexes during gait remains at part unclear, largely for reasons related to the properties of the monosynaptic stretch reflex itself. The monosynaptic reflex system is highly sensitive to small inputs, and its function during gait should, therefore, be restricted to compensation for small ground irregularities. This is consistent with the observation that leg muscle vibration, which excites type Ia afferent input, affects human locomotion only a little.

Cutaneous Reflexes

Muscle responses induced by electrical stimulation of a sensory nerve of a limb are mediated by cutaneous reflexes. These reflex responses appear in different muscles of the limb with a latency that is compatible with a spinal pathway. In human leg muscles, the task-dependency of cutaneous reflexes has been shown in standing versus running or walking, and in cycling versus static contraction. Cutaneous reflexes in leg muscles are sensitive to the specific motor task that is performed, and undergo profound modulation depending on the context in which they are evoked. Furthermore, cutaneous reflex modulation is nerve specific (i.e., it depends on the nerve that is stimulated), and this seems to be important functionally. Certain features of cutaneous-reflex modulation, such as task-dependency, have been suggested to include central-pattern-generating elements. Such influences of the CPG on rhythmical and cyclical leg movements would be parallel with observations made in the cat.

Polysynaptic Reflexes

It has been proposed that polysynaptic reflexes are mediated mainly by muscle proprioceptive input from group II afferent fibers (e.g., from static muscle spindles or skin) and possibly from group III fibers (carrying information mainly from joint capsules and ligaments). Polysynaptic spinal reflexes produce functionally useful compensatory responses during locomotion, which are more complex than simple stretch-reflex responses. As the pathway of this reflex system is polysynaptic, it allows the integration of inputs from muscle, joint, and cutaneous afferents, and convergence with commands from supraspinal centers to common spinal interneurons. In addition, this reflex system has excitatory and inhibitory connections to both extensors and flexors.

The sensory input determines the direction, velocity, and amplitude of the adjustment that is needed to restore the subject's center of gravity over the feet and to generate the required pattern of leg muscle activation. Consequently, this reflex system leads to functional activation of synergistic muscle groups of both legs, and it can clearly be separated from segmental stretch-reflex responses, which affect only individual muscles.

A polysynaptic pathway also mediates the effects of flexor-reflex afferent (FRA) fibers. Although the modulation of flexion reflexes has several similarities to the polysynaptic spinal reflexes discussed above, there are also distinct differences. First, in people with spinal cord lesions, there is a loss of polysynaptic spinal reflexes, but the flexor reflex can still be elicited. Second, the characteristic feature of the flexor reflex is to serve as a withdrawal reflex to noxious stimuli, which is released as a direct response by the CPG, rather than in modulating the locomotor pattern to adapt to irregularities of the ground. Finally, the activity of spinal polysynaptic reflexes depends on the presence of contact forces, whereas flexor-reflex responses can be elicited by tibial nerve stimulation independently of loading.

Two main sources of afferent input are probably integrated in the polysynaptic reflex system: load-related and joint-position-related information. Load receptors, or graviceptors, are thought to signal the influence of gravity on the body to the spinal cord. There is only indirect evidence for such receptors in humans. For many years, the question of how the position of the body's center of mass relative to the feet is signaled to the central nervous system (CNS) has been neglected in most studies of human locomotion. To achieve appropriate gain control of postural reflexes, information is needed that signals the influence of gravity on the body. This information is insufficiently provided by muscle stretch receptors and the vestibular system.

Essential Sources of Proprioception

During locomotion, multisensory proprioceptive feedback is continuously weighted and selected. This process depends on the requirements of a particular locomotor task and the availability of afferent input. Afferent inputs from load receptors and hip joints make essential contributions to the activation pattern of leg muscles during human locomotion. It has been proposed that proprioceptive input from extensor muscles, and probably also from mechanoreceptors in the sole of the foot, provides load information. The afferents that signal hip-joint position mainly come from muscles around the hip. The role of this afferent activity in rhythmic locomotion is to shape the pattern, to control phase transitions, and to reinforce ongoing activity. Simple stretch and cutaneous reflexes might be involved in compensating for irregularities and in adapting to ground irregularities.

Load-Related Afferent Input

Extensor load receptors are suggested to provide essential information about the body's center of gravity during locomotion in humans. Experiments in the cat indicate that these receptor signals might arise from Golgi tendon organs and be carried by type Ib afferents to the spinal locomotor generator. In humans, the influence of load receptors on the regulation of stance and gait became evident from studies of weightlessness induced in adult humans either during space flight or by water immersion. Furthermore, it became clear that body load has an effect on the magnitude of polysynaptic reflex responses. The effect of load-receptor input on leg extensor activation during the stance phase of gait might be reinforced by heteronymous reflexes from ankle dorsiflexors. In addition, extensor muscle activity is reinforced during the stance phase by positive feedback, which contributes to load compensation without leading to instability.

Joint-Position-Related Afferent Input

In the cat, there are two main sources of afferent input that lead to rhythm entrainment and/or resetting of locomotor activity. Such input can either block or induce a switch between the alternating flexor and extensor locomotor bursts. The first of the afferent sources that satisfy these criteria is related to load, whereas the second is related to hip position. The afferents that signal hip-joint position mainly come from muscles that act around the hip. It has been suggested that receptors of the hairy skin can also provide high-fidelity information about knee-joint movements in humans.

Quadrupedal Coordination of Bipedal Locomotion

For most of the basic mechanisms that underlie locomotion, there seems to be no fundamental difference between bipeds and quadrupeds. Essential spinal neuronal mechanisms, such as the afferent inputs that determine the locomotor pattern (including hip-joint-related and load-receptor-related inputs), are probably similar for quadrupedal and bipedal locomotion. The coordination of forelimb and hindlimb rhythmic activities is a main characteristic feature of quadrupedal locomotion. Specialized neural circuits located in the caudal spinal cord (CPG) organize hindlimb locomotor activity, whereas specialized circuits in the rostral spinal cord control forelimb movements. The coordination of both circuits is mediated by propriospinal neurons with long axons, which couple the cervical and lumbar enlargements of the spinal cord.

In many respects, bipedal and quadrupedal locomotion share common spinal neuronal control mechanisms. As in quadrupeds, long projecting propriospinal neurons couple the cervical and lumbar enlargements in humans. Furthermore, the coordination of limb movements during walking is similar in human infants, adults, and quadrupeds. Nevertheless, there are distinct differences also as the upper limb in primates has become specialized to perform skilled hand movements. The evolution of upright stance and gait, in association with a differentiation of hand movements, represents a basic requirement for human cultural development.

In humans there is a task-dependent switch, from a strong direct (i.e., monosynaptic) cortical–motoneuronal control during reaching and skilled hand and finger movements to a more indirect control by cervical propriospinal circuits during locomotion, as it appears to occur in the cat. The neuronal control of human locomotor activities is based on the coordination of the quadruped fore- and hindlimbs, and this phylogenetically older system coexists with the newly developed, direct cortical–motoneuronal control of skilled hand movements. A typical example for such an interlimb coordination in humans represents the alternated movements of the arms during locomotion and its impairment, for example, in Parkinson's disease.

Coordination of Arm and Leg Movements

There is some evidence that interlimb coordination is organized similarly in the lower and upper limbs during cyclic movements of humans and cats. This indicates that the neuronal coordination and patterns of reflex modulation are conserved within the human lumbar and cervical spinal cord. During human bipedal walking, a quadrupedal limb

coordination of upper and lower limb muscles occurs similar to that of fore- and hindlimbs in quadrupeds.

Evidence for Neuronal Coupling

Recent experiments have indicated that there is neuronal coupling of upper and lower limb muscles during various human locomotor activities. A linkage between the cervical and lumbar enlargement of the spinal cord by propriospinal neuronal circuits with long axons can also be inferred on the basis of H-reflex studies. For example, during rhythmic movements of one foot, a cyclic H-reflex modulation was observed in the upper limbs. According to recent studies using functional magnetic resonance imaging, the supplementary motor area might be involved in the supraspinal control of this coupling between upper and lower limb movements.

Task Dependency of Neuronal Coupling

Only during walking do mechanical impulses applied to one leg (in the middle or at the end of the stance phase) evoke distinct bilateral arm EMG responses. Similar interlimb reflex responses were obtained in arm muscles following electrical stimulation of the distal tibial nerve (a mixed nerve, which innervates plantar foot muscles and the skin of the sole) during walking. Correspondingly, arm muscle responses were absent when stimuli were applied either during standing with voluntary arm swing or sitting while writing (i.e., with a comparable background EMG activity). These observations indicate a flexible task-dependent neuronal coupling between upper and lower limbs. The pathway that couples upper and lower limb movements seems to become gated by the activity of the CPG during walking. It has been concluded that a stimulus applied to a leg can exert a direct influence not only on the compensatory leg muscle EMG activity but also, depending on the task, on the neuronal control of upper arm muscles of both sides. The range of movements in which such task-dependent neuronal coupling of upper and lower limb movements occurs has yet to be determined. The stronger impact of leg flexors in interlimb coordination is in line with the increasing evidence that leg flexor and leg extensor muscles are differentially controlled, both in animals and humans.

Recently, it has been shown that arm and leg muscle activity is well coordinated during walking, crawling on all fours, or swimming. In such conditions, arm and leg movements are locked with a fixed frequency-relationship. This indicates a coupling of the neuronal circuits controlling arm and leg movements, which is again under supraspinal control. The frequency relationship characterizing this coordination corresponds to that observed in well-defined biological systems consisting of coupled

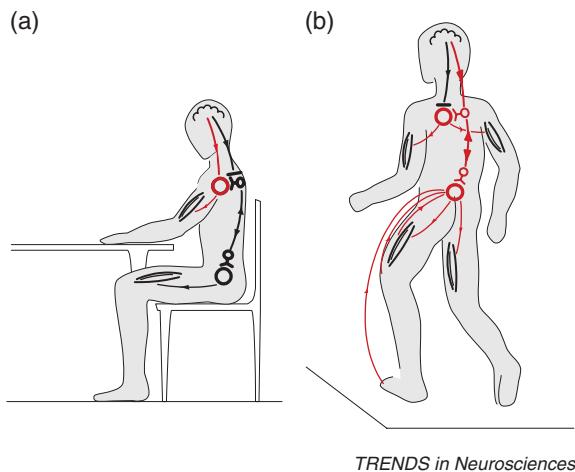


Figure 2 Movement control during different motor tasks. According to the research cited in this article, neuronal control of arm movement is task dependent. (a) During skilled hand movements, strong direct cortical-motoneuronal excitation is predominant (red lines) and the cervical propriospinal neuronal system is inhibited. (b) During locomotion, it is assumed that the brain command is predominantly mediated by interneurons. Cervical and thoraco-lumbar propriospinal systems become coupled and coordinate arm and leg movements (red lines). From Dietz V (2002) Do human bipeds use quadrupedal coordination? *Trends in Neurosciences* 25: 462–467.

oscillators. In addition, during gait, swinging of the arms serves to counteract torsion-related movements of the trunk. Therefore, swinging of the arms can be seen as an integral part of the dynamics of progression.

Differential Control of Upper Limb Movements

The task dependency of the neuronal coupling between upper and lower limbs might be based on a differential neuronal control of upper limbs during skilled hand movements and during locomotion. Direct cortical–motoneuronal connections to hand muscles are thought to determine the degree of dexterity in humans and nonhuman primates. It has been suggested that these phylogenetically new components are integrated into preexisting neuronal circuits. Indirect evidence indicates that propriospinal neuronal circuits corresponding to those described for cats persist, and most likely remain involved in the control of arm movement. It seems possible that there is an indirect corticospinal pathway to upper limb motoneuron pools, in addition to the well-documented direct cortical–motoneuronal pathway.

On the basis of the available evidence, the following hypothesis is put forward. Efficient corticospinal excitation of upper limb motoneurons via propriospinal neurons might occur during automatically performed movements, such as locomotion. By contrast, during skilled hand movements, strong cortical–motoneuronal

input dominates and transmission through the propriospinal system becomes suppressed.

Conclusions

It is proposed that a flexible coupling of thoraco-lumbar and cervical centers allows humans to use the upper limbs for manipulative and skilled movements or, alternatively, for locomotor tasks (Figure 2). This implicates a functional, task-dependent gating of neuronal pathways between the neuronal circuits controlling lower and upper limb muscles during walking, reflected in the arm swing as a residual function of quadrupedal locomotion. Therefore, propriospinal networks linking the thoraco-lumbar and cervical parts of the spinal cord can either function in a coupled way, for example, during locomotion, or an uncoupled mode during goal-directed voluntary movements.

Further studies are required to define more precisely the functions of evolutionarily older and evolutionarily newer neuronal control mechanisms in the broad variety of movements that can be carried out by humans.

See also: Active Avoidance and Escape Learning; Behavioral Planning: Neurophysiological Approach of the Frontal Lobe Function in Primates; Motor Learning in the Vestibulo-Ocular Reflex; Neural Plasticity of Spinal Reflexes; Orientation and Navigation; Parkinson's Disease; Primate Origins of Human Behavior; Voluntary Movement: Control, Learning and Memory.

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Environmental Influences on Adult Neurogenesis

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Glossary

Bromodeoxyuridine (BrdU) – A synthetic thymidine analog that becomes incorporated into a cell's DNA during the S-phase of the cell cycle. BrdU is commonly used to detect proliferating cells in the brain.

Dentate gyrus – Part of the hippocampal formation and one of the select brain structures currently known to have high rates of adult neurogenesis in mammals.

Hypothalamic-pituitary-adrenal (HPA) axis – The combined system of direct and feedback projections between the hypothalamus, the pituitary gland, and the adrenal glands, which is ultimately involved in regulating stress-related homeostasis.

Irradiation – The use of radiation, which is a technique that can be used to block hippocampal neurogenesis.

Neurogenesis – The creation of new neurons.

Environmental influences play a key role in refining the neural circuitry required for normal adult brain function. The hippocampus is a brain region that undergoes structural changes throughout life. The dentate gyrus of the hippocampus has a great capacity to produce new neurons in adulthood. New neurons are produced from progenitor cells residing in the dentate gyrus within the subgranular zone, a region on the border of the granule cell layer and hilus (see **Figure 1**). These cells divide, producing mostly new neurons and a lesser percentage of non-neuronal cells. The new neurons differentiate into granule cells and become functionally integrated into the preexisting neuronal network. The hippocampus may be especially sensitive to environmental perturbations due to a high degree of structural plasticity in this brain region. This article reviews environmental influences on adult neurogenesis in the dentate gyrus with a specific emphasis on the effects of stress.

Stress Effects on Adult Neurogenesis

Although the hippocampus is primarily associated with learning and memory, this structure has also been implicated in the stress response. The hippocampus contains a high concentration of glucocorticoid receptors and stress throughout life has been shown to influence hippocampal biochemistry and function. Likewise, stress during

prenatal, postnatal, and adult life can alter adult neurogenesis in the dentate gyrus.

Prenatal Environment

Prenatal stress predisposes offspring to psychiatric disorders in adulthood. Since adult neurogenesis has been linked to mood disorders, understanding the influence of early aversive experiences on the production of new neurons may shed light on the mechanisms that underlie psychopathology.

Rat offspring exposed to prenatal stress demonstrate permanent behavioral and neurobiological changes. Maternal stress during the last week of pregnancy alters adult neurogenesis in offspring. Regardless of the stressor (repetitive restraint stress; various unpredictable stressors), both male and female offspring showed decreased proliferating cells in adulthood following prenatal stress. The changes in bromodeoxyuridine (BrdU)-labeled cells reflect changes in cell proliferation, as confirmed by a significant reduction in Ki67-labeled cells, an endogenous cell cycle marker, in the dentate gyrus of prenatally stressed rats. Yet, one study only found a significant reduction in hippocampal granule cell neurons among animals exposed to stress at the end of gestation. These different results are likely due to inconsistencies in stress paradigms and methodologies used to visualize and measure neurogenesis.

This finding has been further extended into primates, investigating whether the timing of maternal stress alters postnatal neurogenesis in offspring. Pregnant rhesus monkeys were subjected to 6 weeks of acoustic startle stress, 5 days a week, during early or late gestation. These time periods corresponded with two distinct stages of cell growth and synaptogenesis in the fetal monkey cortex. Both early and late fetal disturbances dramatically decreased the density of new cells in the dentate gyrus of juvenile monkeys, compared to controls. Collectively, these findings indicate that prenatal stress can have lasting negative effects on the rodent and primate brain, including the suppression of neurogenesis later in life. The mechanisms that induce persistent inhibition of postnatal neurogenesis following developmental stress remain unknown, but glucocorticoids are likely to play some role.

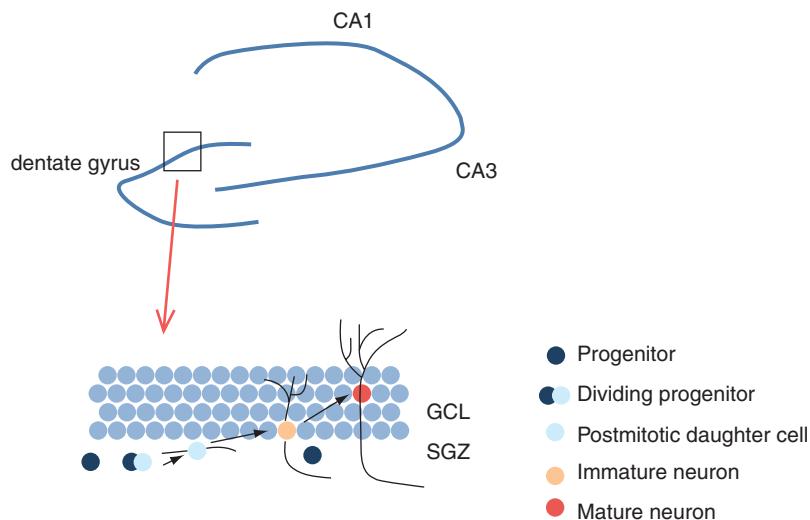


Figure 1 Adult neurogenesis in the dentate gyrus. New neurons develop from progenitor cells in the subgranular zone (SGZ), which divide to form postmitotic daughter cells. The remaining progenitor cell stays in the SGZ and continues to proliferate. Daughter cells migrate into the granule cell layer (GCL) and differentiate into immature neurons. These neurons develop dendrites that reach through the GCL into the molecular layer, and axons that reach into area CA3. New granule cells mature, develop neuronal properties, such as complex dendritic morphology, and become integrated into the existing neuronal circuitry.

Neonatal Environment

While maternal experience is critical to fetal brain development, environmental influences during the postnatal period have equally profound consequences. Early-life stressors activate the hypothalamic–pituitary–adrenal (HPA) axis; this activation can later impact some hippocampal functions, such as learning and anxiety regulation (see below), increase susceptibility to psychopathology, and have lasting effects on adult neurogenesis.

Rats subjected to prolonged maternal separation from postnatal day 1 to postnatal day 14 have significantly fewer proliferating cells and immature neurons in the dentate gyrus in adulthood, compared to control or handled rats (those that experienced a shorter duration of maternal separation). This reduction is similar to cell proliferation effects observed after exposure to an acute stressor. Given that a shorter duration of maternal separation does not alter adult neurogenesis, this suggests that only very extreme changes in mother–pup contact result in alterations to the dentate gyrus. However, the effects of maternal separation on cell proliferation and immature neuron production do not have a permanent effect on the number of new neurons. By 3 weeks after mitosis, the number of new neurons in maternally-separated rats is similar to that of controls, suggesting that compensatory factors may result in a greater proportion of surviving new cells. Interestingly, in mice, maternal separation-induced deficits in adult neurogenesis have also been observed, but following only one 24 hour period of separation on postnatal day 9, suggesting that species differences may play a role in the impact that a single bout of maternal separation can have on adult neurogenesis.

Adult Environment

In adulthood, the hormonal stress response is a critical reaction to demands in the environment that disrupt homeostasis. Activation of the HPA axis, and an increase in the production of glucocorticoids, is associated with both physical and psychological stressors. Both acute and chronic stress paradigms, including subordination, resident–intruder, footshock, restraint, isolation, cold immobilization, cold swim, and predator odor, decrease cell proliferation and neurogenesis in the dentate gyrus. This effect has been shown in at least four species: mice, rats, tree shrews, and marmosets. A single exposure to predator odor decreases cell proliferation in the adult rat, while a single exposure to the resident–intruder paradigm reduces cell proliferation and neurogenesis in the adult tree shrew and marmoset. Similar results have been found with a chronic psychosocial stress paradigm – prolonged social defeat decreases cell proliferation in the adult rat and tree shrew.

It is likely that the effects of stress on cell proliferation are partly related to an increase in glucocorticoids. Glucocorticoid manipulations have many structural effects on the hippocampus, including dendritic architecture, synaptic plasticity, and the rate of cell proliferation and survival. Removal of circulating adrenal steroids by adrenalectomy increases the proliferation of granule cell precursors and the production of new neurons. Furthermore, increased exogenous corticosterone suppresses cell proliferation and cell survival. Since aversive stressors and elevated glucocorticoids have similar effects on adult neurogenesis, it is likely that glucocorticoids are one of the main mediators of these

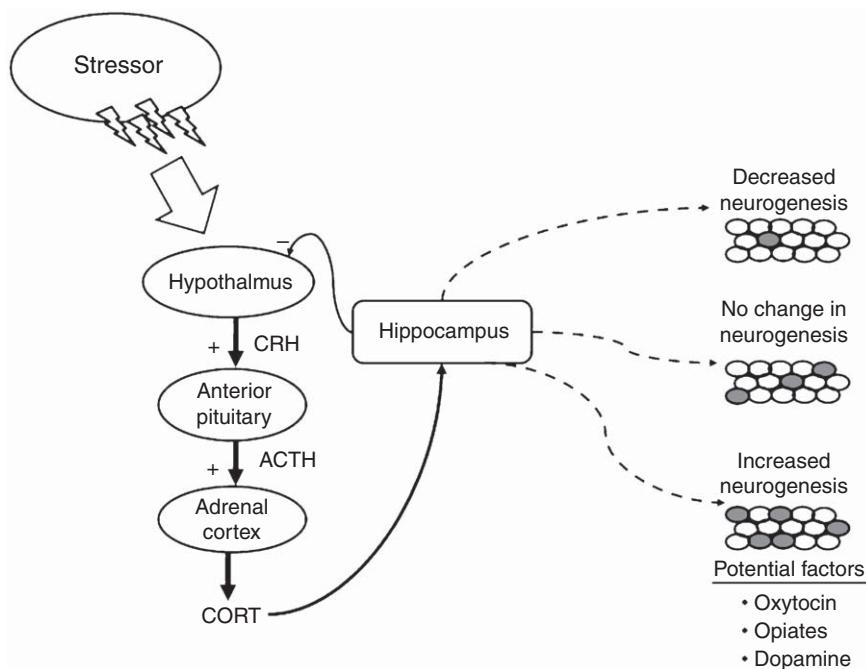


Figure 2 Possible glucocorticoid effects on adult neurogenesis in the hippocampus. Stressors activate the hypothalamic–pituitary–adrenal (HPA) axis, resulting in the release of a cascade of hormones, ultimately ensuring the release of glucocorticoids, such as corticosterone (CORT) in many rodent species. Corticosterones, or glucocorticoids, differentially alter cell proliferation and survival in the dentate gyrus of the hippocampus. It is commonly thought that stress inhibits adult neurogenesis as a result of increased glucocorticoids; however, in some instances, an enhancement in glucocorticoids does not alter adult neurogenesis. Paradoxically, some stressors, such as voluntary exercise and sexual experience, increase circulating glucocorticoids while also enhancing adult neurogenesis. The social or emotional valence of these stressors may play a role in the observed outcomes, as well as potential factors such as oxytocin, opiates, and dopamine.

stress effects. Preventing the stress-induced rise in glucocorticoids has been shown to eliminate the suppression of cell proliferation caused by stress.

Although a clear negative relationship exists between glucocorticoid levels and adult neurogenesis, a broader look at the literature suggests that this relationship is more complex. Some experiences that elevate glucocorticoid levels have no effect on adult neurogenesis, while other stressful experiences increase adult neurogenesis (see **Figure 2**). Active shock avoidance training increases glucocorticoid levels, but has no effect on cell proliferation or survival.

Voluntary exercise and sexual experience increase circulating glucocorticoid levels and enhance adult neurogenesis. Even long-term running has been shown to buffer the negative effects of certain stressors, such as social isolation (see below). Training in certain learning paradigms also increases the number of new neurons, despite elevated corticosterone concentrations. However, extensive training in learning paradigms, such as spatial reference or working memory maze tasks, can increase glucocorticoids and reduce adult neurogenesis. This reduction in the production of new neurons may be due to the intensity of the learning paradigm and the duration of training. The number of training trials has

been shown to determine whether adult neurogenesis is enhanced or diminished by learning. Future studies would be helpful to differentiate between the different phases of learning, such as acquisition and retrieval, when examining the effect of learning tasks on neurogenesis.

While much is known about the effects of stress on adult neurogenesis, there are still many questions that remain unanswered. Predictability and controllability of stress can decrease the negative impacts on neurogenesis. For example, active avoidance learning is a controllable stressor that has no effect on cell proliferation. This raises questions about whether the emotional valence of stressful situations can mitigate the stressors' negative effects on cell proliferation.

Environmental Influences and Neurogenesis

Social Stimulation

Housing

Across laboratories, standard housing conditions can vary. However, what usually remains consistent is the presence of a cage lined with bedding and *ad libitum* access to food

and water. It is also typical that same-sex animals will be group housed. Occasionally single, or isolation, housing is defined as ‘standard housing.’ However, some have found that isolation can have negative consequences on behavior, such as decreased spatial memory and increased aggression.

Four or 8 weeks of isolation housing decreases the number of newly dividing cells in both mice and rats, compared to animals housed in groups; however, the effect of isolation housing on adult neurogenesis is prevented by repeated antidepressant treatment (i.e., fluoxetine) and subsequent group housing. Isolation housing in guinea pigs also reduces the number of proliferating cells in the dentate gyrus, with a smaller percentage of new cells migrating into the granule cell layer.

It has been hypothesized that group housing or social support can interact with stress to ameliorate stress-induced alterations in both brain and behavior. Isolation housing renders the hippocampus more susceptible to the suppressive actions of glucocorticoids on adult neurogenesis; however, the interaction between housing and stress effects on the survival of proliferating cells in the dentate gyrus is dependent on the sex of the animal. Chronic exposure to inescapable footshock differentially affects individually-housed male and female Sprague-Dawley rats. A reduction in cell survival was observed among male rats, an effect mitigated by grouped housing, whereas females were not affected by either the chronic stressor of inescapable footshock or the housing condition. The lack of a chronic stress-induced deficit in cell survival does not indicate that females are not affected by chronic stress. Other indices of stress, such as behavioral alterations, adrenal hypertrophy, and c-fos expression, have been reported following chronic stress in females.

Social dominance

Many animals will develop a dominance hierarchy, particularly when living with conspecifics of the same sex, along with limited resources. Tree shrews, when paired with an animal of the same sex, will rapidly establish a dominant/subordinate relationship that is very stressful to the subordinate animal. The production of cells in the dentate gyrus can be rapidly modulated by subordination – decreased cell proliferation occurs within hours in subordinates, compared to controls. Similarly, rats housed in an apparatus called “the visible burrow system” will quickly establish a dominance hierarchy, characterized by individual differences in offensive and defensive behavior. These behavioral differences, resulting from time spent in the visible burrow system, alter adult neurogenesis in the dentate gyrus. Dominant rats in the visible burrow system demonstrated enhanced survival of new neurons in the dentate gyrus, compared to cage controls and subordinates. This effect persisted even when access to multiple burrows and tunnels was removed, indicating that the effect of dominance on cell survival was not contingent on the complexity of the environment. This

divergence was not due to substantial differences in circulating corticosterone levels in dominant and subordinate rats, which raises the likelihood that mechanisms other than stress may be responsible for the changes in dentate gyrus neurogenesis. One possibility is that dominants produce more new neurons because they have more opportunities for sexual experience, which has also been shown to increase adult neurogenesis.

Exercise

Running is a universally motivating behavior in rodents. Animals housed in cages with access to running wheels engage in a considerable amount of running, up to 4km a night. Although physical exercise is associated with numerous health benefits, some physiological changes that accompany running are indicative of stress, such as increased HPA axis activity. Despite the elevations in glucocorticoid levels, voluntary exercise is associated with positive influences on brain and behavior. Running enhances the number of new neurons in the dentate gyrus of adult rodents, which appears to be a paradox since elevated glucocorticoids have been associated with suppressed neurogenesis.

Most studies examine voluntary exercise in group-housed animals; therefore the social context in which voluntary exercise is experienced may buffer the negative effects of elevated glucocorticoids. Social isolation precludes the positive effects of short-term exercise on adult neurogenesis in the dentate gyrus. Individually-housed runners eventually show an enhancement in adult neurogenesis, but only after a considerably longer period of running, compared to group-housed runners. Therefore, the social context is important when examining the effects of voluntary exercise on adult neurogenesis.

The type of voluntary exercise may influence its effects on adult hippocampal neurogenesis. In studies investigating the effects of water maze training on neurogenesis, yoked controls swam for the same amount of time as animals undergoing water maze training; however, yoked controls did not experience enhanced neurogenesis. Further analysis of these data revealed that due to the short duration of swimming, it could not be concluded that swimming was less efficient than running in augmenting adult neurogenesis in the dentate gyrus. Nevertheless, voluntary exercise has remained one of the best studied paradigms of physical exercise in rodents, in respect to its effects on adult neurogenesis. The effects of voluntary exercise on hippocampal neurogenesis are even transmissible from the exercising pregnant mouse to her offspring.

Unlike voluntary wheel running, where animals can freely run when they so desire, forced or treadmill running only allows animals to run for a limited period of time each day. However, much the same as voluntary wheel running, forced exercise increases neurogenesis in the dentate gyrus. The intensity of forced exercise can alter the effects that treadmill running may have on adult neurogenesis. Low-intensity

forced running increases short-term cell survival more so than moderate- or high-intensity running, although all 3 groups significantly increased cell survival, compared to controls. The effects of forced running intensity on cell survival have produced conflicting results. One study found that only low-intensity exercise on a treadmill resulted in greater neurogenesis in the dentate gyrus, compared to moderate- and high-intensity exercise. Both moderate- and high-intensity treadmill running produced similar numbers of BrdU-labeled cells as controls. Here, the discrepancies most likely revolve around the duration of running and the definition of low-, moderate-, and high-intensity forced running.

Environmental Enrichment

Environmental enrichment refers to various housing conditions, either in the home cage or in exploratory chambers, that increase sensory, motor, and cognitive stimulation, compared to standard housing conditions. Most environmental enrichment paradigms also include more animals per cage, compared to the control setting.

Enriched environments enhance neurogenesis, an effect observed in many species, such as rats and mice. Unlike voluntary exercise, which is able to increase both cell proliferation and cell survival, enriched environments only increase the survival of newly born cells. Although enrichment paradigms vary among laboratories, the effects on adult neurogenesis remain consistent – nearly all enrichment protocols result in enhanced hippocampal neurogenesis. Due to differences in species, gender, standard housing conditions, objects used for enrichment, access to running wheels (see the section on voluntary exercise above), use of food treats, and so on, it is difficult to come to a consensus on which environmental enrichment paradigm is ideal (see **Table 1**). Numerous studies use female mice in enrichment paradigms instead of male mice. Given the tendencies for male mice to become highly aggressive when group-housed, male mice may not be ideal rodents to use in enrichment paradigms. What is clear is that the complexity of the environment, via sensory and motor stimulation, is able to significantly increase adult neurogenesis in the hippocampus.

Parenting

Motherhood is a very enriching experience, accompanied by changes in many nonreproductive functions, such as learning, memory, anxiety, and stress regulation. Lactation suppresses the number of proliferating cells in the dentate gyrus of primiparous and multiparous female rats, compared to nulliparous controls, with this effect lasting until the time of weaning. The effect of lactation on adult neurogenesis is transient – by 2 weeks post-BrdU injection, at a time when most newly born cells have matured, no difference are detectable among groups.

The diminished cell proliferation accompanied by lactation was resultant of elevated glucocorticoids – once pups were removed, basal glucocorticoid levels returned, resulting in no differences in BrdU-labeled cells compared to nonlactating females. Interestingly, nulliparous female rats briefly exposed to pups displayed enhanced cell proliferation in the dentate gyrus. Taken together, these results suggest that reproductive experience and exposure to offspring can significantly alter adult neurogenesis.

Functional Consequences of Adult Neurogenesis

One of the most important and, as of yet, unanswered questions in the field of adult neurogenesis is the functional significance of newly born neurons. Do new neurons replace mature neurons that have died, or do they have a unique function that could not be accomplished by existing mature neurons? For instance, new neurons may be used for processing novel stimuli or for temporary memory storage. While their function remains unclear, there is much evidence that adult-born neurons are involved in certain types of learning and memory, and possibly involved in regulation of anxiety and mood.

Learning and Memory

Not only do many learning paradigms increase adult neurogenesis, but there is also evidence that adult-generated neurons within the hippocampus are important for certain types of learning and memory. For instance, differences between mouse strains in their amount of baseline neurogenesis parallel their learning abilities in spatial navigation tasks; mice with the fewest new neurons performed worse in the Morris water maze. However, no such strain difference has been reported in rats. Experiences that increase adult neurogenesis, like enriched environments and voluntary exercise, also improve learning. Conversely, many of the same factors that decrease neurogenesis, like aversive stress and pharmacological manipulation of glucocorticoids, also impair learning.

There are several methods used to increase or decrease neurogenesis in order to examine the subsequent effects on learning and memory. Because voluntary exercise is known to increase the production of new neurons in the adult dentate gyrus, many studies use running to examine the effects of increased neurogenesis on learning and memory tasks, when compared to sedentary animals. Running-induced increases in neurogenesis enhance performance on learning tasks, such as improved memory acquisition and retrieval in the Y-maze and Morris water

Table 1 Representative selection of environmental enrichment paradigms

<i>Species</i>	<i>Gender</i>	<i>EE conditions</i>	<i>Age; duration of EE</i>	<i>Control housing</i>
C57/Bl6 mice	Female	Housed 10 per cage in large stainless steel cages ($42 \times 50 \times 20$ cm) with activity wheel and swing; toys and bedding changed every 2 days	3 months of age; 8 weeks in EE	Housed 3 per cage in clear plexiglas cages ($20 \times 22 \times 20$ cm)
		Housed 14 per cage (86×76 cm) with toys, tunnels, and running wheels	3 months of age; 2 or 5 weeks in EE	Housed 3–4 per cage in standard cages
		Housed 15 per cage in a 1 m^2 floor space with plastic tubes, a small running wheel, nesting material, and toys	10 months of age; 10 months in EE	Housed 15 per cage in standard cages
129Sv/Ev mice	Female	Housed 8 per cage in two clear plastic cages adjoined by a tube; cages contained a wooden house, hollow wooden log, paper tube, plastic igloo, and paper towels	10 weeks of age; 100 days in EE	Housed 4 per cage in clear plastic cages ($29.2 \times 19 \times 12.7$ cm)
Wistar rats	Male	Housed 5 per cage in a cage with 0.5 m^2 area with plastic tubes, tunnels, bridges, wheels, and food treats all changed daily	6 weeks of age; 11 or 28 days in EE	Housed 1 per cage in cages measuring $45 \times 22 \times 18$ cm
		Housed 8 per cage in a cage ($120 \times 100 \times 60$ cm) containing running wheels, rearrangeable plastic tunnels, an elevated platform, and toys changed every 3–4 days	2 and 25 months of age; 8 weeks in EE	Housed 1 per cage in wire mesh cages
Sprague-Dawley rats	Male	Housed 4–6 per cage in a large box ($1.5 \times 0.8 \times 0.8$ m) with toys (rearranged daily), wooden blocks, climbing platforms, plastic tubes, and small houses	$220\text{--}250\text{g}$; 3 h day^{-1} for 14 days in EE	Housed 2 per cage in standard laboratory cages
Long Evans rats	Both	Housed 4–5 pups plus parents per cage in a large clear plexiglass box ($1 \times 1 \times 1$ m) with a can, piece of wood, plexiglass tube, colored light bulbs, a towel, and a metal lamp shade	15 days of age; 6 or 12 days in EE	Housed 4–5 pups plus parents per cage in a standard laboratory cage of clear acrylic ($30 \times 50 \times 15$ cm)
Mongolian gerbils	Male	Housed 15–20 per cage in two storied cages ($30 \times 20 \times 30$ m) with tunnels, running wheels, and toys. New objects introduced every week and object locations changed $2 \times$ per week	2 months of age; 2 months in EE	Housed 3 per cage in conventional rat cages

EE = Enriched environment.

maze, as well as enhanced freezing response and consolidation in contextual fear conditioning paradigm. Exercise also mitigates the negative effects of hippocampal lesions on learning and memory tasks.

Irradiation, the administration of antimitotic agents, and transgenic knockdown of adult neurogenesis are three techniques used to block the production of new neurons in rodents. Rats and mice with reduced neurogenesis in the dentate gyrus caused by one of these methods exhibit performance deficits in several learning paradigms, such as trace conditioning and delayed non-matching to sample tests, although inconsistencies exist within the literature. In C57BL/6J mice, exercise-induced hippocampal neurogenesis is necessary for enhanced spatial learning, but not motor performance or contextual fear learning. In rats, treatment with the

antimitotic agent, methylazoxymethanol acetate (MAM), caused impaired trace conditioning, but had no effect on spatial learning in the water maze, even though both of these tasks are considered hippocampal dependent. Transgenic mice with ablation of adult-born hippocampal neurons were tested on several spatial tasks of varying complexities: detection of new environments, spatial navigation, and contextual conditioning. Mice with decreased neurogenesis showed impairments in spatial relational memory, but not in simpler forms of spatial knowledge.

All of the methods currently used to reduce adult neurogenesis have possible confounds, including lack of specificity and unwanted side effects. Because irradiation causes inflammation in the brain, the activation of microglia must be monitored to assess whether inflammation,

rather than reduced neurogenesis, is affecting cognition. Administration of MAM at a dose high enough to prevent adult neurogenesis induces physical illness in rats, characterized by weight loss, decreased locomotor activity, and degradation fur degradation. Transgenic knockdowns require long-term treatment with drugs in order to reduce the production of new neurons, causing a lack of temporal resolution. The time period that elapses between treatment and testing associated with these methods is long enough to allow neural reorganization to occur, therefore complicating the interpretation of the results.

Besides methodological problems, other possible explanations for the inconsistencies in the literature include differences in species, gender, timing of BrdU injections, and variations in particular paradigms and training schedules. Although studies often show a correlation between adult neurogenesis and learning and memory tasks, it is difficult to make statements about causation. In order to elucidate this relationship, it will be necessary to use a method that cleanly reduces adult neurogenesis with greater temporal resolution.

Mood Disorders

The hippocampus is implicated in the pathology and treatment of many mood disorders. Stress and animal models of depression cause a decrease in the number of neurons in this structure, as well as reductions in its overall volume. Reduced adult neurogenesis elicits anxiety or depressive-like behaviors in several species. While aversive stress is generally associated with a reduction in adult neurogenesis (see above sections for exceptions), administration of antidepressant drugs increases cell proliferation and neurogenesis. Behavioral studies indicate a possible role of adult neurogenesis in depression, as antidepressants may be able to reverse or prevent stress-induced deficits in neurogenesis. This effect has been shown with a variety of antidepressants, including a selective serotonin reuptake inhibitor (fluoxetine), a specific norepinephrine reuptake inhibitor (reboxetine), a monoamine oxidase inhibitor (tranylcypromine), and a phosphodiesterase-IV inhibitor (rolipram).

Acute antidepressant treatment does not alter cell proliferation, whereas treatment for 14 or 21 days results in the production of new cells. The delayed onset in antidepressant-induced neurogenesis in rodents parallels the chronic administration necessary to obtain therapeutic results in humans, as well as the time point when most newly born cells have matured and integrated into the preexisting hippocampal circuitry.

Although the adult neurogenesis hypothesis of depression has gained momentum in recent years, there are also studies that contradict this hypothesis. In 129/Sv mice, blocking antidepressant-induced neurogenesis through irradiation abolishes the behavioral responses to

antidepressant treatment. However, blocking neurogenesis did not decrease antidepressant effectiveness or the animals' sensitivity to unpredictable chronic mild stress in BALB/c mice. Focal hippocampal irradiation did not prevent the antidepressant-like actions of a corticotrophin releasing factor antagonist or vasopressin 1b antagonist. These inconsistencies could result from the specific type of antidepressant, the length or dosage of drug administration, and inherent strain differences.

Most studies investigate whether antidepressants increase adult neurogenesis in nonstressed animals, but it may be more useful to examine animals undergoing stressful situations to see whether these drugs can reverse or prevent the negative effects of stress. Two commonly used animal models of depression are chronic subordination and learned helplessness, both of which have been associated with reduced adult neurogenesis. Treatment with antidepressants can prevent the decrease in cell proliferation caused by chronic exposure to psychosocial stress. Moreover, inescapable shock (a learned helplessness paradigm) decreases cell proliferation, and leads to behavioral deficits, both of which can be prevented or reversed with antidepressants.

Not only do antidepressants prevent stress-induced reductions in neurogenesis, but they also decrease anxiety-like behavior in animal models. Approach-avoidance tests, including elevated plus maze, novelty-induced hypophagia, novelty-suppressed feeding, and the open field, are often used to measure anxiety. Anxious rodents reduce exploratory behavior due to an inherent fear of open areas. Antidepressant administration decreases anxiety in these tasks, possibly as a result of enhanced neurogenesis. There is suggestive evidence that enhanced neurogenesis may play a role in the therapeutic efficacy of antidepressants, but there is also evidence contradicting this theory. More studies are needed, particularly in humans, to assess the potential links among depression, anxiety, and adult neurogenesis.

Neurodegenerative Disorders

Stroke, the most frequent cause of acute neurodegeneration, affects millions of people per year and has become one of the leading causes of death. Current treatments, such as the use of thrombolytic agents, have had limited impact, as they are only effective within a small temporal window. There are many chronic neurodegenerative diseases, including Parkinson's disease, Huntington's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (ALS). Each is associated with a gradual loss of neurons in certain areas of the nervous system. Except for Parkinson's disease, effective therapies are not readily available to reduce symptoms and restore lost function. Even in the case of Parkinson's disease, pharmacological

interventions are typically only useful for a limited period of time – after their therapeutic window closes, patients often resort to experimental treatments like deep brain stimulation. Given that there is restricted cell loss in many neurodegenerative disorders, restorative therapies are of high interest and worth pursuing.

Toward this end, the ability for the environment to stimulate adult neurogenesis should be considered. Understanding the molecular and cellular effects of the environment on adult neurogenesis may not only provide insight into the pathogenesis of disorders affecting the nervous system, but will also open the door to novel therapeutics that may mimic the effects of environmental enrichment on adult neurogenesis.

See also: Animal Models of Learning and Memory; Animal Tests for Anxiety; Brain Aging: Structural Imaging Biomarkers of Risk of Alzheimer's Disease; Cognition: Learning and Memory: Spatial; Depression; Developmental Neurogenesis; Effects of Stress on Learning and Memory; Eyelid Classical Conditioning; Fear, Anxiety, and Defensive Behaviors in Animals; Fear Conditioning; Genes and Behavior: Animal Models; Hormones and Memory; Mammalian Parental Behavior and Neurohormonal Determinants; Maternal Deprivation; Neural Bases of Defensive Aggression; Neural Substrates of Conditioned Fear and Anxiety; Neural Substrates of Unconditioned Fear, Defense, and Anxiety; Neurogenesis and Exercise; Neurogenesis and Memory; Parental Behavior; Perinatal Influences on Behavior and Neuroendocrine Functions; Parkinson's Disease; Regulation of the HPA Axis by Acute and Chronic Stress; Stress and Brain Morphology; Stress and Social Behavior.

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Neurogenesis and Exercise

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Glossary

Adult neurogenesis – The set of events leading to the production of new neurons in the adult brain, from precursor cell division to functionally integrated survival.

Bromo-deoxyuridine (BrdU) – A thymidine analog incorporated into dividing cells that are in the DNA synthetic phase (S-phase) of the cell cycle. BrdU incorporation can be visualized immunocytochemically. BrdU labeling can be used for quantitative stereological analysis of new cell number. In addition, BrdU labeling can be combined with immunohistological markers for neurons or glia to determine the phenotype of the newly generated cells.

Dentate gyrus – Neurogenic brain region that is one of the three subfields of the hippocampus. The dentate gyrus contains densely packed granule cell bodies in the granule cell layer. The granule cell axons, which are called mossy fibers, project to hilar interneurons and the hippocampal CA3 subfield. The dendritic tree of the dentate gyrus granule cells is contained in the molecular layer.

Growth factors – Neuropeptides that play a role in the following processes: cell proliferation, survival, differentiation, connectivity, and synaptic plasticity.

Long-term potentiation (LTP) – An enduring increase in the amplitude of excitatory postsynaptic potentials as a result of high-frequency (tetanic) stimulation of afferent pathways, considered to be the cellular basis for memory formation.

Progenitor cell – A mitotic cell with a fast cell-division cycle that retains the ability to proliferate and to give rise to terminally differentiated cells but that is not capable of indefinite self-renewal.

Retrovirus – RNA virus that uses reverse transcriptase to convert its RNA into DNA. It consists of *gag*, *pol* and *env* genes, which are translated as polyprotein precursors and are later processed to yield internal structural proteins, enzymes, and the envelope glycoproteins, respectively. The Moloney MLV retrovirus only infects dividing cells, can be made to express a fluorescent reporter, and is used to label stem and progenitor cells *in vitro* and *in vivo*.

Stem cells – Cells that have both the capacity to self-renew (make more stem cells by cell division) as well as to differentiate into mature, specialized cells.

Adult Neurogenesis

Traditionally, genesis of new neurons was considered to be restricted development. However, it is now established that neurogenesis occurs in two brain areas: the subventricular zone of the lateral ventricles/olfactory bulb and the dentate gyrus of the hippocampus (Figure 1). In initial studies in the 1960s, using tritiated thymidine autoradiography, continued cell division in the postnatal and adult brain was observed. This work was largely ignored due to lack of proof that the adult-generated cells were neurons. In the mid-1980s, it was discovered that neurogenesis occurs in the bird brain in areas that mediate song-learning and seed-storing behavior. The avian results and the isolation of cells with stem cell properties from the adult mouse brain led to a revived interest in adult mammalian neurogenesis. Subsequent work using the thymidine analog 5-bromo-2'-deoxyuridine (BrdU), in combination with specific neural markers, showed that newborn cells could become neurons in the rodent and human hippocampus. Conclusive evidence that the new neurons are functionally integrated into the hippocampal circuitry was obtained by injecting retrovirus specific for dividing cells and express green fluorescent protein (GFP) into the dentate gyrus. This approach revealed the morphology and electrophysiological characteristics of newly born neurons in the adult rodent hippocampus (Figure 1).

The process of proliferation, migration, differentiation, and survival of new neurons *in vivo* has become well-defined in recent years. Two types of neural stem cells have been identified in the subgranular zone of the dentate gyrus. Type 1 cells have a long radial process and express nestin and glial fibrillary acidic protein (GFAP), even though they are not astrocytes. Type 2 cells have short processes, can self-renew, as well as proliferate and differentiate into new neurons. Type 2 cells go through a series of developmental steps, beginning with axonal and dendritic growth, followed by spine formation and maturation over the course of 2 months. These cells express a sequential series of markers during maturation, starting with nestin, doublecortin, and finally the mature neuronal marker Neun. Similar to newborn neurons in the embryonic brain, new granule cells in the mature brain are initially depolarized by γ -aminobutyric acid (GABA). The transmitter becomes hyperpolarizing at about 2–4 weeks after cell genesis, concurrent with the development of dendritic spines and glutamatergic activity. Although the newborn neurons

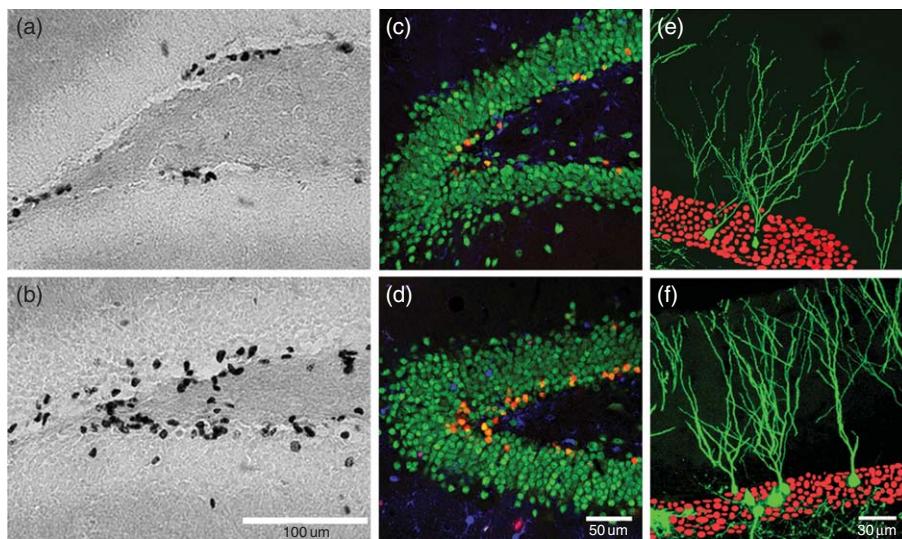


Figure 1 Regulation of cell proliferation and neurogenesis by exercise. (a, b) Photomicrographs of BrdU+ cells 1 day after the last of a series of 12 BrdU injections (50 mg kg^{-1} per day). Mice housed with a running wheel (b) have more BrdU+ cells than sedentary mice (a), showing that running increases cell proliferation. (c, d) Confocal images of sections that were immunofluorescently triple-labeled for BrdU (red), NeuN-indicating neuronal phenotype (green), and S100 β -selective for glial phenotype. BrdU+ neurons are orange (red + green). These images demonstrate that the survival and neuronal differentiation of the cells are enhanced 4 weeks after the last BrdU injection in runners (d) relative to control (c) mice. (e, f) Retroviral labeling of new neurons. Retrovirus-expressing green fluorescent protein (GFP) was injected into the dentate gyrus of sedentary (e) and running (f) mice. Confocal images show more GFP+ new neurons in running than in sedentary mice 4 weeks after virus injection. The red ovals (e, f) were added to illustrate the location of the granule cell layer.

are similar to mature granule cells in their basic physiological properties, they continue to mature for several months and show transiently enhanced synaptic plasticity between 4 and 8 weeks of age.

Exercise and Hippocampal Neurogenesis

Adult neurogenesis has been shown to be highly regulatable by both extrinsic and intrinsic, genetic and epigenetic factors. In general, an enhancement of cell genesis has been correlated with improved learning and memory, whereas decreased neurogenesis is associated with aging, stress, and depression. The initial study that showed enhancement of adult neurogenesis used environmental enrichment. In an enriched environment, mice are housed in a larger cage with toys, running wheels, and more opportunity for social interaction. The mice were housed in enriched or control conditions for 40 days and given a series of daily BrdU injections for the first 12 days. Animals were sacrificed either immediately after the last injection to study cell proliferation, or 1 month later to investigate cell survival. It was found that cell neurogenesis and survival were enhanced, but not cell proliferation. Mice were also tested in a spatial memory task, the Morris water maze, in which mice are trained to find a platform hidden under the surface of a pool using visual cues on the walls of the room. The enriched mice learned faster

than controls, raising the possibility that the new neurons contribute to enhanced cognition.

In a subsequent study, it was determined which aspect of the environment—increased opportunity for learning, socialization, or physical activity—may mediate the neurogenic effect. The avian literature at the time indicated that neurogenesis was correlated with food storing, and therefore learning was considered to be an essential factor of enrichment. However, to represent all aspects of the enriched environment, mice were tested in five different conditions: water maze learning, yoked swim controls, wheel running, enrichment (positive control), or standard housing (negative control). Surprisingly, voluntary exercise in a running wheel enhanced both proliferation and survival of newborn neurons in the dentate gyrus, indicating that physical activity was the critical neurogenic factor. Physical activity also improved hippocampus-dependent memory function. The strong correlation between increased neurogenesis and learning following exercise, suggested that the new neurons might play an important role in cognition.

Exercise, Neurogenesis, and Brain Function

Enhancement of hippocampal neurogenesis by running is a robust phenomenon that has been replicated by many different laboratories. The onset of the effect is rapid

regardless of whether the animals are housed individually or in groups. Running induced cell proliferation peaked after 3 days of running and was still significantly enhanced at 10 days. After 32 days of running, the proliferative effect had returned to baseline. Interestingly, the number of new neurons continued to increase. Recent research has shown that the benefits of exercise for new neurons are also qualitative. Using a retroviral labeling strategy (**Figure 1**), it was shown that exercise enhances the maturation of newborn neurons. Specifically, the density of mushroom spines is enhanced and spine motility is decreased during development of new neurons in the adult brain, even though the total number of dendritic protrusions does not change.

Synaptic Plasticity

Exercise-induced increase in cell genesis is associated with enhanced hippocampal synaptic plasticity. In particular, long-term potentiation (LTP), a physiological model of certain forms of learning and memory, is influenced by physical activity. In an initial study, field excitatory postsynaptic potential (fEPSP) amplitudes, as well as LTP, were compared in hippocampal slices from running and control mice. The fEPSPs were unchanged in both groups. However, LTP amplitude was enhanced in the dentate gyrus in slices from running mice as compared with controls. Recordings from another hippocampal subfield, area CA1, showed no change in LTP in response to running. In subsequent studies, dentate gyrus LTP was studied *in vivo* in anesthetized rats that had been housed with a running wheel or given forced treadmill exercise. In both voluntary and forced exercise conditions, LTP was increased.

Changes in synaptic plasticity associated with exercise occurred in the same region where neurogenesis was stimulated by running, suggesting that newborn cells have a functional role in this process. Although the new cells are a small percentage of the granule cell layer, several studies have indicated that they have greater plasticity than do mature cells. Indeed, in immature rats, dentate gyrus LTP lasts longer than in adults. In another study, properties of granule cells from the inner and outer layers of the dentate gyrus were compared. Inner layer cells were considered to be young cells and the outer layer to be old cells. It was found that putative young cells had a lower threshold for LTP and were unaffected by γ -aminobutyric acid-A (GABA_A) inhibition, indicating enhanced plasticity in the young cells. In a subsequent investigation, recordings were made from young neurons identified by electrophysiological criteria established during early postnatal development of dentate gyrus neurons, immunoreactivity for immature neuronal markers, and developing dendritic morphology. It was shown that LTP could be induced more easily in young neurons than

in mature neurons under identical conditions. Recently, using retroviral labeling, it was reported that individual new neurons have increased LTP amplitude and decreased induction threshold between 1 and 1.5 months of newborn neuron age. A proposed mechanism is increased dependence of LTP on N-methyl-D-aspartic acid (NMDA) NR2B receptors during this critical developmental period.

Cognition/Learning and Memory

The enhanced synaptic plasticity of individual newborn neurons and the exercise-associated increase in the number of new dentate granule cells may mediate some of the beneficial effects of exercise on learning and memory in humans and animals. Indeed, exercise has been shown to improve learning in human subjects and is correlated with an increase in hippocampal volume. In rodents, both voluntary wheel running and forced treadmill training have been shown to enhance spatial learning using different types of mazes (water maze, y- and radial arm maze). Running also improves performance in other hippocampus-dependent tasks, such as contextual fear conditioning and novel object recognition.

The correlation between neurogenesis, exercise, and cognitive function is maintained during normal aging. Physical activity has been shown to protect against age-related cognitive decline and brain atrophy in aging adults. Neurogenesis declines to low levels with aging in rodents as well as in nonhuman primates and has been associated with cognitive deficits. The age-dependent reduction in cell genesis can be partially prevented when animals are housed with running wheels over a 6-month period. In addition, the decline in neurogenesis and cognition associated with normal aging can be reversed in part by the onset of wheel running late in life. In a recent study, normal aged mice that had been sedentary until they were 18 months of age were housed in a cage with a running wheel for 1 month and tested for spatial memory using the Morris water maze. It was found that exercise significantly improved neurogenesis as well as acquisition and retention of the water maze task in aged runners.

Mechanisms Underlying Exercise-Induced Neurogenesis

Adult neurogenesis in the hippocampus is an extremely dynamic process, and the neurogenic effects of exercise add a level of complexity to the molecular mechanisms mediating this phenomenon. Dentate granule cells receive inputs from many areas of the brain and from the periphery through different neurotransmitters, neural

peptides, and growth factors. Therefore, the complexity of the circuitry and factors in the neurogenic niche must be taken into account for an accurate description of the regulation of cell genesis by exercise.

Neurotransmitters

Acetylcholine

Exercise influences the firing rate and theta rhythm in hippocampal cells through a cholinergic mechanism. In addition, lesion studies in rodents have shown that acetylcholine is required for the proliferation and survival of new neurons. Lesions of the forebrain cholinergic input in rats reduced dentate gyrus neurogenesis. Systemic administration of the cholinergic agonist physostigmine increased neurogenesis. Furthermore, mice lacking the β -2 subunit of the neural nicotinic acetylcholine receptor have significantly decreased cell proliferation in the hippocampus.

Glutamate

The NMDA receptor (NMDA-R) subtype of glutamate receptors also plays a key role in regulation of adult hippocampal neurogenesis. Under exercise conditions, it was found that after 3 days of running both NR2A and NR2B subunits of the NMDA receptor were elevated, and that after 7 days of running only NR2A gene expression remained elevated. It was also shown that exercise leads to an increase in NR2B subunit mRNA levels. Both low- and moderate-intensity treadmill running was proven to enhance NMDA receptor 1 (NMDAR1) mRNA levels. These results are also supported by the lack of exercise-induced neurogenesis in NMDAR1 knockout mice. Interestingly, transgenic mice that overexpress NR2B subunits show enhanced LTP and increased performance on learning and memory tasks. There does not appear to be any significant functional expression of NMDA-R on early adult progenitor cells. However, NR1 has been indicated as important for the survival of young newborn neurons.

γ -Aminobutyric acid (GABA)

GABAergic inputs onto hippocampal progenitor cells have been shown to promote activity-dependent differentiation of progenitor cells in culture. GABA also promotes the synaptic integration of newly generated neurons in the adult brain. The release of GABA from hippocampal interneurons is regulated by a number of other neurotransmitters, including 5-hydroxytryptamine (5-HT; serotonin) and cannabinoids. It was shown that embryonic and adult hippocampal neural precursor cells are immunoreactive for cannabinoid receptors 1 (CB1). Indeed, chronic administration of cannabinoids has been shown to promote neurogenesis in the dentate gyrus of adult rats. These findings are in agreement with other studies showing that

mice lacking the CB1 receptor have significantly decreased neurogenesis.

Monoamines

Monoamines such as noradrenaline and 5-hydroxytryptamine (5-HT; serotonin) are activated by exercise. The dentate gyrus is enriched with 5-hydroxytryptamine (5-HT; serotonin) 1A (5-HT1A) receptors and receives serotonergic innervations from the brainstem. A considerable amount of evidence suggests that 5-HT may stimulate the production of neurons; antidepressants such as selective serotonin re-uptake inhibitors (SSRIs) and 5-HT1A receptor agonists stimulate adult hippocampal neurogenesis, whereas lesions of serotonergic projections from the raphe nuclei (RN) significantly reduced the accumulation of new hippocampal neurons. Inhibition of serotonin synthesis is associated with a decreased number of newly generated cells in the dentate gyrus. 5-HT can also promote the survival of neurons in the adult brain, as demonstrated by the ability of a 5-HT receptor agonist to protect neurons against excitotoxic and ischemic injury. The proposed mechanism is that exercise can elevate the levels of tryptophan hydroxylase, the enzyme involved in the rate-limiting step for the synthesis of 5HT in the RN. Projections from the RN to the hippocampus can influence hippocampal activity. The altered expression of the 5-HT precursor tryptophan may lead to increased serotonin synthesis.

Opiates

Exercise enhances endogenous levels of the opioid systems. For example, in humans, it was shown that the level of β -endorphins increases after exercise. In fact, β -endorphins are a candidate mechanism to explain why people become addicted to running. Recent studies have shown that an increase in cell proliferation can be produced by the direct infusion of opiates, and that opiate receptor antagonists decrease cell proliferation in the dentate gyrus. Interestingly, endorphins and enkephalins stimulate cell genesis *in vitro*. Moreover, administration of the mu receptor antagonist naltrexone decreased running-induced cell proliferation.

Although these neuromodulatory signals trigger proliferation, the direct mitogenic stimulus to the progenitor cells appears to be mediated through growth factors.

Growth Factors

Running wheel exercise increases both central and peripheral levels of key growth factors. In the periphery, trophic factors are released predominantly from muscle tissue during exercise. Growth factors provide important extracellular signals regulating proliferation and differentiation of stem and progenitor cells. Trophic factors that influence adult neurogenesis include brain-derived

neurotrophic factor (BDNF), basic fibroblast growth factor (bFGF-2), epidermal growth factor (EGF), insulin-like growth factor I (IGF-I), and vascular endothelial growth factor (VEGF).

BDNF

BDNF fits particularly well into the concept of function-dependent regulation of adult neurogenesis, because stress and glucocorticoids, which decrease adult hippocampal neurogenesis, also downregulate BDNF, whereas physical activity upregulates BDNF and neurogenesis in the hippocampus. It is known that BDNF supports the survival and growth of many neuronal subtypes, including glutamatergic neurons. With exercise, levels of BDNF mRNA in the hippocampus increased rapidly and remained elevated for weeks. Specifically, it was shown that several days of voluntary wheel running increased levels of BDNF mRNA in the hippocampus, particularly in neurons of the dentate gyrus, hilus, and CA3. Researchers found that 4 weeks of wheel running increase hippocampal BDNF mRNA levels in rats aged 2, 15, or 24 months; the highest level of BDNF was found in 2-month-old rats. BDNF protein levels also increased with running.

BDNF heterozygous mutant mice exhibited a 20% reduction in hippocampal stem cell proliferation. In addition, in BDNF knockout mice, no enhancement of hippocampal neurogenesis following environmental enrichment was observed. It was also shown that knockout of one BDNF allele leads to severe deficits in both LTP and experience-dependent synaptogenesis. Interestingly, selective knockout of the tropomyosin receptor kinase B (trkB) in the hippocampal neural progenitor cells prevented the exercise-induced increase in neurogenesis. Furthermore, intracerebral infusion of BDNF increased cell genesis in the dentate gyrus, but not in the subventricular zone, consistent with data showing that the neurogenic effect of exercise is limited to the hippocampus. BDNF may therefore have a key role in bridging the effects of complex environments from the molecular scale to the level of cellular plasticity. Altogether, these data suggest that exercise might be an effective method for increasing BDNF, which, in turn, can enhance the survival of newborn cells in the hippocampus.

FGF-2 and EGF

Growth factors such as FGF-2 and EGF are also potent factors for the maintenance of adult neural stem cells. FGF-2 was the first molecule identified to promote the proliferation of adult neural progenitor cells, because it was necessary for expansion in culture. These *in vitro* mitogenic effects of FGF-2 are mirrored *in vivo*. FGF-2 mRNA levels are upregulated in the hippocampus following exercise. *In vivo* FGF-2 appears to promote proliferation and differentiation of neural stem cells.

Intracerebroventricular (ICV) infusion of FGF-2 and EGF results in increased neurogenesis in the subventricular zone (SVZ) and in the dentate gyrus. The rate of hippocampal neurogenesis declines with age and intracerebroventricular (ICV) FGF-2 infusion counteracts this reduced neurogenesis.

IGF-1 and VEGF

In recent years, there has been a growing interest in the relationship between angiogenic factors and neurogenesis. In the dentate gyrus, new cells are clustered close to the blood vessels and proliferate in response to vascular growth factors. These observations lead to the hypothesis that neural progenitor cells are associated with a vascular niche and that neurogenesis and angiogenesis are closely correlated. Vasculature changes associated with exercise have been shown to occur in the brain and may be mediated by IGF-1 and VEGF.

Running enhances IGF-1 gene expression and protein levels in the hippocampus, and serum levels of both IGF-1 and VEGF. IGF-1 is a polypeptide hormone that induces neurogenesis in the adult mammalian brain both *in vivo* and *in vitro*. Peripheral infusion of IGF-1 increased adult neurogenesis and reversed the aging-related reduction in new neuron production. Blockade of peripheral IGF-1, on the other hand, inhibited the exercise-induced increase in hippocampal neurogenesis.

VEGF links exercise and adult hippocampal neurogenesis. An increase in levels of VEGF can be measured in exercising individuals. In experiments using local perfusion into the brain, VEGF has been shown to increase neurogenesis in the dentate gyrus. VEGF has also been shown to stimulate neuronal precursors in murine cerebral cortical neurons and *in vivo* in adult rat brain. The trophic factor primarily increased cell proliferation, whereas no alterations in cell survival were observed. Hippocampal gene transfer of VEGF in adult rats resulted in approximately doubling of the number of new neurons in the dentate gyrus and improved cognition. When VEGF is secreted into the blood, it can stimulate the formation of new blood vessels. Therefore, VEGF seems like an ideal trophic factor to induce the changes in angiogenesis and cell proliferation that occur with exercise. Indeed, peripheral blockade of VEGF by use of soluble VEGF receptor led to an inhibition of the effect of running on neurogenesis in the adult dentate gyrus. Similar findings were reported for IGF-1.

It is interesting that exercise increased cell proliferation in the hippocampus, but both exercise and environmental enrichment increased cell survival. The reason might be that exercise induces cell proliferation due to the action of IGF-1 and FGF-2, the expression of which is not altered following environmental enrichment.

Intracellular Pathways

A variety of intracellular mechanisms has been implicated in the regulation of adult neurogenesis. Among these, several transcription factors have been shown to play critical roles in postnatal neurogenesis. TLX, an orphan nuclear receptor, and Bmi-1 are required for the maintenance of adult forebrain neural stem cells. Interestingly, in an inducible knock-out model of TLX, which showed a marked reduction in BrdU-positive cells, voluntary exercise still stimulated cell proliferation. Another transcription factor, Pax6 promotes neuronal differentiation of SVZ progenitors, whereas Olig2 has an opposite effect. In addition, genes involved in cell-cycle regulation, DNA repair, and chromosome stability are required for the proper functioning of adult neural progenitors. The early process of adult neurogenesis might also be influenced by somatic gene insertions, such as the retrotransposon long interspersed nuclear element-1 (*LINE-1*), which is expressed in adult hippocampal neural progenitors *in vivo*, and upregulated by physical activity.

Exercise activates the intracellular pathways that were thought to play a role in cell survival. BDNF, IGF-1, and NGF activate the inositol trisphosphate (IP₃) and the mitogen-activated protein kinase (MAPK) pathways, and the former pathway appears to be essential for cell survival. A common downstream effector of the IP₃ pathways is a serine-threonine kinase, Akt, which has been shown to be activated by exercise and is instrumental in BDNF and IGF-1-induced cell survival. Therefore, exercise might augment the activity of Akt to promote cell survival through NGF.

See also: Aging and Cognition; Animal Models of Learning and Memory; Developmental Neurogenesis; Environmental Influences on Adult Neurogenesis; Memory and Aging, Neural Basis of; Neurogenesis and Memory; Neurotransmitters and Neuromodulators Regulating Sleep and Wakefulness.

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Neurogenesis and Memory

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Glossary

Bromodeoxyuridine (BrdU) – An analog of thymidine, which may be dissolved in saline and injected into animals. It crosses the blood–brain barrier and is incorporated into cells that are in the S-phase of mitosis at time of injection. *Postmortem*, cells containing BrdU are visualized using immunohistochemistry techniques.

Delay conditioning – The conditioned and unconditioned stimulus overlap and co-terminate in time. Learning this task neither requires an intact hippocampus nor rescues new cells from death.

Eyeblink conditioning – A form of classical conditioning in which stimulation of the eyelid, or unconditioned stimulus, occurs at the same time as an auditory tone, or conditioned stimulus. The unconditioned response in this learning model is closing the eyelid. After many trials, the subject learns to accurately time the closing of the eyelid. The precisely timed eyeblink is the conditioned response.

Morris water maze – A spatial navigation task that is widely used for rats and mice. A tank is filled with water which is made opaque with nontoxic paint. A platform is submerged just below the surface of the water. Animals are placed in the tank from various starting points. They use external cues in the room to find their way to the platform. The time it takes the animal to reach the platform, referred to as escape latency, is used as a measure of learning.

Trace eyeblink conditioning – A 500-ms stimulus-free interval occurs after the conditioned stimulus (auditory tone) and before the unconditioned stimulus (eyelid stimulation) is presented. Learning this task requires an intact hippocampus and rescues 1-week-old hippocampal cells from death.

Introduction

The production of a new neuron involves multiple stages and is regulated by several exogenous and endogenous factors. In the broadest sense, neurogenesis, or the generation of new neurons, includes two processes: (1) proliferation, where the number of new cells increases as they undergo repeated cell divisions, and (2) survival, the

fate of a single cell to either develop into a mature neuron or instead to become apoptotic and die. In the adult mammalian brain, one of the most active regions of neurogenesis is found in the dentate gyrus of the hippocampal formation. The hippocampal formation is critical for learning and memory processes. Humans with damage in this brain region exhibit severe and specific deficits in certain types of learning. In animals as well, learning tasks can be categorized as hippocampal dependent or not. In 1999, it was discovered that hippocampal-dependent learning enhances the probability that a newly generated neuron in the hippocampus will survive.

Adult Neurogenesis in the Hippocampal Formation

Newly generated neurons in the adult brain have been identified in several brain regions including the amygdala, hypothalamus, and neocortex, with much larger populations along the wall of the lateral ventricle (subventricular zone), the olfactory bulbs, and the hippocampal formation. The dentate gyrus of the hippocampal formation is among the most active neurogenic regions. These neurons arise from the border between the granule cell layer and the hilus. The precise cell lineage of hippocampal cells is not entirely clear; however, evidence suggests that these neurons arise from a constitutively active population of radial glial cells. Data are derived from a combination of techniques, including retroviral labeling and immunohistochemistry, to selectively label newly generated cells, and laser and electron microscopy to visualize morphological changes across development. When proliferating hippocampal cells were selectively destroyed, the first new cell type to arise was glial fibrillary acidic protein (GFAP)-expressing cells which later expressed neuron-specific proteins. GFAP is used to identify glial cells. While glial cells, or GFAP-expressing progenitors, represent one putative source of new neurons, other possibilities exist. For instance, when a cluster of new neurons is identified, there is often an increased density of blood vessels nearby. A positive correlation between neurogenesis and angiogenesis, the birth of new vasculature, occurs in both mice and humans. Furthermore, running, which increases cerebral blood flow, in turn, increases cell proliferation quite

dramatically. An intriguing possibility is that bidirectional communication between blood and nerve cells leads to the synthesis of neural progenitor cells *de novo*. Indeed, the angiogenic protein, vascular endothelial growth factor (VEGF), promotes the growth of vascular endothelial cells and also stimulates the growth of neuronal precursors both *in vitro* and *in vivo*. Although speculative, perhaps the delivery of lipids, proteins, and carbohydrates across blood and nerve cell membranes provide the building blocks of a new cell or otherwise contribute to adult neurogenesis in the hippocampus. Regardless of the mechanism for synthesis, newly generated cells that give rise to neurons in the adult hippocampus are found in the dentate gyrus of the hippocampal formation.

Newly generated hippocampal cells exhibit significant growth between 4 weeks and 4 months. Throughout this period, the size of the soma, total dendritic length, dendritic branching, and spine density all increase by about 60%. During the first 3 days after mitosis, apical dendrites extend through the granule cell layer at a rate of $10 \mu\text{m day}^{-1}$. When the cells are 4 and 5 days old, the dendrites double in length each day as they continue to extend toward the molecular layer. Between 6 and 10 days after mitosis, new granule cells extend axons to the CA3 layer of the hippocampal formation; just as the existing, fully mature dentate granule cells do. Basal dendrites appear early in cell development but are no longer apparent as the cell matures, a feature also observed in the young, developing brain. Another property of new cells in the adult brain, which is also observed in the young brain, is the manner in which they respond to the amino acid neurotransmitter, γ -aminobutyric acid (GABA). Initially, diffuse, tonic GABA surrounding the cell is adequate to induce intracellular changes in the new cell. As the cell develops, it is no longer responsive to ambient GABA, rather it requires synaptic-mediated input. It depolarizes in response to the binding of GABA, then as the cell matures, it hyperpolarizes in response to the binding of GABA. This is the same stereotypical pattern observed in development, and in both cases, it is attributed to the sequential expression of chloride transporters. Ultimately, newly generated cells in the dentate gyrus of the adult brain are completely integrated into the hippocampal network. They form functional synapses and are capable of firing action potentials.

Identifying New Neurons

The ability to label newly dividing cells and track their survival *in vivo* is critical to understanding the role of neurogenesis in memory. The most widely used technique to do this is immunohistochemistry with a synthetic thymidine analog. Bromodeoxyuridine (BrdU) is the thymidine analog most frequently used. It is incorporated into DNA during the S-phase of the cell cycle, during

which DNA is single stranded and BrdU can bind to the thymidine base pair, adenine. As this only occurs during a fraction of the cell cycle, BrdU only labels a fraction of mitotic cells. BrdU is dissolved in saline, is injected into the intraperitoneal cavity (i.p.), crosses the blood–brain barrier, and can label mitotic cells which are in S-phase for up to 2 h after injection. Once incorporated inside cells of the adult brain, BrdU remains there for several cell divisions without substantial dilution. This is different from the postnatal brain, where cell division of neurons occurs at an exponential rate and therefore, substantial dilution occurs in a relatively short amount of time. After animals are euthanized, brain tissue is fixed with paraformaldehyde. The brain is then cut into thin sections and mounted onto glass slides. Cells containing BrdU are visualized using standard immunohistochemical techniques. Essentially, the tissue is treated with an antibody that was created to bind specifically to BrdU. The antibody complex is then reacted with a peroxidase which gives a cell containing BrdU a distinct brown color (**Figure 1**). Alternatively, a BrdU-containing cell is labeled with a fluorescent secondary antibody (**Figure 2**). Fluorescent immunohistochemical techniques are beneficial because they allow for labeling of more than one antigen inside of a single cell. Several methods for labeling new neurons are becoming increasingly available. For instance, additional thymidine analogs, such as iododeoxyuridine and chlorodeoxyuridine, may also be used. In addition, new cells may be selectively labeled using a retroviral vector that expresses green fluorescent protein. In this case, the vector is typically injected into the brain using stereotaxic procedures. This approach has been critical in demonstrating that newly generated neurons *in vivo* possess electrophysiological properties, such as the firing of action potentials, which are similar to those of mature granule neurons.

Learning-Sensitive Period during Cell Development

The age of the cell at the start of the training experience appears to be a critical factor for learning-induced survival. Majority of new neurons in the adult hippocampus begin to die about 1–2 weeks after they are generated. If animals are exposed to a hippocampal-dependent task, or to an enriched environment, at the time when these cells normally die, then the fate of these cells is altered and they survive. Recent data indicate that nearly all of the cells that are available at the onset of training are rescued from death, provided that the animal learns and learns well; once rescued, they remain in the hippocampus for at least 2 months after training. These findings indicate that learning increases the number of surviving neurons in the hippocampus and thereby alters the existing circuitry of the hippocampal formation.

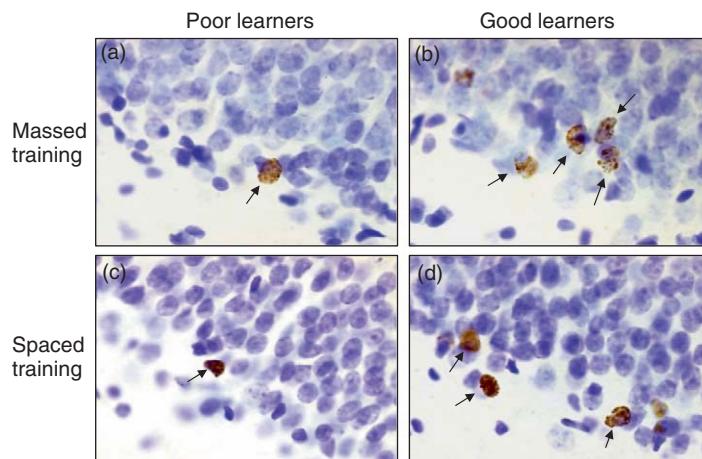


Figure 1 Mitotic cells that were in the S-phase at the time of injection incorporate BrdU. After the brains are dissected, immunohistochemical procedures are used to change the color of cells that contain BrdU. In this image, BrdU-labeled cells are brown. Purple cells were stained with cresyl violet; these are mature cells, as well as cells that may have been synthesized two or more hours after injection with BrdU. Number of BrdU-labeled cells in the dentate gyrus depends on how well the animal learned. An example of an animal trained with massed trials that learned poorly (a), massed trials that learned well (b), spaced trials that learned poorly (c), and spaced trials that learned well (d). With permission from Sisti HM, Glass AL, and Shors TJ (2007) Neurogenesis and the spacing effect: Trials distributed over time enhance memory and cell survival. Learning and Memory 14(5): 368–375.

Learning and New Neurons

In general, the term ‘learning’ is used to imply a change in cognitive state or behavior as a result of acquiring new information. The primary distinction between learning and memory is a temporal one. That is, learning is the acquisition of a new task or skill, whereas memory is the retention and retrieval of that newly acquired information. Learning processes engage different brain regions to varying degrees depending on the type of task: (1) learning that involves fearful and threatening situations is highly dependent on amygdala activity; (2) learning that involves associations between overlapping and discrete sensory stimuli, as occurs in the conventional delay eyeblink conditioning task, depends on an intact cerebellum; and (3) learning that involves trace associations or spatial tasks depends on the hippocampus. As the hippocampus is among the most active neurogenic regions in the adult brain, it was hypothesized that learning that involves the hippocampus could rescue newly generated cells from death.

Learning processes that require the hippocampus tend toward those that require considerable effort and cognitive engagement. Some of these processes include: learning to associate events across time (trace conditioning); learning to navigate and remember locations in three-dimensional space without any obvious visual or olfactory cues (spatial learning); learning the sequence of events (relational learning); and learning to associate the environment with an event (contextual learning). To be explicit, learning is dependent on the hippocampus if

lesions to all of the neurons within it prevent or severely retard learning; learning is not dependent on the hippocampus if learning still occurs even though all the neurons within the hippocampus do not exist (both old and new neurons).

To determine whether hippocampal-dependent learning could rescue new cells from death, animals were trained with two tasks: spatial learning using the Morris water maze and associative learning using eyeblink conditioning. For each task, a hippocampal-dependent and hippocampal-independent version was used. In the Morris water maze, when the platform is hidden just below the surface of the water, the hippocampus is needed; however, when the platform is made visible, the hippocampus is not needed. That is, animals with a damaged hippocampus can navigate toward the visible platform just as well as control animals with no hippocampal damage. For eyeblink conditioning, both trace (hippocampal-dependent) and delay (hippocampal-independent) tasks were used. In delay conditioning, the conditioned stimulus (CS), usually an auditory cue, overlaps and co-terminates with the unconditioned stimulus (US), an airpuff to the eye. Thus, the two stimuli are contiguous in time. In the trace version of this task, the conditioned and (USs) are separated by a stimulus-free interval, for example, the stimuli are discontiguous. Acquisition of the trace task depends on an intact hippocampus. Hippocampal-lesioned animals can learn the delay task as well as controls, but they are unable to learn the trace task, even after many trials. Animals were injected with BrdU 7 days before the start of training.

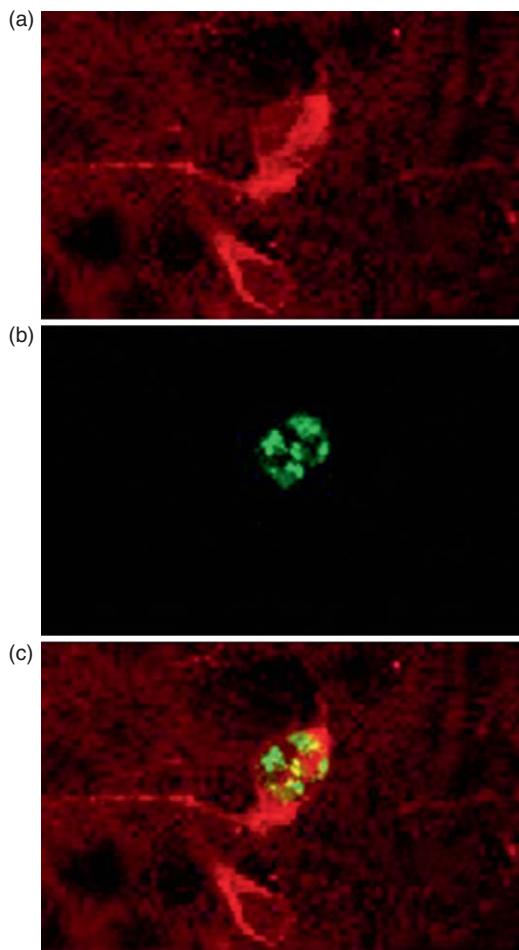


Figure 2 Newly generated cells may be visualized using fluorescent immunohistochemistry and confocal microscopy. Here, the BrdU-labeled cell is green (a). The cells in red express doublecortin (b). The cell expressing both DCX and BrdU is a new neuron, whereas the cell expressing only doublecortin (red only) is a new neuron that was synthesized outside the 2 hour time-window of BrdU injection (c). With permission from Sisti HM, Glass AL, and Shors TJ (2007) Neurogenesis and the spacing effect: Trials distributed over time enhance memory and cell survival. Learning and Memory 14(5): 368–375.

Learning which requires the hippocampus, regardless of whether it was spatial or associative, enhanced the probability that new neurons would survive. Animals trained using the hippocampal-dependent version of either the Morris water maze or eyeblink conditioning had higher numbers of BrdU-labeled cells compared to hippocampal-independent versions of both tasks. Animals trained with the visible platform or with delay conditioning had similar number of new neurons as animals that received no training. Thus, if animals are exposed to a hippocampal-dependent learning task at the time when new cells would normally die, then their fate is altered and the new cells survive. In other words, learning can rescue newly generated neurons from death.

Learning versus Performance

In addition to hippocampal activation, in order to rescue new neurons from death it is important that new learning indeed occurs. Merely exposing animals to a task and all relevant training stimuli is not sufficient to rescue new neurons from death. For instance, for over a century it has been recognized that memory is longer lasting when the new information is learned over time compared with the same amount of information learned in a very brief period of time. This phenomenon has been known alternatively as the ‘spacing effect,’ ‘distribution of practice,’ or colloquially as ‘cramming.’ The role of new neurons in the spacing effect was investigated using a spatial learning task. One group of animals was trained with 16 trials distributed, or spaced, across 4 days. A second group was trained with 16 trials all in 1 day (massed training). Generally, animals trained with spaced trials learned better and had a longer-lasting memory compared with animals trained with massed trials. However, a few animals in the spaced condition learned poorly compared to those in the massed condition, despite having an advantageous training protocol. When the number of BrdU-labeled cells was measured, good learners retained more 1-week-old cells than poor learners, regardless of training condition (Figure 1).

The importance of learning *per se* in rescuing 1-week-old neurons from death also occurs in associative learning tasks. Trace conditioning, which enhances cell survival, was compared with another task, contiguous trace conditioning (CTC). This task is similar to trace except that the CS, or tone, is presented a second time during which it coincides with the US. Unlike the trace conditioning task, the CS and US are presented contiguously. Only when the CS and US are presented simultaneously, animals generally learn poorly. When the number of learned responses was compared between animals trained with either trace or CTC, there were both good and poor learners in each training condition. Animals that learned well had higher numbers of new cells than those that learned poorly, regardless of the training condition.

Task Difficulty

Generally, tasks are considered more difficult to learn if more training trials are necessary to reliably and accurately express a learned response. For example, learning to emit an eyeblink response during delay conditioning (which does not depend on the hippocampus) requires many fewer trials than learning to emit a similar eyeblink response during trace conditioning, which does depend on the hippocampus. If a typical delay conditioning task is rendered more difficult, as in very long delay conditioning, learning this new task will rescue new neurons from death. In contrast, if a trace conditioning task, which is

usually quite difficult to learn, is made easier to learn, learning the new task does not increase the number of surviving cells. In the end, it appears that tasks that are more difficult to learn are also more likely to rescue new neurons from death. However, as stated previously, it is also important that the animals actually learn. That is, animals that learn best tend to possess more cells after training than those that are trained but do not learn or do not learn very well.

Are New Neurons Necessary for Learning?

Eliminating new neurons induces deficits in some types of learning tasks, while leaving most types of learning intact. To test if new neurons are necessary to learn a given task, newly generated cells must be selectively destroyed before animals begin training. It is somewhat challenging to prevent new cell proliferation *in vivo* without any other detrimental consequences; however, pharmacological agents and focused irradiation have been successfully used. For instance, a drug used to treat cancer patients, methylazoxymethanol acetate (MAM), blocks neurogenesis without obvious debilitating side effects in animals. Animals injected with MAM were tested for pain sensitivity, motor activity, stress hormone levels, hippocampal volume, and excitatory postsynaptic potentials in CA1 of the hippocampal formation. On all measures, MAM-treated animals were similar to saline-treated controls. They were then trained on either a hippocampal-dependent (trace eyeblink conditioning) or a hippocampal-independent (delay eyeblink conditioning) task. Both groups could learn the delay task. However, when number of learned responses on the trace task was compared, animals with relatively few new cells could not learn the trace task. The MAM-treated animals emitted few learned responses compared with saline controls. Furthermore, when MAM treatment was stopped, the ability to learn trace conditioning was recovered.

Focused irradiation selectively reduces ongoing neurogenesis in the dentate gyrus without any detectable damage to mature neurons or reduced cell proliferation in the subventricular zone. Low-dose irradiation has been used to inhibit hippocampal cell proliferation of animals which were then tested on the Morris water maze. Training began 4 weeks after irradiation treatment, so that new neurons 4 weeks or younger were not available during training. Animals could learn the task and even remember it when tested 1 week later. However, when tested 2 and 4 weeks later, irradiation-treated animals had no memory of the platform location, whereas controls did. Therefore, new neurons were not necessary in spatial learning or short-term memory of the platform location; however, new neurons were necessary for long-term retrieval of the spatial memory. This is consistent with previous experiments in which MAM-treated rats could

learn the location of a hidden platform in the Morris water maze as well as control animals. Spatial learning is not the only task that can be performed well without neurogenesis. Learning simple associations between aversive events and contextual or environmental cues also does not require new neurons.

There has been much speculation about what the new cells do once they become incorporated as mature neurons into the hippocampus. Some argue that the numbers are too few to matter. This may be the case, but most of the cells that remain in the hippocampus after learning are still there 2 months later. Within that time period, each neuron can develop thousands of spines. If tens of thousands of new neurons are generated each week, the number of potential connections could increase dramatically over a relatively short period of time. In addition, the new cells are located in a prime location – at the first major synapse in the hippocampal formation. Additional neurons will impact neuronal activity within the dentate gyrus, not to mention efferent synapses and other brain regions. Whether these neurons are then used to retrieve the memory of the task by which they were rescued seems unlikely. The hippocampus is mostly involved in learning and only temporarily involved in the retention of most memories. Within weeks of learning, animals without a hippocampus can clearly remember the context in which an aversive event occurred, and they readily express a memory for the trace interval. If the hippocampus is not required to express these types of memory, then new neurons within the hippocampus cannot be necessary either, irrespective of any effect that learning may have on them. Most likely, learning-induced cell survival represents a way in which this brain region is rewired to encode new information. The phenomenon is specific to the type of learning and to the brain regions involved and seems to occur in an activity-dependent manner.

Potential Mechanism of Action

While several theories have been put forth regarding the mechanism by which new learning rescues cells from death, the precise cascade of events remains to be determined. Generally, it is likely that neural progenitor cells of the dentate gyrus receive either mitogenic or anti-apoptotic signals from adjacent mature granule cells as new hippocampal-dependent learning occurs. If the animal has already learned the task, or if the task is very easy to learn, then such signals simply may not be expressed. However, when learning is sufficiently difficult, then the neural mechanisms implicated in learning may result in the induction of specific pathways that lead to the neuronal differentiation of newly generated cells. The most likely candidates in regulating learning-induced cell survival may be found in those pathways which are critical for

both hippocampal-dependent learning and neuronal differentiation. For instance, the acquisition of the conditioned eyeblink response depends on activation of *N*-methyl-D-aspartic acid (NMDA)-receptors and NMDA receptors are critical in regulating adult neurogenesis. NMDA receptors have been localized on newly generated neurons. When glutamate, the excitatory amino acid which activates NMDA receptors, is applied to hippocampal neural progenitor cells *in vitro*, the proportion of cells that differentiate into neurons is increased. Regardless of the mechanism by which new neurons are rescued from death, new learning can predict cell survival.

Conclusions

The ability of learning to rescue new neurons from death has important implications for both the healthy and diseased brain. Even throughout adulthood, the brain continues to generate new neurons.

Current evidence suggests that patients with dementia continue to produce new neurons in their hippocampus, but the numbers are few and most do not mature. It is perhaps paradoxical that even the few new neurons patients produce may not survive simply because the individuals are not able to learn. Determining precisely how learning rescues new neurons from death will be an important advancement in behavioral neuroscience and may translate to widespread therapeutic interventions in the damaged brain.

See also: Attention and Speed of Information Processing; Cognition: Learning and Memory: Pavlovian; Cognition: Learning and Memory: Spatial; Environmental Influences on Adult Neurogenesis; Eyelid Classical Conditioning; Hormones and Memory; Implicit Learning and Memory: Psychological and Neural Aspects; Knock-Outs: Learning and Memory; Learning and Memory: Computational Models; Memory and Aging, Neural Basis of; Memory Consolidation; Neural Basis of Classical Conditioning; Neural Basis of Working Memory; Role of Gene Transcription in Long-Term Memory Storage; Short-Term Memory: Psychological and Neural Aspects.

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Neuron Excitability and Learning

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Glossary

Accommodation – A neuronal response to a sustained depolarizing stimulus where the neuron accommodates to the depolarization and stops or reduces its action potential generation (firing). It is also referred to as ‘spike-frequency adaptation.’

Afterhyperpolarization – Membrane hyperpolarization, mediated mainly by calcium-dependent potassium currents, that follows a train of action potentials, acting to reduce a neuron’s propensity to fire.

Calcium-dependent potassium current – An outward current triggered by elevation of calcium ions in the cytosol of the neuron that causes membrane hyperpolarization mediated by potassium ions leaving the neuron and thus reducing its membrane potential.

Conditioned stimulus (CS) – A neutral stimulus used in learning paradigms that does not elicit the reflexive behavior (measured to reflect learning and memory) prior to training.

Eyeblink conditioning – A classical or Pavlovian learning task designed around the reflexive closure of the eyelid. The subject learns to associate a CS with an unconditioned stimulus (US), with repeated pairings, to anticipate the US after the onset of the CS. After conditioning, the subject closes the eyelid prior to the onset of the US. A simple, yet powerful learning task first used with humans, it has been used extensively to examine the role of the cerebellum and the forebrain, including the hippocampus, in learning and memory storage.

Fear conditioning – A task in which the subject learns to associate a neutral CS with a fear evoking US. This paradigm has been extensively used to examine the learning-related changes in the amygdala, the hippocampus, and the prefrontal cortex.

Hippocampus – A portion of the ancient cortex buried in the medial temporal lobe that was brought to scientific prominence by a landmark study reported by William Scoville and Brenda Milner in 1957. They described profound memory impairments caused by bilateral removal of the hippocampus and surrounding areas in patients with intractable seizure, depression, or paranoid schizophrenia.

Memory consolidation – A stage in the temporal progression of memory formation. The hippocampus and its associated areas are critical when declarative learning (knowing that) is taking place and memories are

initially being formed. Once formed, the memory is distributed and hypothesized to be stored in the neocortical regions.

Unconditioned stimulus (US) – A stimulus used in learning paradigms that evokes a reflexive behavior, for example, an eyeblink response to a puff of air on the eye. After pairing with the neutral CS, the learned conditioned response (CR) has components of the reflexive behavior caused by the US.

What happens when you learn something and it becomes part of your memory? What and where are the changes that must take place in your brain for learning to occur? An alteration in the strength of synaptic contacts between neurons which has been postulated by Donald Hebb is commonly thought to occur with learning. Neuronal excitability, an intrinsic property of neurons, also changes during learning and is our focus. Pyramidal neurons in a portion of the ancient cortex buried in the temporal lobe, the hippocampus, show alterations in the postburst afterhyperpolarization (commonly referred to as AHP) as memories are formed and stored.

Before delving into the learning-related alterations in the AHP, a brief discussion of the well-defined learning tasks used to analyze these changes with a systematic experimental approach is necessary.

The Morris water maze and fear-conditioning tasks are two behavioral assays used by numerous laboratories. The water maze tests the spatial navigation learning ability of rodents that relies on the proper function of the hippocampus. In this task, the animal learns that a hidden platform for getting out of the water in which it is swimming is located in a fixed location in the pool. By learning the location of the platform relative to the visual cues around the water maze, the animal quickly escapes the water in the pool by swimming directly to and climbing onto the escape platform. The fear-conditioning task relies on the proper function of the amygdala and the neocortex. It can also be hippocampus dependent by having the rodents learn the context (the spatial cues) of the fear training environment, or by introducing a temporal gap between the offset of the conditioning cue and the onset of the mild, aversive shock, such as the case in the trace fear-conditioning task.

Eyeblink conditioning is a simple, yet powerful tool to assess the proper function of the various neural networks

involved in learning this task. The subject has to learn to associate a conditioned stimulus (usually a tone) that precedes the airpuff unconditioned stimulus to the eye that causes the reflexive response of eyelid closure. After learning, presentation of the tone causes eyeblinks that occur prior to or even without the air puff. Due to its similarity to Pavlov's experiments with dogs (ringing the bell before giving food reward), eyeblink conditioning is often called a Pavlovian learning task. This remarkably simple sounding task can be made challenging by inserting a temporal gap between the tone (the predictor) and the airpuff (the reinforcer): the resulting paradigm is called 'trace eyeblink conditioning' because it requires that a short-term memory trace be formed to allow the correct prediction of when the airpuff will occur. Many studies, including those from our laboratory, have demonstrated that trace eyeblink conditioning relies on the proper function of the hippocampus and is sensitive to aging. More importantly, results from animal experiments with trace eyeblink conditioning are directly applicable to humans, because results from human experiments on trace eyeblink conditioning are similar to those found with animals: humans with hippocampus damage (and thus amnesia) and the elderly are impaired in learning the association between the tone and the airpuff. Most of the experiments that illustrate the learning-related AHP reductions observed in hippocampal pyramidal neurons are from animal studies using trace eyeblink conditioning.

The Postburst AHP

In the brain and the spinal cord, relay of information from one neuron to another is achieved by action potentials (APs) (Figure 1). In most neurons, especially pyramidal neurons, an AP is followed by a hyperpolarization (commonly referred to as 'the refractory period') that makes it difficult for the neuron to fire another AP in quick succession. However, when a neuron fires a burst of APs, the result is a prolonged, postburst AHP that increases in size with increasing number and frequency of APs in the burst (Figure 1). Thus, the AHP plays a central role in determining whether or not a neuron will relay a message to other neurons within and between the cortical neural networks.

Three Phases of AHP: Fast, Medium, and Slow

The fast AHP

The fast AHP is part of the repolarizing phase of an AP (Figure 1). Comprised of multiple potassium currents (I_{C} , I_{M} , and I_{A}), it plays a significant role in shaping the AP. When these potassium currents are active, the repolarization of the membrane is accelerated, and as a consequence, it limits the influx of calcium ions (Ca^{2+})

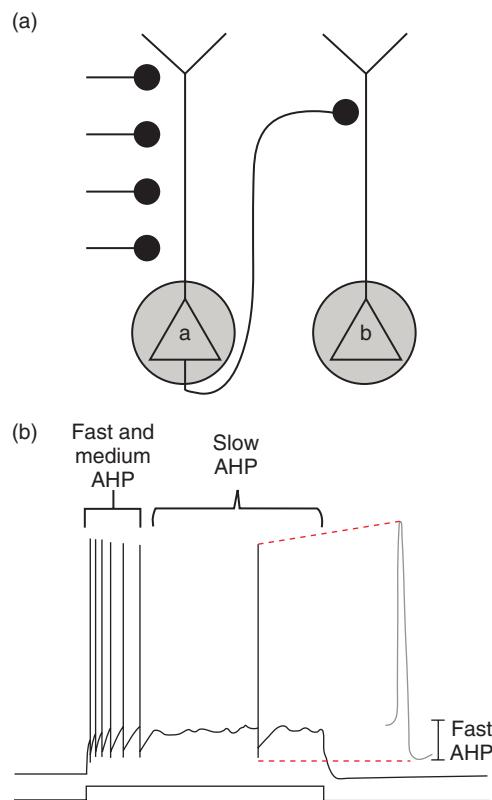


Figure 1 The postburst AHP plays a critical role in determining transmission of information from one neuron to the next. (a) Highly simplified diagram of two neurons that are in a neural circuit. Neuron 'a' receives synaptic input from various sources in the neural circuit and integrates it in the soma (triangle). When the synaptic inputs exceed the threshold for the neuron, an action potential (AP) is generated in the axon initial segment, and information is relayed to the next neuron 'b'. The hypothesized location of the slow AHP channels is the shaded circle around the soma. Due to its localization, the size of the slow AHP plays a critical gatekeeper role in determining whether or not an AP will be generated. A large slow AHP can shunt depolarizing inputs arriving to the soma from the dendrites and effectively prevent these depolarizing signals from being passed on to the axon initial segment. (b) An example of CA1 pyramidal neuron's response to a prolonged depolarizing input is illustrated. The neuron initially responds with a number of APs. With time, the frequency of the APs reduces, to a point where there is a cessation of AP for a long duration. This reduction of APs is called 'spike-frequency adaption', or simply 'accommodation', and has been found to be highly correlated to the size of the slow AHP. The fast and medium AHPs dictate the frequency of APs that can be generated during the initial depolarization. The slow AHP dictates the neuronal response to the sustained depolarization. The inset illustrates where the fast AHP is measured after an AP.

into the cell by removing the depolarization necessary for the voltage-gated calcium channels (VGCCs) to work. Conversely, when the fast AHP is reduced or inhibited, the AP becomes broader and allows Ca^{2+} influx through the VGCCs. By having a large fast AHP, the membrane potential is repolarized to a point further away from AP threshold and prevents the neuron from firing

immediately after repolarization. When the fast AHP is reduced or inhibited, the membrane potential is near the AP threshold upon repolarization, and the neuron can subsequently generate another AP with a depolarizing stimulus. It is this balancing act of regulating the rate and degree of membrane repolarization after an AP that allows the fast AHP to help determine the frequency of AP generation.

The medium AHP

The medium AHP, I_{AHP} , is a calcium-dependent, but not voltage-dependent, potassium current that results from a burst of APs and lasts for 50–125 ms during which the peak of the AHP occurs. Originally identified by its sensitivity to a neurotoxin from bee venom, apamin, it is generally accepted that the medium AHP is mediated by SK2 channels in most cortical neurons, including hippocampal pyramidal neurons. However, contrary data reported by Johan Storm and colleagues suggest that an apamin-sensitive AHP does not exist in hippocampal pyramidal neurons and that the medium AHP reflects activation of I_M and I_h in these neurons.

The slow AHP

The slow AHP, sI_{AHP} , is mainly a calcium-dependent, voltage-independent, potassium current that can last for seconds after a burst of APs and that is modulated by a wide variety of neurotransmitters and peptides. It is hypothesized that the channels mediating the slow AHP are located on the soma and the dendrites near the soma of pyramidal neurons; however, the channel identity has yet to be discovered. Due to its proposed location and long-lasting duration, the slow AHP plays an important role in signal transduction and plays a role akin to a gatekeeper. When the slow AHP is large, the region surrounding, and including, the soma is at a hyperpolarized state far away from AP threshold. Thus, unless the neuron is bombarded with synaptic signals that can significantly depolarize the cell, the large slow AHP will prevent further relay of synaptic information that arrives at the cell. On the flip side, when the slow AHP is small, the soma and its surrounding region will be less hyperpolarized and in a state closer to AP threshold. Thus, synaptic information that arrives at the cell will more likely be relayed to the next neuron in the neural circuit ([Figure 1](#)).

Learning-Related Alterations in the AHP

Daniel Alkon and colleagues demonstrated a learning-related alteration of an intrinsic membrane property of a neuron in invertebrates. They showed that I_A and a calcium-dependent potassium current were significantly reduced in isolated type B photoreceptors from

Hermissenda crassicornis trained to avoid a lighted area: *Hermissenda* normally move toward light. The resulting reduction in these AHP currents led to increase in excitability of the type B photoreceptors after training.

Disterhoft, Coulter, and Alkon reported that an intrinsic neuronal property, the postburst AHP, is altered by associative learning in vertebrates. They demonstrated that learning the classic, delay eyeblink conditioning task is correlated with an increase in neuronal excitability resulting from a reduction in the postburst AHP in CA1 hippocampal pyramidal neurons in brain slices taken from rabbits. The AHP reduction was an alteration in the intrinsic membrane property as the learning-related AHP reduction was still evident after synaptic transmission in the hippocampal slice was blocked. More importantly, the AHP reduction was a learning-specific phenomenon as the postburst AHPs in neurons from control animals given unpaired delay eyeblink training were not different, and nearly identical, to that measured in neurons from naive, untrained animals. However, all these observations were made in CA1 hippocampal pyramidal neurons after the animals learned the delay eyeblink conditioning task; which engages the hippocampus, but can be learned without it.

Building upon these initial findings, several studies have demonstrated that the postburst AHP is reduced in pyramidal neurons in various cortical regions that are necessary for learning a specific task. The postburst AHP and the currents mediating it (I_{AHP} and sI_{AHP}) are reduced in dorsal CA1 hippocampal pyramidal neurons after spatial water maze learning in rats ([Figure 2](#)). Learning trace eyeblink conditioning task is correlated with smaller postburst AHP in hippocampal pyramidal neurons of rats and rabbits ([Figure 3](#)). Recently, Elizabeth Matthews in our laboratory demonstrated a learning-related reduction in I_C (BK channel-mediated fast AHP) in hippocampal CA1 pyramidal neurons after rats learned the trace eyeblink conditioning task. Smaller postburst AHP in piriform cortical neurons after odor discrimination learning in rats has been demonstrated by Edi Barkai and colleagues. All of these learning-related AHP reductions lead to enhanced neuronal excitability of the neurons.

However, reduced AHP and the resulting increase in neuronal excitability are not always appropriate for all learning tasks. Proper modulation of the postburst AHP is essential for learning to take place. Recent work by James Porter and colleagues demonstrate this point. In their experiments, rats are trained on auditory fear conditioning. This task relies on the proper function of the amygdala and the prefrontal cortex for the rats to learn that a tone will be followed by a shock. In a naive state, the activity in the amygdala is repressed or kept in check by efferent activity of the prefrontal cortical neurons that act to inhibit

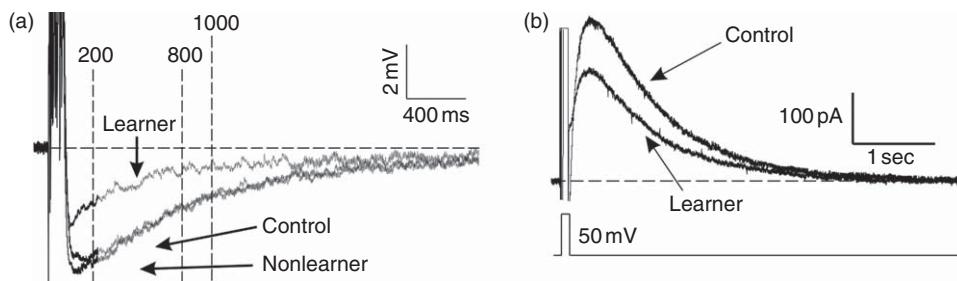


Figure 2 The postburst AHP and the currents underlying it were reduced in CA1 pyramidal neurons from rats that learned the hidden platform location. (a) Typical examples of the postburst AHP from dorsal CA1 neurons of learner, nonlearner, and controls are illustrated. These neurons were current clamped to near -68 mV (horizontal dashed line). The vertical dashed lines illustrate the time points of AHP measurements at 200, 800, and 1000 ms after the pulse offset. (b) Examples of isolated I_{AHP} - S/I_{AHP} currents from dorsal CA1 neurons of a learner and a control rat are illustrated. The horizontal dashed line indicate the holding current used to hold the neurons at -55 mV ; that is, the relative zero for the measurements. Reproduced with permission from Oh MM, Kuo AG, Wu WW, Sametsky EA, and Disterhoft JF (2003) Watermaze learning enhances excitability of CA1 Pyramidal neurons. *Journal of Neurophysiology* 90: 2171–2179, copyright The American Physiological Society.

amygdala neuron firing rate. However, for fear learning to take place, the activity in the amygdala increases as a result of reduced activity in the prefrontal pyramidal neurons. Porter and colleagues observed a significant reduction of activity in infralimbic pyramidal neurons of the prefrontal cortex in rats trained on the fear-conditioning task that was mediated by an enlargement of the postburst AHP in these neurons. Subsequently, they observed that the postburst AHP in these infralimbic neurons was reduced and restored to a naive-like (pretraining) state when the auditory fear memory was extinguished in the trained rats: that is, the increased activity resulting from the decreased AHP in the prefrontal cortex keeps the amygdala in check to prevent expression of the fear memory. Thus, the proper modulation of the postburst AHP is essential for expression of the appropriately learned behavior.

This leads to the question of where memory is stored. Experimental evidence points to the neocortex as the site for permanent memory storage. However, memory for even the simplest learned event appears to be distributed throughout the cortex. What we do know is that memory for a learned association, such as trace eyeblink conditioning, is not stored permanently in the hippocampus as demonstrated by two important studies.

First, in a series of experiments by Moyer, Thompson, and Disterhoft, the transient nature of the learning-related postburst AHP reduction was demonstrated in hippocampal pyramidal neurons after trace eyeblink conditioning in rabbits. The significantly smaller postburst AHPs in the pyramidal neurons were maintained for about a week after the animals learned the task (Figure 3). This does not mean that the rabbits forgot the learned association. Once the rabbits learned the trace eyeblink conditioning task, the

memory of the conditioned stimulus–unconditioned stimulus association is maintained for months after the last training session; although experimentally untested, the memory may last the lifetime of the subject. More importantly, the learning-related postburst AHP reduction is not merely a by-product of sensory experience during training. To demonstrate this, the rabbits were trained to a learning criterion and left undisturbed in their home cages for 2 weeks, after which they were given another training session. As expected, these rabbits showed excellent retention of the learned trace eyeblink conditioning response. However, the biophysical properties, including the postburst AHP, of the pyramidal neurons from these rabbits were no different than those from neurons in naive and control rabbits (Figure 3). Apparently, hippocampal neurons show AHP reductions early in training when the association is being acquired and initially consolidated or stored in neocortex. After the neocortical storage has occurred, the conditioned response is demonstrated independent of the hippocampus and neurons there do not show AHP reduction after further training sessions.

In another series of experiments, Kim, Clark, and Thompson demonstrated the critical time window of hippocampal engagement for learning and retaining the memory for the trace eyeblink conditioning task. Briefly, they trained rabbits on the trace eyeblink conditioning task and chose two different times to remove the hippocampus bilaterally. In one group of rabbits, they removed the hippocampus 1 day after the rabbits achieved a learning criterion. These animals failed to recall the learned behavior and failed to relearn the trace eyeblink conditioning task. Notably, these rabbits did learn the cerebellum dependent but hippocampus independent, delay eyeblink conditioning task. In another group of

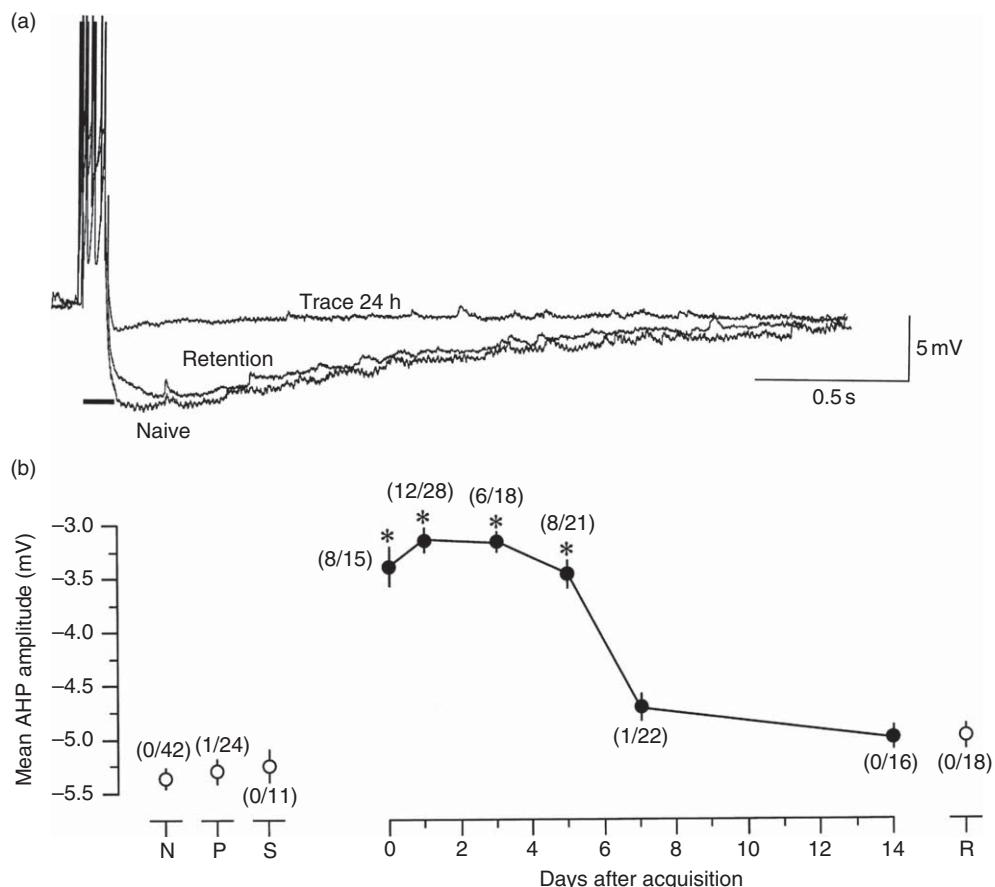


Figure 3 The postburst AHP was transiently reduced in CA1 pyramidal neurons while performance remained persistent after learning trace eyeblink conditioning in young adult rabbits. (a) Voltage trace shows an overlay of recordings of the postburst AHPs in CA1 neurons from a naive rabbit (naive) and from trace-conditioned rabbits studied 24 h after initial learning (trace 24 h) or 24 h after receiving an additional training session given 14 days after initial learning (retention). The resting membrane potentials for these cells were approximately -66 mV, with APs truncated for visualization of the AHP. The AHP was measured for 5 s beginning after a 100 ms depolarizing current injection (solid black line), with minimal current (~ 0.6 nA) required to reliably evoke a burst of four APs. (b) Learning-related reductions of the AHP amplitude were transient, lasting ~ 1 week in slices prepared at various times after learning [1 hr (0 d), 1 d, 3 d, 5 d, 7 d, or 14 d]. Such changes were not observed in: N, naive; P, pseudoconditioned; or S, slow-learning control rabbits. Numbers in parentheses indicate the ratio of individual cells with reduced AHPs to number of cells studied in that group. Slow learners were defined as rabbits that did not reach criterion within 15 training sessions, and that exhibited $<30\%$ conditioned responses on the last training session. R, retention rabbits received an additional 80-trial training session on the 14th day after initial learning. Asterisks indicate data significantly different from all three control groups: $*p < 0.001$. Reprinted with permission from Moyer JR, Jr., Thompson LT, and Disterhoft JF (1996) Trace eyeblink conditioning increases CA1 excitability in a transient and learning-specific manner. *Journal of Neuroscience* 16: 5536–5546, copyright The Society for Neuroscience.

rabbits, Thompson and colleagues removed the hippocampus 1 month after the rabbits met the learning criterion. These rabbits were able to produce the learned trace eyeblink responses at a high level without their hippocampus.

The results from the studies by Disterhoft and Thompson and colleagues provide further evidence that the hippocampus and the learning-related modification of the postburst AHP play a critical role in learning and consolidation of associative tasks. Importantly, the learning and memory detriment observed with hippocampal lesions 1 day after the rabbits learned the task coincides with the time point when

maximal learning-related postburst AHP reduction is observed after training. The lack of the learning-related postburst AHP change when measured 2 weeks after learning and the lack of behavioral consequence of hippocampal removal a month after learning provide further evidence that the hippocampus is necessary to learn the associative task, but is not the site of memory storage. These findings in animals are consistent with neuropsychological observations of hippocampus lesioned amnesic subjects, with HM being the most famous case study, who cannot store new declarative memories but have access to information stored prior to their amnesic insult.

Does Size Matter?

Considerable evidence for the postburst AHP in hippocampal pyramidal neurons suggests that AHP size is an important determinant of hippocampal function. This can be illustrated by examining the relationship between the size of the postburst AHP and learning with normal aging.

The postburst AHP in CA1 hippocampal pyramidal neurons is significantly enlarged with normal aging, as first reported by Landfield and Pitler (1984) and later replicated by others including our laboratory. The channel that mediates the slow AHP has not been identified; so much attention has been focused on alterations of calcium with aging, because the postburst AHP is mainly a calcium-dependent potassium current. This attention also arises from the calcium hypothesis of aging. From many studies that examined the alteration of potential sources of calcium with aging, it has been shown that there is an enhancement of calcium influx into neurons via the L-type voltage-gated calcium channels and an increase in calcium-induced calcium release from the endoplasmic reticulum with normal aging. Both of

these phenomena contribute to the enlarged postburst AHP observed in hippocampal pyramidal neurons from aged animals.

Learning various associative tasks has been shown to be progressively more difficult with normal aging. Specifically, spatial learning in the water maze and temporal eyeblink conditioning have been used extensively to demonstrate that aging subjects, including humans, have impaired learning. For example, studies in our laboratory have demonstrated that nearly half of the aging rabbits and rats fail to learn the trace eyeblink conditioning task. For those subjects that do meet the learning criterion, it takes them many more trials to do so as compared to the young.

The surprising observation concerns the size of the postburst AHP in CA1 pyramidal neurons from aging animals that successfully learn the trace eyeblink conditioning task. The CA1 neurons from aging learners had significantly smaller postburst AHPs as compared to those from aging animals that failed to learn (**Figure 4**). The animals that failed to learn the task had enlarged postburst AHPs-like those in neurons from naïve or control aging subjects. The size of the

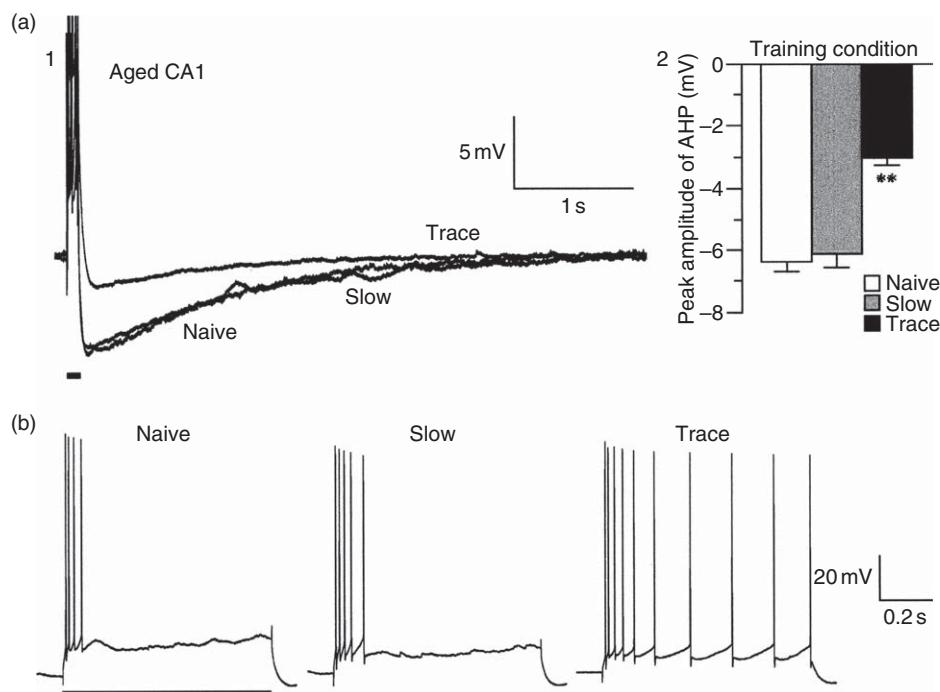


Figure 4 Acquisition of hippocampus-dependent trace EBC increased excitability of aging rabbit hippocampal CA1 pyramidal neurons. (a) Effects of trace conditioning on the size of the postburst AHP. (1) Overlay of voltage recordings of the postburst AHP in CA1 neurons from an aging naïve rabbit (naive), an aging rabbit that showed <15% conditioned responses after 15 sessions (slow), and an aging trace-conditioned rabbit (trace). Duration of current injection is indicated by the bar below the trace. (2) Mean effects of trace EBC on postburst AHP amplitude in aging rabbit CA1 neurons. Notice that after learning the AHP was significantly reduced compared with naïve and slow-learning aging controls. (b) Typical examples of accommodation responses in CA1 pyramidal cells from aging naïve, aging slow-learning and aging trace-conditioned rabbits. Reproduced with permission from Moyer JR, Jr., Power JM, Thompson LT, and Disterhoft JF (2000) Increased excitability of aged rabbit CA1 neurons after trace eyeblink conditioning. *Journal of Neuroscience* 20: 5476–5482, copyright the Society for Neuroscience.

postburst AHPs from the aging subjects that did learn were nearly identical to that found in CA1 neurons from young animals that learned the task. These findings strongly suggest that proper modulation of the intrinsic neuronal properties, mainly those that control the postburst AHP, may be a key to successfully learning associative tasks.

Cause or Result

Thus far, we have reviewed experimental results that illustrate a correlation between successful learning and alteration in the postburst AHP. However, it remains to be determined if the alteration in the postburst AHP leads to successful learning, or if learning leads to the change in the postburst AHP.

In an attempt to address this question, Tombaugh, Rowe, and Rose designed a set of experiments that took advantage of the heterogeneity of learning ability in aging rats. They separated the aged rats into two groups after spatial water maze training: those that learned the location of the hidden platform; and those that failed to learn. After the behavioral assay, they allowed the aged rats to remain in their home cages for at least 2 weeks to ensure that any learning-related alteration in the postburst AHP will return to a pretraining level, based on the timeline identified by Moyer, Thompson, and Disterhoft (**Figure 3**). They observed that the postburst slow AHPs of CA1 pyramidal neurons from aged animals that successfully learned the water maze task were nearly identical to that found in young rats, while the learning impaired rats had CA1 neurons with large AHPs (**Figure 5**). More importantly, they found an inverse relationship between the size of the slow AHP and performance on the water maze task: better performers had smaller postburst AHP.

Thus, a cascade of events that may underlie the age-related learning impairment of hippocampus-dependent tasks is as follows. Increase in the postburst AHP of hippocampal pyramidal neurons is part of the normal aging process. This increase in the AHP leads to significantly reduced excitability of the pyramidal neurons. The reduced excitability of the pyramidal neurons prevents them from participating in the neural network involved in learning. AHPs from those aging animals able to learn, even after more training trials, show reductions similar to those from young animals.

If the pyramidal neurons need to be in a young-like state to participate in the neural network for successful learning, are there interventions that allow this state be achieved?

We have addressed this issue with pharmacological experiments using potential therapeutic compounds developed to ameliorate the cognitive deficits observed

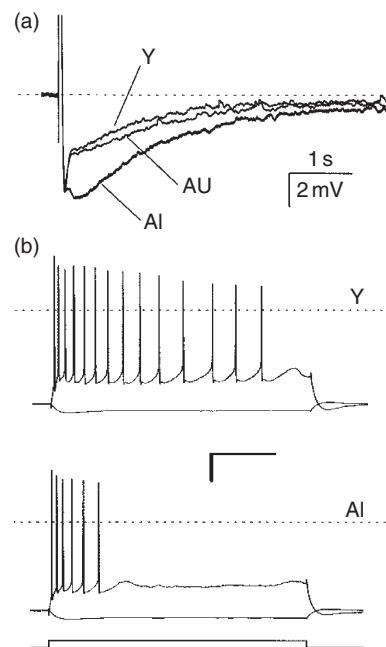


Figure 5 Aged rats that are impaired in learning the spatial water maze task have greater AHP and accommodation in CA1 neurons than do young rats and aged rats that learned the task successfully. (a) Representative waveforms taken from a Y, young rat; AU, an aged unimpaired rat; and AI, an aged impaired rat. In each case, the AHP was evoked with a 50 ms current pulse sufficient to trigger four APs. The broken line indicates holding potential. (b) Representative recordings from young and aged impaired CA1 neurons during tonic current injection. The broken lines represent 0 mV. Scale bar: 20 mV and 0.2 s. Reproduced with permission from Tombaugh GC, Rowe WB, and Rose GM (2005) The slow afterhyperpolarization in hippocampal CA1 neurons covaries with spatial learning ability in aged Fisher 344 rats. *Journal of Neuroscience* 25: 2609–2616, copyright the Society for Neuroscience.

in Alzheimer's disease. As an example, we have tested the impact of a cholinesterase inhibitor, metrifonate, on aging rabbits. Metrifonate significantly ameliorated the learning impairments on trace eyeblink conditioning in aging rabbits. Importantly, when we pretreated another group of aging rabbits with metrifonate and examined the biophysical properties of neurons from these subjects, we found that metrifonate treatment significantly increased the neuronal excitability of CA1 neurons from these aging naive animals. The basal activity level of the CA1 neurons from these metrifonate treated, naive aging rabbits was similar to that found in naive young rabbits. Thus, metrifonate treatment restored the aging CA1 neurons to a young-like state. We hypothesize that it is this rejuvenation of hippocampal pyramidal neurons with the various potential therapeutics (cholinesterase inhibitors, L-type calcium channel blocker, and muscarinic agonist) that we have tested which ameliorated the age-related learning impairments on the trace eyeblink conditioning task in aging rabbits.

Thus, our current working hypothesis is that the modulation of the postburst AHP (depending on the neural circuit, the AHP will need to be enhanced or reduced) precedes and is a prerequisite for learning to occur in young and aging subjects. In addition, further potential modification of the postburst AHP may also occur as learning occurs.

Potential Mechanism for Learning-Related AHP Reduction

The postburst AHP of hippocampal pyramidal neurons is reduced by nearly all known neuromodulators and neurotransmitters that, for the majority, act via second messengers involving activation of protein kinases. Notably, activation of the same kinases (e.g., protein kinase A, PKA) that reduce the AHP also activates various signaling cascades that lead to activation of cAMP response element binding (CREB) and gene transcription that lead to altered synaptic connectivity during learning. Barco and colleagues have demonstrated that activation of CREB also results in a reduction of the postburst AHP in CA1 pyramidal neurons.

Recently, we examined the potential role of PKA and another kinase, protein kinase C (PKC), in mediating the learning-related AHP reduction observed in CA1 pyramidal neurons after trace eyeblink conditioning in young adult rats. One day after the rats met our learning criterion; we prepared hippocampal slices and measured the postburst AHP in CA1 neurons. As previously reported and reviewed in this article, we observed a learning-related AHP reduction in CA1 neurons from rats that learned the trace eyeblink task. When the recorded neurons were challenged with a PKA activator, we found that the postburst AHP was only reduced in CA1 neurons from control animals, but not in neurons from animals that learned the task. Thus, learning-related AHP reduction occluded the PKA effect on the AHP in CA1 neurons from animals that learned. Contrary to the results observed with PKA, when the recorded neurons were challenged with a PKC activator, the postburst AHP in CA1 neurons from both trained and control animals was significantly reduced. While not ruling out the involvement of PKC during initial stages of learning, these data suggest that the activity of PKA maintains the learning-related AHP reduction observed in CA1 neurons at a time point after successful learning.

Conclusion

In this article, we have reviewed the learning-related alteration in the intrinsic membrane property of pyramidal neurons, the postburst AHP, mainly by using

examples from our studies examining hippocampal pyramidal neurons after trace eyeblink conditioning. We have also reviewed the fact that this learning-related AHP modulation is observed in other brain regions (such as the prefrontal and piriform cortices) after a subject has learned a task. But the story does not end here. Much more needs to be done to understand the biological mechanisms of learning. For example, using the new technologies of multiphoton imaging and gene silencing with siRNAs, questions regarding the role and sources of calcium that may also be altered by learning can be addressed. Furthermore, learning-induced intracellular signaling cascades involved in gene transcription and translation that lead to alterations in the intrinsic membrane properties of the neuron have yet to be fully explored. No matter where these studies lead, it is clear now that alteration in intrinsic neuron excitability is most certainly one cellular fingerprint of learning.

See also: Aging and Cognition; Analysis of Learning in Invertebrates; Animal Models of Learning and Memory; Cerebellum: Associative Learning; Cholinergic Systems in Aging and Alzheimer's Disease: Neurotrophic Molecular Analysis; Cognition: Learning and Memory: Pavlovian; Cognition: Learning and Memory: Spatial; Cognitive Decline in Laboratory Animals: Models, Measures, and Validity; Declarative Memory; Episodic and Autobiographical Memory: Psychological and Neural Aspects; Eyelid Classical Conditioning; Fear Conditioning; Genes and Behavior: Animal Models; Genetics of Memory in *Drosophila*; Knock-Outs: Learning and Memory; Learning and Memory: Computational Models; Mechanisms of Memory Formation and Storage in *Hermissenda*; Memory and Aging, Neural Basis of; Memory Consolidation; Memory in *Caenorhabditis elegans*; Memory in the Honeybee; Neural Basis of Classical Conditioning; Neural Basis of Working Memory; Neural Substrates of Conditioned Fear and Anxiety; Neurogenesis and Memory; Neurotransmitters and Neuromodulators Regulating Sleep and Wakefulness; Protein Synthesis and Memory; Role of Gene Transcription in Long-Term Memory Storage; Sleep: Learning and Memory; Synapse Formation and Memory; Synaptic Mechanisms for Encoding Memory; Temporal Lobe and Object Recognition; Transgenic Technologies and Their Application to the Study of Senile Dementia; Value of Animal Models for Predicting CNS Therapeutic Action.

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Neurotransmitters and Neuromodulators Regulating Sleep and Wakefulness

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Glossary

Electroencephalogram (EEG) – The electrical activity of the cerebral cortex measured by placing electrodes on the skull. The different stages of sleep are defined, in part, by the characteristic pattern of cortical electrical activity.

Gamma-aminobutyric acid (GABA) – The major inhibitory neurotransmitter in the brain. The binding of GABA to its receptor on the surface of a neuron reduces the probability that the neuron will discharge.

Laterodorsal and pedunculopontine tegmental nuclei (LDT/PPT) – Brainstem nuclei located at the border between the midbrain and pons which contain cholinergic neurons that play an important role in the generation of REM sleep.

Non-rapid eye movement (NREM) – The majority of sleep time is spent in NREM sleep, which is divided into four stages. Stages 1 and 2 are lighter states of sleep, whereas stages 3 and 4 are deeper and more restorative. Stages 3 and 4 together are also called slow-wave sleep because the cortical electroencephalogram is characterized by slow-frequency (0.5–4 Hz) waves. Normal sleep is characterized by an orderly progression from stage 1 to 4 and then back up to stage 2, from which REM sleep is entered.

Pontine, an adjective of the term pons – It is normally used to describe neurons that are located in the part of the brainstem called the pons.

Pontine reticular formation (PRF) – A group of brainstem neurons that forms the pontine component of the ascending reticular activating system.

Rapid eye movement (REM) – A state of sleep characterized by skeletal muscle atonia punctuated by phasic bursts of muscle activity, an activated cortical electroencephalogram, rapid phasic eye movements, autonomic dysregulation, and vivid dreaming.

made since the discovery of rapid eye movement (REM) sleep in 1953 by Eugene Aserinsky and Nathaniel Kleitman. A mechanistic understanding is emerging from studies that have identified sleep-related brain regions, neurotransmitters, and receptors. This article highlights some of the key neurotransmitters involved in the process of sleep-cycle control, with an emphasis on sleep-state-dependent changes in neurotransmitter release. **Figure 1** shows the location of the neurons that synthesize these neurotransmitters, and schematizes some of their projection pathways relevant for sleep.

Acetylcholine

Acetylcholine (ACh) was the first neurotransmitter discovered and is an important mediator of wakefulness, REM sleep, motor control, and the autonomic system. The brain contains two major cholinergic projection systems, both of which play a role in generating states of sleep and wakefulness. ACh-containing neurons in the basal forebrain project to the neocortex and hippocampus, where acetylcholine functions to activate the electroencephalogram (EEG), mediate focused attention, and consolidate memories. Cholinergic neurons in the basal forebrain fire fastest during wakefulness and REM sleep, and discharge more slowly during non-REM (NREM) sleep. This discharge pattern of basal forebrain cholinergic neurons is consistent with the ACh release pattern measured in the cerebral cortex (**Table 1**). The degeneration of basal forebrain cholinergic neurons that occurs in Alzheimer's disease contributes to memory loss, daytime sleepiness, and disrupted nighttime sleep in these patients. A second group of ACh-containing neurons is found in the laterodorsal and pedunculopontine tegmental nuclei (LDT/PPT) of the brainstem. LDT/PPT neurons project up to the thalamus, hypothalamus, and basal forebrain, as well as down to numerous brainstem nuclei, including the pontine reticular formation. One group of cholinergic LDT/PPT neurons discharges selectively during REM sleep, consistent with the finding that ACh release in the pontine reticular formation is greatest during REM sleep (**Table 1**). A second group of LDT/PPT neurons fires fastest during wakefulness and REM sleep, consistent with the pattern of relatively high ACh release in the thalamus during wakefulness and REM sleep and relatively low thalamic ACh release during NREM sleep (**Table 1**).

Neurotransmitters and Neuromodulators Regulating Sleep

Sleep neurobiology aims to elucidate the mechanisms by which states of sleep and wakefulness are generated by the brain. Great progress in achieving this aim has been

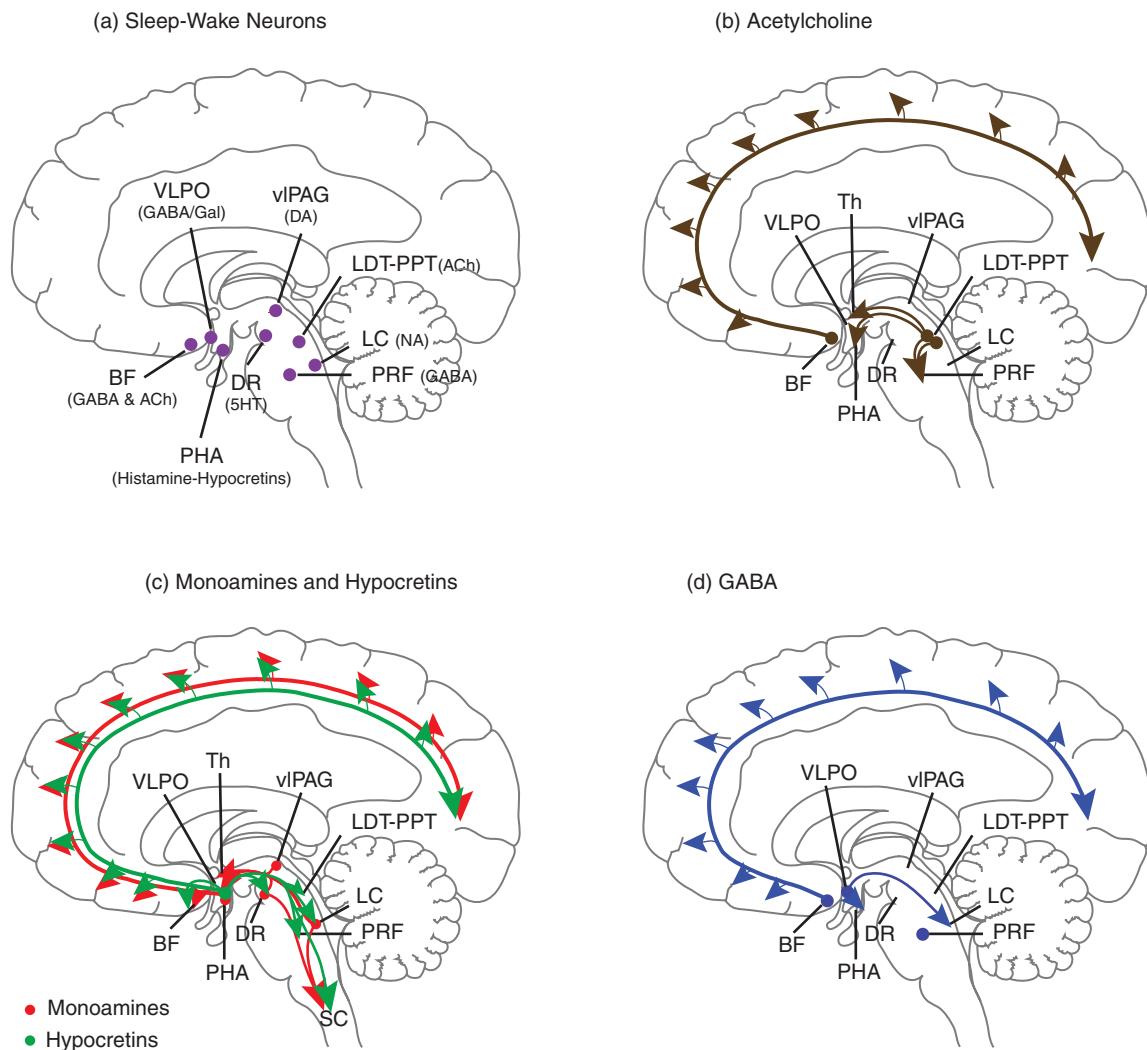


Figure 1 Localization of the major neuronal groups and pathways regulating sleep and wakefulness on a drawing of the human brain. (a) shows the major sleep–wake related neurons and their neurotransmitters. (b) indicates cholinergic projections from the basal forebrain and the brainstem. (c) schematizes the monoaminergic and hypocretinergic wakefulness-promoting neurons and their projections. (d) draws the projections from GABAergic neurons in the basal forebrain and preoptic area. ACh, acetylcholine; BF, basal forebrain; DR, dorsal raphe nucleus; DA, dopamine; Gal, galanin; LC, locus coeruleus; LDT-PPT, laterodorsal and pedunculopontine tegmental nucleus; NA, noradrenaline; PHA, posterior hypothalamic area; SC, spinal cord; 5-HT, serotonin; Th, thalamus; vIPAG, ventrolateral periaqueductal gray; VLPO, ventrolateral preoptic area. We thank Dr. Giancarlo Vanini for contributing this figure.

Although ACh release during sleep and wakefulness differs according to brain region, one consistent finding is that in all of the brain regions analyzed, ACh levels are greatest when the EEG is activated, such as during REM sleep and wakefulness. During NREM sleep when the EEG is deactivated, ACh release is at its lowest (**Table 1**).

ACh exerts its effects by activating two major classes of receptors called nicotinic and muscarinic. Nicotinic receptors are ligand-gated ion channels and exist as 17 subtypes. Activating nicotinic receptors in the thalamus contributes to cortical activation by suppressing slow-

wave activity characteristic of NREM sleep. There are five muscarinic receptor subtypes. M₁, M₃, and M₅ receptors are coupled to excitatory G proteins, whereas M₂ and M₄ receptors are coupled to inhibitory G proteins. Acetylcholinesterase hydrolyzes ACh to choline and acetic acid. Nerve gases inhibit acetylcholinesterase to increase ACh levels, which leads to muscle paralysis and death.

Additional insights concerning the endogenous neurotransmitter regulating sleep have come from administering drugs into specific brain regions of experimental animals while recording sleep and wakefulness.

Table 1 State-dependent changes in acetylcholine release vary according to brain region

Brain region	Acetylcholine release	Species	Reference
Cerebral cortex	Wake = REM > NREM	Cat	<i>Science</i> 192: 161–162, 1971
DTF	REM > Wake = NREM	Cat	<i>Neuroscience Letters</i> 114: 277–282, 1990
Caudate nucleus	Wake = REM > NREM	Cat	<i>ibid</i>
NMC	Wake = REM > NREM	Cat	<i>Brain Research</i> 580: 348–350, 1992
NPM	REM > Wake = NREM	Cat	<i>ibid</i>
Thalamus	Wake = REM > NREM	Rat	<i>Journal of Neuroscience</i> 14: 5236–5242, 1994
Cerebral cortex	Wake \geq REM > NREM	Cat	<i>Brain Research</i> 671: 329–332, 1995
Hippocampus	REM > Wake > NREM	Cat	<i>ibid</i>
PRF	REM > Wake = NREM	Cat	<i>Journal of Neuroscience</i> 17: 774–785, 1997
Basal forebrain	REM > Wake > NREM	Cat	<i>American Journal of Physiology. Regulatory, Integrative and Comparative Physiology</i> 280: 598–601, 2001

DTF, dorsal tegmental field, part of the pontine reticular formation; NPM, nucleus paramedianus, part of the medullary reticular formation; NMC, nucleus magnocellularis, part of the medullary reticular formation; PRF, pontine reticular formation.

Delivering drugs into the brain is possible, in part, because the brain has no pain receptors. Thus, sleep can be studied humanely in animals following drug administration into specific brain regions. These types of studies provide insights into the sleep-related functions of specific brain regions and receptor subtypes localized within those brain regions. For example, in the pontine reticular formation, microinjection of drugs that mimic the effects of ACh causes an increase in REM sleep. These data fit well with the observation that ACh release in the pontine reticular formation is increased during REM sleep (Table 1). In the pontine reticular formation, drugs that are effective for increasing REM sleep include those that inhibit the breakdown of endogenous ACh and drugs that activate M2 muscarinic cholinergic receptors. Studies in humans have shown that administering low doses of acetylcholinesterase inhibitors also causes an increase in REM sleep. Taken together, these data support the interpretation that the cholinergic transmission in the pontine reticular formation contributes to the generation of REM sleep.

Serotonin

Serotonin is a monoamine neurotransmitter that contributes to the regulation of appetite, emotion, vascular smooth muscle tone, and wakefulness. Serotonin is

synthesized by the raphé nuclei of the brainstem and these nuclei project widely to the rest of the brain. The dorsal raphé nucleus has been most thoroughly studied with respect to sleep and wakefulness. The firing rate of serotonergic dorsal raphé neurons is fastest during wakefulness, slower during NREM sleep, and slowest during REM sleep, suggesting that serotonin promotes wakefulness and is inhibitory to REM sleep. Fourteen serotonin receptor subtypes have been identified, and are expressed throughout the brain and periphery. Clinically, serotonin receptor agonists are used for treating migraine, headache, and preventing nausea. Serotonin reuptake inhibitors, which block uptake of serotonin from the synapse to increase serotonin levels, are used to treat depression. A consistent finding across most brain regions studied to date is that serotonin levels are highest during wakefulness, lower during NREM sleep, and lowest during REM sleep (Table 2). These measures of serotonin release support the interpretation that high serotonin levels are important for wakefulness, and that a decrease in serotonin release promotes sleep.

Norepinephrine

Norepinephrine promotes wakefulness and is an important mediator of autonomic function and muscle tone.

Table 2 Serotonin release is greater during wakefulness than during sleep, independent of brain region

Brain region	Serotonin release	Species	Reference
PRF	Wake > NREM > REM	Cat	<i>Neuroscience Research</i> 18: 157–170, 1993
Dorsal raphé	Wake > NREM > REM	Rat	<i>Neuroscience</i> 83: 807–814, 1998
Frontal cortex	Wake > NREM > REM	Rat	<i>ibid</i>
Dorsal raphé	Wake > NREM > REM	Cat	<i>Brain Research</i> 648: 306–312, 1994
PPT	Wake > NREM > REM	Cat	<i>Sleep Research Online</i> 2: 21–27, 1999
Amygdala	Wake > NREM = REM	Cat	<i>Brain Research</i> 860: 181–189, 2000
Locus coeruleus	Wake > NREM > REM	Cat	<i>ibid</i>
Hippocampus	Wake > NREM > REM	Rat	<i>European Journal of Neuroscience</i> 17: 1896–1906, 2003

PRF, pontine reticular formation; PPT, pedunculopontine tegmental nucleus.

Neurons that synthesize norepinephrine are concentrated in the brainstem nucleus called the locus coeruleus. These neurons project rostrally to the forebrain and cerebral cortex, as well as caudally to the brainstem and spinal cord. Similar to the serotonergic neurons described above, noradrenergic neurons fire at their fastest rates during wakefulness, slow their discharge during NREM sleep, and cease firing during REM sleep. This ‘wake-on/REM-off’ discharge pattern is unique to monoaminergic neurons and is consistent with the wakefulness-promoting role of norepinephrine. As a result of the unique state-dependent discharge pattern, norepinephrine release is higher during wakefulness than during sleep in all brain regions studied (**Table 3**).

Clinically, norepinephrine has many uses including the treatment of shock because it acts as a vasoconstrictor. Norepinephrine binds to and activates α and β receptors. There are two α noradrenergic receptors, $\alpha 1$ and $\alpha 2$. The $\alpha 2$ receptors function as autoreceptors to regulate the release of norepinephrine. The $\alpha 2$ receptor agonist dexmedetomidine is used frequently in the intensive care unit setting to provide sedation. Xylazine is an $\alpha 2$ receptor agonist used clinically in veterinary practice for sedation. Preclinical studies have shown that dexmedetomidine inhibits the firing of wakefulness-promoting locus coeruleus neurons.

Histamine

Histamine is a monoamine synthesized in the tubero-mammillary nucleus of the posterior hypothalamus. These neurons send projections throughout the brain, including the sleep-promoting anterior hypothalamus, the arousal-promoting LDT/PPT, and the neocortex. Similar to the other monoamines serotonin and norepinephrine, histaminergic neurons fire with a wake-on/REM-off discharge pattern and function to promote

wakefulness. Histamine also modulates the immune system and gastric acid secretion. Histamine is synthesized by the enzyme histidine decarboxylase. Knock-out mice lacking the gene responsible for histamine synthesis show slow frequencies in the EEG during wakefulness, suggesting that they are less alert than control mice. In addition, mice that cannot synthesize histamine do not show the normal increased wakefulness in response to a novel environment.

There are three known subtypes of histamine receptors, H1–H3, which are coupled to G proteins. H1 and H2 receptor antagonists are used clinically to block allergic responses and gastric acid secretions, respectively. First-generation antihistamines cause drowsiness, and H1-receptor knock-out mice show a decreased waking response to the arousal-promoting peptide hypocretin (orexin). Less is known about H3-receptor subtype function except that it is an autoreceptor and modulates the release of histamine. Histamine release has only been studied in a few brain regions during sleep and is highest during wakefulness, similar to serotonin and norepinephrine (**Table 4**).

Inhibiting tubero-mammillary neurons with the GABA_A receptor agonist muscimol causes a decrease in wakefulness and an increase in sleep, consistent with a wakefulness-promoting role for histamine and a sleep-promoting role for GABA. Microinjection of an H1-receptor agonist into the LDT also causes an increase in wakefulness, as does administering histamine into the basal forebrain. Consistent with these findings are data showing that increasing histamine levels in the tubero-mammillary nucleus by blocking the enzymatic degradation of histamine also increases wakefulness and decreases both NREM sleep and REM sleep. In general, the wakefulness-promoting effects of histamine are mediated by the H1 receptor, whereas increases in sleep are produced by drugs that activate H2 or H3 receptors.

Table 3 Norepinephrine release is greater during wakefulness than during sleep, independent of brain region

Brain region	Norepinephrine release	Species	Reference
Locus coeruleus	Wake > NREM > REM	Cat	<i>Brain Research</i> 860: 181–189, 2000
Amygdala	Wake > NREM > REM	Cat, rat	<i>Brain Research</i> 860: 181–189, 2000; <i>Journal of Korean Medical Sciences</i> 17: 395–399, 2002
Nucleus accumbens	Wake > NREM = REM	Rat	<i>Journal of Neuroscience Research</i> 81: 891–899, 2005
Prefrontal cortex	Wake > NREM = REM	Rat	<i>ibid</i>

Table 4 Histamine release is greater during wakefulness than during sleep

Brain region	Histamine release	Species	Reference
Preoptic hypothalamic area	Wake > REM > NREM	Cat	<i>Neuroscience</i> 114: 663–670, 2002
Prefrontal cortex	Wake > Sleep	Rat	<i>Neuroscience Research</i> 49: 417–420, 2004

Dopamine

Dopamine is the precursor of norepinephrine and mediates mood, motor activity, addiction, and the secretion of prolactin. The D1 family (D1, D5) of dopamine receptors is coupled to stimulatory G proteins to activate adenylcyclase. The D2 family (D2, D3, D4) of dopamine receptors is coupled to inhibitory G proteins that inhibit adenylcyclase. Similar to norepinephrine, dopamine is used clinically to treat shock. Dopamine neurons of the substantia nigra selectively degenerate in Parkinson's disease, which is characterized by excessive daytime sleepiness and disrupted nighttime sleep. Dopamine-receptor agonists are widely used to treat Parkinson's disease. The firing rates of dopaminergic neurons in the ventral tegmental area and substantia pars compacta do not vary across sleep–wake states. However, dopamine levels do vary across sleep–wake states in the frontal cortex, nucleus accumbens, and spinal cord (Table 5). The locus coeruleus does not appear to be a target region for dopaminergic regulation of arousal state as dopamine levels do not show state-specific changes.

Hypocretins

Hypocretins (also known as orexins) are neuropeptides discovered by separate groups in 1998 and play diverse roles in physiology by regulating arousal, feeding, nociception, energy homeostasis, neuroendocrine, and cardiovascular functions. The two subtypes of hypocretins, hypocretin-1 (orexin A) and hypocretin-2 (orexin B) are synthesized in the lateral, posterior, and perifornical hypothalamus and project widely throughout the brain. There are two receptor subtypes, hypocretin receptor-1 and hypocretin receptor-2. Hypocretin receptor-2 binds both peptides equally, whereas hypocretin receptor-1 is selective for hypocretin-1. Hypocretin deficiency underlies the sleep disorder narcolepsy, which is characterized

by excessive daytime sleepiness and disrupted nighttime sleep, and can be accompanied by cataplexy, or the sudden loss of muscle tone.

One normal, physiological role of the hypocretins is the promotion of wakefulness. Hypocretin levels are greater in the hypothalamus and the basal forebrain during wakefulness and REM sleep (when the EEG is activated) when compared to NREM sleep (Table 6). These data fit nicely with the increase in firing rate of basal forebrain neurons observed during wakefulness and the increase in activity seen from PET imaging in the basal forebrain. Hypocretinergic neurons in the lateral hypothalamic area also increase firing rate during wakefulness. Interestingly, hypocretin levels in locus coeruleus (wakefulness-promoting region) do not vary across sleep–wake states even though this is where the greatest number of hypocretin-1 receptors are located. Hypocretin levels are greater during wakefulness than during sleep in the lateral hypothalamus and medial thalamus (Table 6).

Microdialysis studies suggest a causal relationship between neuromodulator levels and states of wakefulness, NREM sleep, and REM sleep. Pharmacological approaches further describe the sleep-related roles of these sleep modulators in different brain regions. Understanding the interactions between sleep modulatory neurochemical systems in different brain regions as well as deciphering the effects of specific neurotransmitter receptor subtype on sleep–wake states will pave the way for better pharmacological therapeutics for disorders of sleep and mental health.

GABA

GABA is the major inhibitory neurotransmitter in the brain and is synthesized from glutamate. Unlike the neurotransmitters discussed above, GABA is released from glial cells as well as from neurons. These glial cells, or

Table 5 State-dependent changes in dopamine release vary according to brain region

Brain region	Dopamine release	Species	Reference
Locus coeruleus	Wake = NREM = REM	Cat	<i>Brain Research</i> 860: 181–189, 2000
Frontal cortex	Wake > NREM = REM	Rat	<i>Journal of Neuroscience Research</i> 81: 891–899, 2005
Nucleus accumbens	Wake = REM > NREM	Rat	<i>ibid</i>
Spinal cord	Wake = NREM > REM	Cat	<i>Journal of Neurophysiology</i> 100: 598–608, 2008

Table 6 Hypocretin release is lowest during NREM sleep

Brain region	Hypocretin/orexin levels	Species	Reference
Lateral hypothalamus	Wake > Sleep	Rat	<i>European Journal of Neuroscience</i> 14: 1075–1081, 2001
Hypothalamus	Wake = REM > NREM	Cat	<i>Journal of Neuroscience</i> 22: 5282–5286, 2002
Basal forebrain	Wake = REM > NREM	Cat	<i>ibid</i>
Locus coeruleus	Wake = NREM = REM	Cat	<i>ibid</i>

astrocytes, are in close proximity to synapses and modulate synaptic transmission. Furthermore, GABA transporters reside on both neurons and astrocytes; so GABA is taken up into astrocytes.

GABA binds to three types of receptors, GABA_A, GABA_B, and GABA_C. GABA_A and GABA_C receptors are ligand-gated ion channels and GABA_B is a G protein-coupled receptor. The GABA_A receptor has binding sites for a variety of molecules that produce sedation, sleep, or general anesthesia. Benzodiazepines also act via the GABA_A receptor to reduce anxiety. Almost all brain regions contain GABAergic interneurons. The basal forebrain also contains GABAergic projection neurons, which provide input to the cerebral cortex. GABA levels in the cortex during sleep and wakefulness have not been measured. GABAergic neurons in the anterior hypothalamus send projections to the wakefulness-promoting monoaminergic neurons in the posterior hypothalamus, locus coeruleus, and dorsal raphe, as well as the cholinergic LDT/PPT neurons. GABA levels in the dorsal raphe, locus coeruleus, and posterior hypothalamus are higher during sleep than during wakefulness (Table 7). GABA levels in the LDT/PPT have not been measured during sleep and wakefulness, but GABAergic interneurons in the LDT/PPT are active during REM sleep. The GABA_A-receptor agonist muscimol causes sleep when microinjected directly into the posterior hypothalamus, but causes wakefulness when delivered directly into the anterior hypothalamus. These findings are consistent with the interpretation that GABA in these brain areas contributes to sleep generation by inhibiting wakefulness-promoting neurons.

GABA levels in the pontine reticular formation are greater during wakefulness than during NREM sleep, REM sleep, or general anesthesia. These data indicate that GABA in the pontine reticular formation contributes to the generation of wakefulness. A wakefulness-promoting role for GABA is also supported by data showing that wakefulness is either increased or decreased by drugs that either increase or decrease, respectively, pontine reticular formation GABAergic transmission. GABA and ACh interact in the pontine reticular formation to regulate REM sleep. Activation of GABA_A receptors in the pontine reticular formation decreases REM sleep, in part, by decreasing ACh release. Similarly, blocking GABA_A

receptors in the pontine reticular formation increases ACh release and increases REM sleep. In summary, GABAergic inhibition either inhibits or promotes wakefulness, depending upon the brain region. In brain regions that promote wakefulness, GABAergic inhibition functions to increase sleep. In brain regions that promote sleep, GABAergic inhibition contributes to an increase in wakefulness.

Glutamate

Glutamate is the major excitatory neurotransmitter in the brain and is the precursor to GABA. Similar to GABA, glutamate is synthesized and released from neurons and glial cells. Glutamate exhibits its actions by activating ionotropic NMDA, AMPA, or kainate receptors and metabotropic glutamatergic receptors. Little is known about the role of glutamate in sleep-cycle control. Glutamate is excitatory, and in most brain areas, glutamate levels are highest during wakefulness or REM sleep, both of which are activated brain states (Table 8).

The results from studies that have microinjected glutamatergic drugs into the brain and quantified the effects on sleep and wakefulness have shown that glutamate promotes either wakefulness or REM sleep, depending on the brain region where glutamate is administered. Glutamate at lower doses, delivered directly into the PPT, causes REM sleep and at higher doses, causes wakefulness. The increase in REM sleep is mediated by kainate receptors and the increase in wakefulness results from activating NMDA receptors. Furthermore, activating the metabotropic glutamate subtype II receptor decreases wakefulness and increases NREM sleep. These data demonstrate that endogenous glutamate is an important regulator of arousal and suggest that in the PPT, activation of kainate and NMDA receptors is important for inducing sleep whereas activating metabotropic subtype II receptors is important for inducing wakefulness. Administration of glutamate to the dorsal raphe or basal forebrain increases wakefulness and decreases sleep.

Ketamine is an NMDA-receptor agonist and is used clinically to produce a state of dissociative anesthesia. The term dissociative arose from the finding that when ketamine is used for surgical procedures, sensory

Table 7 State-dependent changes in GABA levels

Brain region	GABA levels	Species	Reference
Dorsal raphe	REM > Wake = NREM	Cat	<i>American Journal of Physiology</i> 273: R451–R455, 1997
Locus coeruleus	REM > NREM > Wake	Cat	<i>Neuroscience</i> 78: 795–801, 1997
Posterior hypothalamus	NREM > Wake = REM	Cat	<i>American Journal of Physiology. Regulatory, Integrative and Comparative Physiology</i> 271: 1707–1712, 1996
Spinal cord	REM > Wake = NREM	Cat	<i>Journal of Neurophysiology</i> 100: 598–608, 2008

Table 8 Glutamate levels vary across the sleep–wakefulness cycle in a brain-region-specific manner

Brain region	Glutamate levels	Species	Reference
NMC	REM > NREM = Wake	Cat	<i>Brain Research</i> 780: 178–181, 1998
NPM	Wake = NREM = REM	Cat	<i>ibid</i>
NAcc	Wake > NREM = REM	Rat	<i>Journal of Neuroscience Research</i> 81: 891–899, 2005
Prefrontal cortex	Wake = NREM = REM	Rat	<i>ibid</i>
oFC	REM > Wake > NREM	Rat	<i>Archives of Medical Research</i> 38: 52–55, 2007
Spinal cord	REM > NREM = Wake	Cat	<i>Journal of Neurophysiology</i> 100: 598–608, 2008
PH/TMN	Wake = REM > NREM	Rat	<i>American Journal of Physiology. Regulatory, Integrative and Comparative Physiology</i> 295: R2041–R2049, 2008

NPM, nucleus paramedianus; NMC, nucleus magnocellularis; NAcc, nucleus accumbens; oFC, orbital frontal cortex; TMN, tuberomamillary nucleus.

information reaches the cortex but is not accurately perceived as painful, thus causing a dissociation between objective and subjective experience. When given intravenously or directly into the pontine reticular formation, ketamine suppresses REM sleep and decreases the release of the REM sleep-promoting transmitter ACh.

Adenosine

Adenosine is a purine nucleoside that is derived from the breakdown of ATP, and adenosine has many physiological functions. The function of adenosine most relevant for this volume is as an endogenous promoter of sleep. There are four adenosine receptor subtypes, A1, A2a, A2b, and A3, which are G protein-coupled receptors. A1 and A3 receptors inhibit adenylyl cyclase activity whereas A2a and A2b receptors activate adenylyl cyclase. One of the most widely used legal drugs in the world is caffeine, which promotes wakefulness and increases alertness by blocking adenosine receptors. Adenosine levels in the brain increase during wakefulness and decrease during sleep (**Table 9**).

Adenosine levels in different brain regions have been quantified during sleep deprivation and subsequent recovery sleep. These studies show that changes in adenosine levels during sleep deprivation and recovery sleep were different depending on the brain region analyzed. For example, adenosine levels in the cortex and basal forebrain accumulate during prolonged wakefulness and fall during recovery sleep. In contrast, adenosine levels do

not increase in the dorsal raphe or pedunculopontine tegmental nucleus during sleep deprivation. Furthermore, adenosine acting on A1 receptors inhibits wakefulness-promoting neurons in the basal forebrain. Adenosine also inhibits wakefulness-promoting histaminergic and hypocretinergic neurons in the posterior and lateral hypothalamus, and increases the activity of anterior hypothalamic GABAergic neurons that promote sleep.

Pharmacological approaches have provided novel evidence that adenosine is sleep promoting. Adenosine A1-receptor antagonists, delivered directly into the pontine reticular formation or the prefrontal cortex, shorten recovery time from general anesthesia, whereas A1 receptor agonists increase anesthesia-recovery time. Administering adenosine into the lateral dorsal tegmental nucleus decreases wakefulness and increases sleep. Adenosine A1-receptor activation in the pontine reticular formation increases REM sleep and decreases wakefulness. Microinjection of A2a agonist into the pontine reticular formation increases ACh release and increases REM sleep. Taken together, current data demonstrate that although adenosine levels are highest during wakefulness (**Table 9**), adenosine is a sleep-promoting agent.

Concluding Remarks

Sleep is important for many physiological functions, including the immune system, cognition, learning and

Table 9 Adenosine levels increase during wakefulness and decrease during sleep

Brain region	Adenosine levels	Species	Reference
Basal forebrain	Wake > NREM = REM	Cat	<i>Neuroscience</i> 99: 507–517, 2000
Cortex	Wake > NREM = REM	Cat	<i>ibid</i>
Thalamus	Wake > NREM = REM	Cat	<i>ibid</i>
Preoptic area	Wake > NREM = REM	Cat	<i>ibid</i>
Dorsal raphe	Wake = NREM = REM	Cat	<i>ibid</i>
PPT	Wake = NREM = REM	Cat	<i>ibid</i>

PPT, pedunculopontine tegmental nucleus.

memory, and mental health. As of 2006, over 70 million Americans suffer from sleep-related disorders, costing an estimated 15.9 billion dollars. The elderly population is increasing, and the incidence of sleep disorders increases with aging. Understanding how sleep is regulated by neurotransmitters and neuromodulators, and discerning how these transmitter and modulator system interact will improve the medications prescribed for sleep disorders. In 2008, a study demonstrated that the inverse histamine-3 receptor agonist tiptolosant reduces excessive daytime sleepiness in narcoleptic patients and in hypocretin-deficient mice. Modafinil is another drug used to reduce excessive daytime sleepiness, and although the mechanism of action is not well understood, modafinil acts through multiple neurotransmitter systems. Olanzapine is an atypical antipsychotic that improves sleep in schizophrenics and acts at several neurotransmitter receptors. Future studies will need to focus more on elucidating interactions between multiple neurotransmitter systems and on interacting sleep-related brain regions.

See also: Circadian and Ultradian Clocks/Rhythms; Conscious and the Unconscious; Neuropsychology of Sleep; Sleep Genetics; Sleep: Learning and Memory; Sleep: Medical Disorders; Sleeping, Waking, and Dreaming.

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Plasticity in the Primary Auditory Cortex: Substrate of Specific Long-Term Memory Traces

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Glossary

Best frequency (BF) – The frequency that produces the best cellular response to a frequency at a given sound-level intensity.

Conditioned response (CR) – A behavioral response that is elicited by a conditioned stimulus as a result of classical or instrumental conditioning.

Conditioned stimulus (CS) – An initially neutral stimulus that elicits a behavioral response after it has been associated with an unconditioned stimulus.

Frequency receptive field (frequency RF) – The portion of the acoustic spectrum to which a cell in the auditory system is sensitive. Frequency RFs are often called ‘tuning curves.’

Local field potentials (LFPs) – Responses of sensory (auditory) cortex to sensory (acoustic) stimuli, which are comprised of evoked voltage changes in the low-frequency range (1–300 Hz). They generally index excitatory postsynaptic potentials recorded in extracellular space with a large electrode on the surface or in the depths of the cortex.

Tonotopic map – The progressive distribution of best frequencies across primary auditory cortex. The posterior-to-anterior axis has a systematic range of BFs, from lower to higher, respectively.

Unconditioned response (UR) – An innate behavioral response that is elicited by a stimulus in the absence of conditioning.

Unconditioned stimulus (US) – A stimulus that elicits a UR.

represented and stored has languished. The two domains of neglect are related, because sensory systems, particularly sensory cortex, appear to be sites of the specific storage of experiences. They are not the only sites of storage, but they provide a particularly advantageous means of locating storage sites and they have now been successfully exploited. The primary auditory cortex (A1) is perhaps that sensory region for which research has yielded the greatest dividends.

This article concerning learning-based plasticity in A1. It will summarize the evidence for specific long-term memory traces. But in so doing, it will more broadly examine the usually implicit assumptions about brain-behavior organization, concluding that the schema, which has served behavioral neuroscience since its inception, is no longer valid.

The Dominant Model of Brain-Behavior Relationships

Scientific study of brain-behavior relationships may be traced back to a sensory-motor conception of the nervous system in the early nineteenth century. Bell and Magendie separately discovered (*c.* 1812–30) that the dorsal roots of the spinal cord are sensory, while the ventral roots are motor. These seminal findings provided the first brain-behavior, structural-functional organizational principle. Much of the research program for the rest of the century concerned the extent to which the entire neuraxis was organized on sensory-motor principles. The last 30 years of the nineteenth century witnessed the discovery of the motor cortex, and the approximate delineation of sensory cortices based on modality-specific sensory deficits following cortical ablations.

Still, sensory and motor areas did not comprise the entire neocortex. Could an overarching principle of cortical organization be discovered? In 1901, Fleischsig reported that sensory and motor cortices exhibited myelination at birth, while other areas could require as long as one postnatal month to myelinate. Further, his observations of fibers in the internal capsule led him to the erroneous conclusion that only the sensory and motor cortices received subcortical projections; the association areas were thought to receive inputs only from other cortical regions. Thus, Fleischsig provided an anatomical

Introduction

The search for the neural substrates of learning and memory has been extremely biased in at least two dimensions: brain structures under investigation and aspects of memory under consideration. With regard to functional anatomy, most attention has been focused on structures such as the hippocampus, amygdala, striatum, cerebellum, frontal, and the so-called association fields of the cerebral cortex. In contrast, the sensory systems have been relatively ignored. Concerning memory itself, interest has been focused on the processes that enable memory storage while inquiry into how the contents of memory are

basis for the distinction between lower (i.e., sensory-motor) and higher psychological functions.

It remained only to specify in greater detail the nature of these lower and higher functions. This was supplied in the anatomical studies of AW Campbell. Purely on the basis of cytoarchitectonics (i.e., study of the fine structure of the cortex), Campbell divided sensory cortical fields into two types, such as primary visual cortex (V1) ‘visual sensory’, and nearby regions (e.g., areas 17 and 18) ‘visual psychic’. Similarly, the region now known as A1 was termed ‘auditory sensory’ while adjacent areas, in modern parlance, auditory belt areas, were ‘auditory psychic.’ In so doing, Campbell executed an almost metaphysical leap that has plagued brain-behavior research to the present day, because he cleaved sensory processes from cognition. Subsequent neurophysiological studies of sensory cortices operated within this conceptual constraint, so that the analysis of sounds, sights, and touches was assumed to occur in the primary sensory fields, while interpretation of the behavioral meaning or comprehension of the external world was assigned to nonprimary sensory areas, that is, to the psychic regions. A major implication of this schema was removing learning and memory from primary sensory cortices.

Once learning, memory, and other cognitive processes were removed from the primary sensory regions, without any experimental support, the dominant brain-behavior paradigm was essentially in place. So imbued into neuroscience as to have gone largely unquestioned, it holds a three-stage processing chain: sensory–association–motor. In short, primary sensory cortex analyzes stimuli, association and related cortex comprehend the stimuli, in large part based on past experiences, and send the results to the motor cortex which is responsible for actual behavior.

Note that this S–A–R theory comprises a reflex model of brain-behavior substrates. In reality, this is an extended model of the functional operation of the spinal cord. As this model still dominates neuroscientific thinking, it would seem that we have not achieved fundamental conceptual advances. This would not be a problem if the S–A–R model was adequate. That it cannot account for the role of A1 in learning and memory will soon become apparent.

In closing this section, we would do well to not look at Pavlovian conditioning as a willing accomplice of this reflex-based model. Pavlov himself emphasized the association between two stimuli, for example, the conditioned and unconditioned stimuli (CS and US, respectively). Such CS–US learning (i.e., stimulus–stimulus, S–S) contrasts with stimulus–response learning (S–R) that has come to dominate much thinking. S–R conditioning is, of course S–A–R in reality, for no one holds that Pavlovian conditioning consists of a monosynaptic connection between the CS and the conditioned response (CR). While both S–S and S–R learning certainly occur,

the former appears to develop within a few trials, before the appearance of specific motor CRs. Indeed, conditioning seems to be at least a two-stage process, in which CS–US (S–S) associations first enable animals to predict the future, followed by the formation of CS–CR (S–R) links that enable an animal to take overt behavioral action based on this knowledge. Certainly, S–R conditioning is of great importance and its circuitry has been well delineated, to an extent unmatched for other forms of learning. However, S–S conditioning remains of central importance. Contemporary understanding of Pavlovian conditioning views it as a general process for determining the ‘causal fabric’ of the environment, a general-purpose mechanism for making the best possible predictions in the service of adaptive behavior. Only when Pavlovian conditioning is narrowly construed as S–R, and labeled ‘procedural learning’ in contradistinction to ‘declarative learning’, can one conclude that it supports the S–A–R model. Unfortunately, such a construal is all too popular and grows more so as workers minimally versed in these matters attack the neurobiology of learning and memory.

Learning, Memory, Plasticity, and the Auditory Cortex: The Foundational Period

Introduction

Vast majority of investigations into the neurophysiology of learning and memory and A1 have studied associative learning, in the form of either Pavlovian (classical conditioning) or instrumental conditioning. This is not a constraint because the formation of associations is so fundamental. Associative processes can account for a surprisingly wide range of learning, memory, and other cognitive processes, far transcending traditional narrow construes of conditioning. Studies during the foundational period, roughly 1956–85, focused on the question of whether associative learning involved (e.g., was accompanied by) associative plasticity in A1.

Conditioning

Galambos and colleagues published the first Western study on Pavlovian conditioning and plasticity in A1. Cats received an auditory (click) CS paired with an immediately following puff of air to the face (US), resulting in significantly larger local field potentials (LFPs) to the CS click. The authors validated learning by noting the development of behavioral CRs. Furthermore, they also controlled for CS constancy at the ear by obtaining the same findings with subjects under neuromuscular blockade, thus preventing head and pinna movements and possible contractions of the middle ear muscles that can alter effective sound levels at the tympanic membrane. Interestingly, the authors failed to include a

nonassociative control, such as a group that received the CS and US unpaired. However, subsequent investigations did include proper controls, confirming the associative nature of increased response magnitude of LFPs in A1. LFP research was extended to different conditioning tasks and Pavlovian processes, with essentially the same findings of enhanced responses to sounds that became behaviorally important. Similar results were obtained for instrumental avoidance learning.

In addition to the use of LFPs, workers began recording cellular discharges in A1. Most studies recorded from clusters of cells (multiple-unit activity). The results generally were the same, that is, increased discharges to an acoustic CS as animals formed CRs. This increase in response was fairly consistent, both in simple (one-tone) conditioning and in two-tone discrimination training. Reversal was also obtained when the CS⁺ and CS⁻ acoustic stimuli were interchanged after initial learning. Moreover, acoustic CS⁺ stimuli acquired the ability to elicit responses in the sensory cortex of the shock US, that is, primary somatosensory cortex.

Such cluster recordings have the advantage over single-unit recordings of yielding good data over many hours or days. They have the disadvantage of being unable to determine if single cells develop different directions of plasticity, that is, either increased or decreased responses. In other words, if associative processes produce both increased and decreased responses to the CS, but increased responses dominate, then the decreased responses would not be detected.

Study of single units in auditory cortex during learning also found plasticity. However, as suspected, despite the detection of many cells that developed increased responses to the CS, a substantial number developed decreased responses, and yet others exhibited no change. Similar heterogeneity of unit discharge plasticity was also found in secondary auditory cortex (A2). Such divergent results were not attributable to inadvertent changes in effective acoustic stimulus level in the auditory periphery, undetected movements, muscle contractions, or feedback from muscle spindles, because the same results were obtained when animals were trained while under neuromuscular blockade.

Although single-unit plasticity was shown to be associative, the findings of opposite sign made little functional sense. Thus, while recording in A1 during training trials had provided fundamental information, this approach appeared to be yielding diminishing returns after almost 30 years of research. Nonetheless, research during the foundational period had sufficiently answered the original question in the affirmative: "Yes, associative learning does involve associative plasticity in the primary auditory cortex." Thus, a major crack in the foundation of the S-A-R model was found. Primary sensory (auditory) cortex does not only analyze stimuli but also changes its response to

the same physical stimuli when they acquire behavioral importance. That is, the responses of the same cells in A1 are governed by at least two factors: physical acoustic parameters of sounds and their acquired meaning. Campbell would not have approved.

Contemporary Approaches: A Synthesis of Two Disciplines

Introduction: Toward Specificity of Plasticity, Memory Content and Memory Codes

The contemporary era may be considered to have been initiated in the 1980s when a new question was posed. Instead of asking whether A1 is involved in learning and memory, focus shifted to the functional significance of associative plasticity. For example, do associative processes change the large-scale functional organization of the auditory cortex? If so, then sensory analysis and learning-induced plasticity do not merely coexist, but rather sensory analysis is shaped by learning processes. Moreover, if learning shapes sensory representations of experience in primary sensory cortex, then it may also store them in primary sensory cortex.

In addressing this previously ignored issue, inquiry transcended associativity to reach the domain of specificity of neuronal plasticity, which is necessary to understand how the brain represents and stores the details of experience, that is, the actual contents of memories. While much research has been devoted to the processes that enable memory and to demonstrations of plasticity in learning, there had been virtually no focus on the information itself that is stored or on the memory codes that transform neural activity into an accurate neural representation of a memory.

For example, learning and memory could have caused increased responses to CS frequencies in either of two ways: first, they could have increased responses not only to the CS but also to most other frequencies; or second, they could have specifically increased responses to CS frequencies but had no effect or even decreased responses to other frequencies. In the latter case, learning could have shifted the tuning of cells to the CS frequency, hence revealing that associative processes actually shape sensory analysis within primary sensory cortex. These alternatives cannot be resolved during training trials because the other test tones either would have to be followed, or not followed, by the US. Both situations would constitute new learning tasks so that one could never find out what happens in simple associative learning. The solution was to determine frequency tuning before and after conditioning. To accomplish this goal, it was necessary to combine two fields that had developed along parallel, seldom-intersecting trajectories, auditory neurophysiology and the neurobiology of learning and memory ([Figure 1](#)).

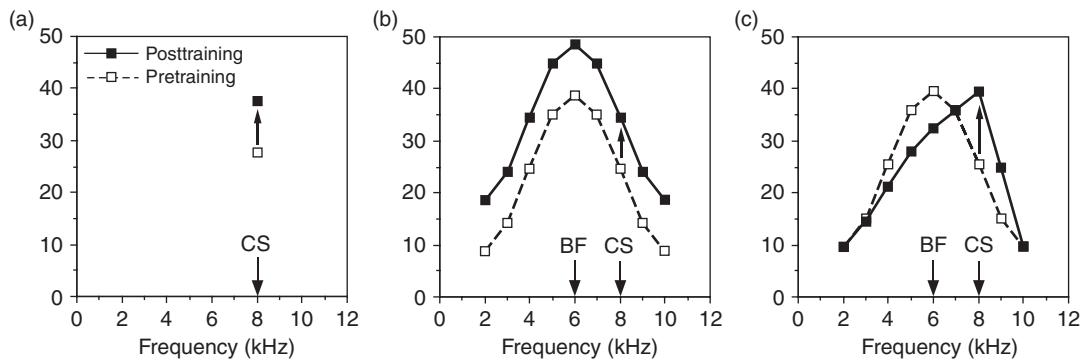


Figure 1 Receptive field analysis reveals whether learning-induced plasticity is general to the dimension of a CS or specific to the value of the CS. (a) During training trials, one can determine whether or not responses to the CS changed; in this case, they increased. However, both general and specific plasticity could produce this result. (b) A general change revealed by receptive field analysis before and after conditioning. (c) A CS-specific instance of associative representational plasticity, in which responses to many non-CS frequencies are reduced, producing a shift in tuning to the frequency of the CS.

Conditioning

Gonzalez-Lima and Scheich used a variant of the new approach. They trained gerbils (tone and aversive stimulation of the mesencephalic reticular formation) and later determined the metabolic response (uptake of 2-deoxyglucose, 2-DG) of the auditory cortex to the CS frequency. Paired animals developed both the behavioral index of learning, that is, conditioned bradycardia, and an increase in the area of A1 with metabolic response to the CS frequency. The absence of similar effects in control groups (CS-US unpaired, CS alone, and US alone) showed that the CS-specific plasticity was associative. These findings indicate that associative processes can enlarge CS-response areas and strongly imply that the underlying neurons have shifted their tuning toward or to the CS frequency within the tonotopic map.

At about the same time, direct evidence of receptive field plasticity was found by obtaining RFs before and after learning. Cats received a single session of tone-shock pairing and behavioral learning was validated by the formation of the pupillary dilation CR. The tuning of single units in two nonprimary auditory fields, A2, and ventral ectosylvian (VE) cortices developed CS-specific plasticity in the paired group but not a control group. Extinction (tone alone) produced a reversal of the RF plasticity, indicating that A1 plasticity reflects the current state of behavioral salience of sound.

Studies then focused on A1 of the guinea pig, with behavioral validation of associative learning, for example, conditioned bradycardia. Following determination of frequency receptive fields, the frequency to be used as the CS was then selected to not be the best frequency (peak of the tuning curve), in order to determine if conditioning caused tuning shifts within the tonotopic map. Animals then received a single session of tone paired with shock. A comparison of posttraining with pretraining RFs revealed increased responses to the CS frequency accompanied by

decreased responses to the pretraining best frequency and many other frequencies. These opposing changes were often sufficiently large to produce frank shifts of tuning toward, and even to, the frequency of the CS, which could become the new best frequency (**Figure 2(a)**). RF plasticity was found to be associative, as it required stimulus pairing; sensitization training produces only a general increase in response to all frequencies across the RF.

CS-specific shaping of frequency tuning also can develop when receptive fields are complex and even nonexistent (i.e., below threshold). **Figure 2(b)** shows an example of a pretraining double-peaked frequency RF. The CS frequency was selected to be in the valley between the peaks. For posttraining, the maximum change was an increase in response at the CS frequency. **Figure 2(c)** illustrates a case in which there was no response to any frequency before conditioning. Nonetheless, postconditioning observations revealed a clear excitatory response to the previously ineffective CS frequency, alone.

Several other attributes of RF plasticity make it an attractive candidate for a process that operates in normal concert with sensory coding processes to subserve the storage of behaviorally relevant auditory information. First, RF plasticity is highly specific to the CS frequency; responses to frequencies a small fraction of an octave away are attenuated. Second, it exhibits generality across different types of training, for example, instrumental avoidance conditioning, two-tone classical discrimination training (i.e., increased responses to the CS⁺ frequency but decreased responses to the CS⁻, BF, and other frequencies), and discriminative instrumental avoidance conditioning. Third, RF plasticity develops very rapidly, after only five training trials, as rapidly as the first behavioral (e.g., cardiac) signs of association. Fourth, RF plasticity exhibits consolidation, that is, continues to develop increased responses to the frequency of the CS

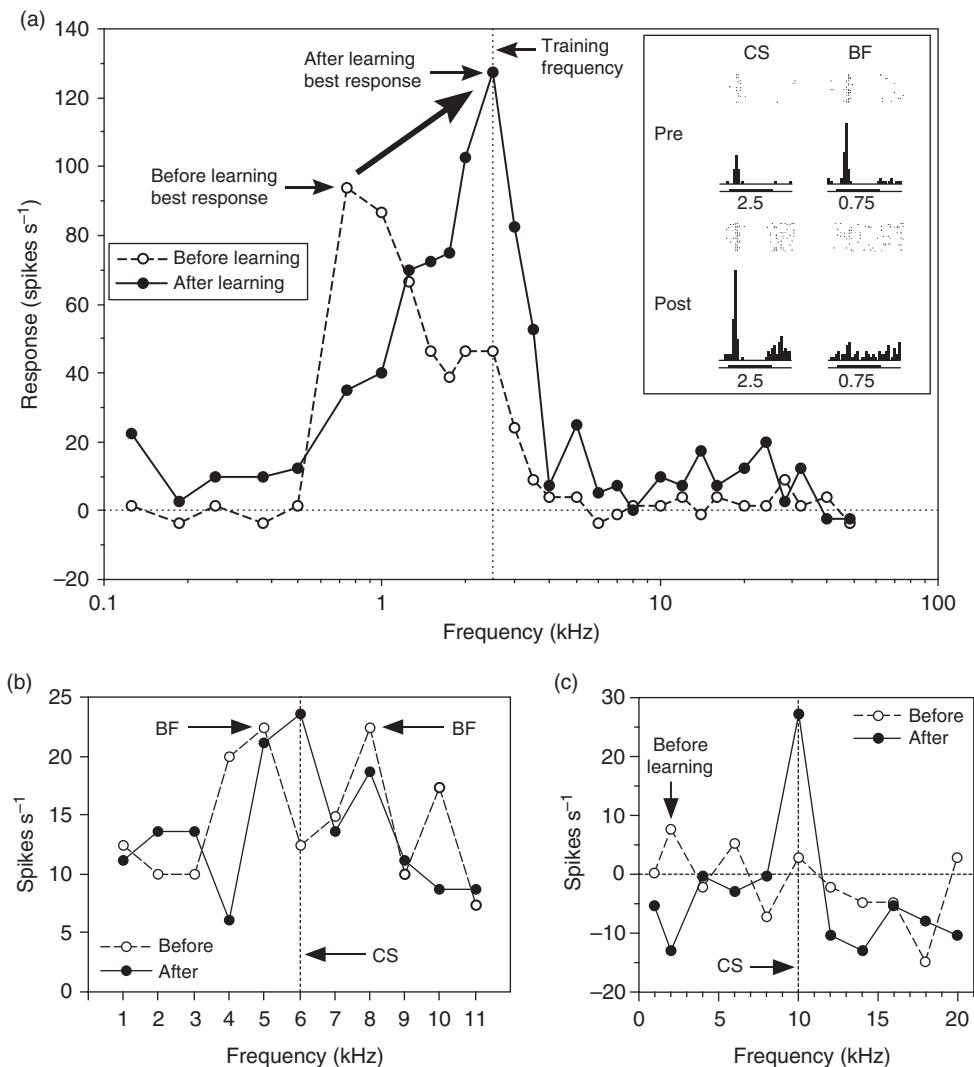


Figure 2 Classical conditioning produces CS-specific facilitation and tuning shifts. (a) An example of a complete shift of frequency tuning of a single cell in A1 of the guinea pig, from a pretraining best frequency (BF) of 0.75 kHz to the CS frequency of 2.5 kHz after 30 trials of conditioning. Inset shows pre- and posttraining poststimulus time histograms (PSTHs) for the pretraining BF and the CS frequencies. (b) Double-peaked tuning, with pretraining BFs at 5.0 and 8.0 kHz. The CS was selected to be 6.0 kHz, a low point. After conditioning (30 trials), responses to the CS frequency increased to become the peak of tuning. (c) A cell that exhibited minimal or no response to tones before tuning developed tuning specifically to the CS frequency after conditioning (30 trials).

versus decreased responses to other frequencies in the absence of further training over hours and days. Fifth, RF plasticity exhibits long-term retention, enduring for the longest periods tested, up to 8 weeks after a single 30-trial conditioning session.

Specific associative RF plasticity is not an artifact of spontaneous changes in tuning because tuning is stable prior to conditioning and shifts are CS directed. Neither is it an artifact of state. Whereas animals exhibit arousal and related responses to sustained (e.g., 2–5 s) CS frequencies during training trials, they do not exhibit any behavioral (e.g., cardiac) responses to the frequency of the CS when it is presented as one of a number of rapidly presented, brief (e.g., 200 ms) sequential tone pips during

RF determination. Moreover, animals trained in the waking state exhibit RF plasticity when tested under deep general anesthesia.

CS-specific associative tuning shifts develop in the A1 of all species studied to date: guinea pig (*Cavia porcellus*), echolocating big brown bat (*Eptesicus fuscus*), cat (*Felis catus*), and rat (*Rattus rattus*). Additionally, CS-directed tuning shifts should increase the representational area of CS frequencies in the tonotopic map of A1. This has been found in instrumental learning in both the owl monkey (*Aotus trivirgatus boliviensis*) and the rat.

Learning-induced tuning plasticity is not limited to animals. The same paradigm of classical conditioning (tone paired with a mildly noxious stimulus) produces

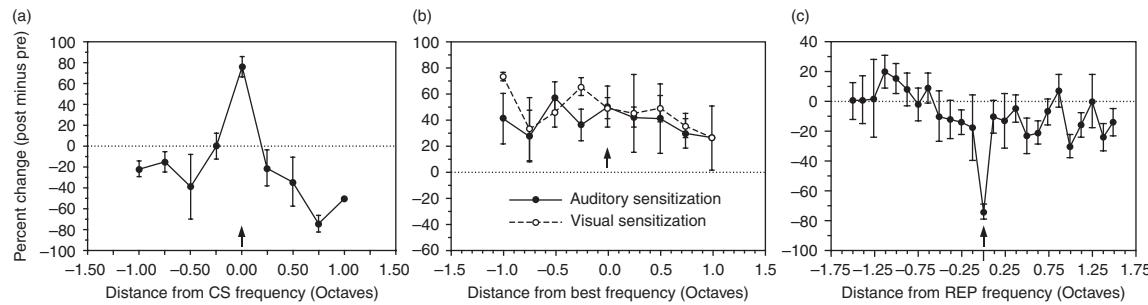


Figure 3 Summary of the effects of (a) conditioning, (b) sensitization, and (c) habituation on frequency receptive fields in the primary auditory cortex (A1) of the guinea pig. Data are normalized to octave distance from (a) the CS frequency, (b) the presensitization best frequency, or (c) the repeated frequency. Note that conditioning produces a CS-specific increased response whereas sensitization (tone-shock or light-shock unpaired) produces general increases across the spectrum. Habituation produces a frequency-specific decreased response.

concordant CS-specific associative changes in A1 of humans (*Homo sapiens*).

In summary, CS-specific RF plasticity has major characteristics of associative memory. It is not only associative, but is also highly specific, discriminative, rapidly acquired, develops consolidation over hours and days, is retained at least for many weeks, and exhibits generality across training tasks, types of motivation, and species. Additionally, when the same tone is repeatedly presented without reinforcement, subjects habituate, that is, learn that the tone does not signal reward or punishment. Habituation produces the opposite effect of associative learning, that is, a frequency-specific decrease in the response of A1 to the repeated sound. **Figure 3** summarizes changes in tuning for conditioning, sensitization, and habituation.

Beyond Specific Plasticity of Acoustic Frequency

Although the plasticity of acoustic frequency has been most extensively studied, specific associative changes in the processing of sound are not confined to this feature of sound. For example, rats were trained in a sound maze in which food reward was contingent upon successful navigation using only auditory cues. In this task, the repetition rate of noise pulses increased as the distance between the rat and target location decreased. After subjects had learned this maze, A1 cells exhibited enhanced responses to high-rate noise pulses and stronger phase locking of responses to the stimuli. In other words, learning produced a shift in tuning to high-repetition rates, that is, the stimulus features that were most closely associated with procurement of food. Similarly, owl monkeys trained to detect an increase in the envelope frequency of a sinusoidally-modulated 1 kHz tone developed increased sensitivity to small changes in envelope frequency. Even the processing of sound intensity (level) is specifically

shifted by associative processes. Rats were trained in a sound maze to move to a place in a small arena guided only by changes in the changing loudness of ongoing sound bursts. A1 responses became selective to relevant sound levels. Moreover, attentional demands can switch plasticity between stimulus parameters, such as frequency and intensity.

These findings are illustrative and we can expect further demonstrations that sensory processing in A1 is shaped by learning. It is likely that most, if not all, acoustic parameters are subject to specific associative plasticity. Moreover, other sensory systems are undoubtedly operating on the same principle: “Behaviorally relevant stimuli receive preferential processing and gain both processing capacity via tuning shifts and representational area, within primary sensory cortices.”

Additional Cognitive-Based Plasticity

The A1 is involved in many other cognitive processes. Space limitations permit only a listing. They include specific neurophysiological correlates of working memory, reference memory, attention, concept formation, and expectancy.

Moreover, cross-modality interactions are prevalent in A1. For example, in monkeys trained in a complex auditory discrimination task, the cue light that signaled trial availability developed the ability to elicit responses in A1. In humans, the sight of speech without sound elicits neural activity in A1. A recent anatomical study in the gerbil may provide an anatomical basis for some cross-modality effects. The authors found a surprisingly large number of inputs to A1 from nonauditory regions of both the cortex and the thalamus.

Perhaps one of the most surprising findings is that learning strategy, rather than the type or amount of learning determines auditory cortical plasticity. Thus, different groups of rats learned to bar press for water reward

contingent on the presence of a tone. Although both groups achieved high levels of correct performance, specific plasticity in A1 (CS-specific decrease in threshold and bandwidth) developed only for subjects that used a strategy based on attention to tone onset rather than tone duration.

Does the Auditory Cortex Form and Hold Specific Long-Term Memory Traces?

A specific long-term memory trace (SMT) is an enduring neural record of a particular aspect of experience. How might one determine if specific RF plasticity does index actual long-term memory traces? One might expect that destruction of A1 should remove its long-term memory traces, which would in turn be revealed by behavioral tests showing a specific loss of auditory memories. This apparently simple and decisive test proves to be neither because A1 is both sensory and mnemonic. Lesions will therefore interfere with both processes, so that any deficits cannot be attributed to either.

If not lesions, then what might be done? One approach is to attempt to defeat the proposal that long-term memory traces form in A1. As A1 does form associative plasticity (above), what line of attack might be taken? It could be argued that in addition to such plasticity, SMTs should possess the major characteristics of behavioral associative memory itself. This would impose a second level of criteria that have not previously been demanded of any neurophysiological studies of learning and memory. Nonetheless, this is not an unreasonable demand.

What are these characteristics? In addition to being associative, SMTs should also exhibit specificity, fairly rapid formation, long-term retention, and even consolidation, that is, continued strengthening over time after training in the absence of additional reinforcement. Another feature of memory is that it can be formed in a wide variety of learning tasks, rather than being confined to, for example, classical conditioning. A further key feature of memory is that it transcends a particular type of motivation, but develops in both appetitive and aversive situations. Additionally, one would expect SMTs to be manifest for whatever the type of CS or signal stimuli used in training, as is the case for genuine associative memory. That is, SMTs should not be limited to plasticity of frequency representation but should develop for any acoustic parameter that can serve as a signal for reward or punishment. Finally, as in the case of memory, SMTs should be biologically conserved, that is, develop across diverse taxa.

Convergent findings reviewed above show that specific plasticity in A1 meets all of these criteria. Moreover, as this is an active area of inquiry, new acoustic parameters are continually being studied. Although this article can

never be up-to-date, at least one prediction can be made: If an acoustic parameter can serve as a signal or gain behavioral relevance through learning, then its processing in A1 will develop specific representational plasticity.

Conclusions

A1 can no longer be considered to function merely as an acoustic analyzer. Nor is it likely that either the primary visual or primary somatosensory cortices differ fundamentally from A1. The auditory cortex is simply that sensory structure which has been most extensively investigated in learning and memory. In fact, both primary visual and somatosensory fields do exhibit some specific learning-based associative plasticity.

The findings reviewed above should be understood to transcend most approaches to neurophysiological correlates of learning and memory. Of course, demonstrations of such correlates are a necessary starting point but it is not in, and of, itself an ending point. In the absence of another level of inquiry, which seeks to understand the functional significance of learning-related plasticity, we are left largely with a list of plastic places and justified, but not fully satisfying conclusions, that these regions are involved in learning and memory. The evidence to date strongly suggests that at least one of the functions of specific associative tuning shifts and related plasticity in A1 is to store information, that is, serve as locus of specific long-term memory traces. To be sure, most memory substrates probably involve widespread networks of neurons. However, that in no way lessens the need to identify a component of such a network. By both determining the degree of specificity of A1 plasticity and delineating its attributes and characteristics in detail, it is possible to meet the criteria for identifying specific long-term memory traces.

The larger-scale implication of a modern concept of A1 is that the S-A-R model is no longer tenable. Despite understandable attempts to separate the analysis of stimuli from the comprehension and assignment of behavioral importance to stimuli at the level of the cerebral cortex, this approach simply cannot account for the data. It is time to seek a new brain-behavior organizational principle.

See also: Active Avoidance and Escape Learning; Animal Models of Learning and Memory; Cholinergic Systems in Aging and Alzheimer's Disease: Neurotrophic Molecular Analysis; Drug Cues: Significance of Conditioning Factors in Drug Abuse and Addiction; Fear Conditioning; Fear, Anxiety, and Defensive Behaviors in Animals; Memory Consolidation; Neural Basis of Classical Conditioning; Neural Basis of Working Memory; Neural Substrates of Conditioned Fear and Anxiety.

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Synapse Formation and Memory

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Glossary

Cortex – The outside layer of an organ, in this case the brain.

Dendritic spine – A tiny protrusion that extends from the dendrite of a neuron which is used to receive synaptic input from another neuron.

Estrogens – Steroid compounds which function as the primary female sex hormone.

Golgi stain – A histological technique first developed over 100 years ago by Golgi and Ramon y Cajal that uses metallic impregnation to randomly label a small number of cells in their entirety so that detailed information regarding dendritic branching, length, and spine density can be measured.

Hippocampus – A brain structure located deep in the medial temporal lobe which plays a critical role in some processes of learning and memory, especially those related to trace conditioning, declarative memory, and spatial navigation learning.

NMDA receptor – Binds the excitatory neurotransmitter glutamate.

Postsynaptic density – Otherwise known as PSD, these cytoskeleton specializations are located at the membrane of a postsynaptic neuron and consist of receptors, ion channels, and signaling systems involved in synaptic transmission.

Sex differences – Systematic difference between individuals of a different sex but within the same species.

The capacity to learn and remember is undeniably critical for the survival of virtually all organisms. However, the neurobiological mechanisms that underlie the formation of memories, their encoding, and storage remain largely unknown. While changes in the efficacy of synaptic transmission are now widely accepted to play a major role in learning, such changes are transient and thus insufficient to explain the stable nature of memory. Because memories persist long after the molecular events involved in their induction have degraded, changes in structural plasticity and, in particular, changes in synaptic architecture are also likely to be necessary. Cajal and others first proposed, more than a

century ago, that memory processes might involve anatomical modifications:

... one can admit as very likely that mental activity provokes a greater development of the dendritic appendages and axonal collaterals in the part of the brain most utilized. In this way, preexisting connections between groups of cells could be notably reinforced by multiplication of the terminal branches of the dendritic appendages and nerve collaterals; and, in addition, novel intercellular connections could be established thanks to the new formation of collaterals and dendrites. (Santiago Ramon y Cajal, 1894)

Despite a long history of speculation, only recently has evidence accumulated to support this idea. The lag in empirical investigation was likely due to the belief that unlike development, which is characterized by massive synaptogenesis and other growth, the adult brain was structurally stable. Moreover, the search for structural change following learning and memory was thought to constitute a needle-in-the-haystack problem, given both the complexity of behavior and the underlying neural circuitry. However, these assumptions have proven to be incorrect. Not only does the brain retain the capacity for structural change throughout life, there is also a growing body of data showing a relationship between synapse formation and cognitive function. These topics are the focus of this article.

Dendritic Spines

Dendritic spines – small protrusions found on the shaft of dendrites in the mammalian brain – are one of the most studied aspects of cellular anatomy that may be associated with memory formation (Figure 1). Because a typical mature spine has a single glutamatergic synapse on its head, measurements of spine number or density provide an estimate of synapse density. Moreover, since dendritic spines represent the main locus of postsynaptic excitatory input, they can regulate the amount of excitatory neurotransmission in a particular brain region and presumably the processing of information by that region.

Although various methods have been used to examine dendritic spines, most studies until recently have used light-microscopic imaging of Golgi-stained material.

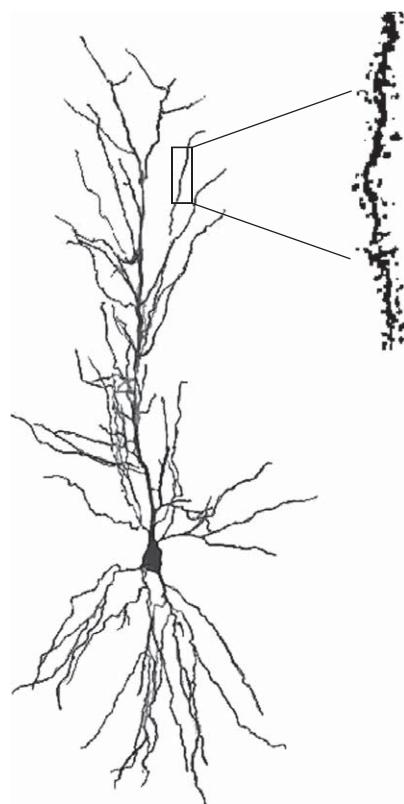


Figure 1 A pyramidal neuron from the hippocampus with a dendritic segment shown at higher magnification. Dendritic spines stud the surface of the dendrite.

Such studies have provided valuable information with regard to alterations in spine density, but due to limits in resolution are generally unable to reveal subtle changes in spine morphology. More recent work has employed fluorescent labeling of neurons followed by confocal microscopy, which can produce spine images of a better resolution. Quantitative immunohistochemical analysis of synapse-associated proteins is another way to identify changes in synaptogenesis. However, electron microscopy is ultimately necessary for the analysis of three-dimensional ultrastructure. Even so, it is limited in that it gives a static view of dendritic spines. The use of two-photon laser scanning microscopy in combination with fluorescent molecular tools has allowed for high-resolution, time-lapse imaging of spines in living slices. At present, it is even possible to study dendritic spines *in vivo* using transgenic mouse lines that sparsely express genetically encoded fluorescent proteins in specific neuronal types. By creating a ‘window’ in the skulls of these mice and positioning them under a two-photon laser scanning microscope, the same dendritic segments can be imaged over extended periods of time, as well as before and after exposure to stimuli. This form of imaging has been used primarily to visualize neurons in the most superficial layers of the cortex, although preparations for *in vivo* imaging of dendrites and spines in the hippocampus

have been reported. One limitation of this technique is that, because anesthesia is required, examination of structural plasticity in an animal as it actively engages in specific behaviors cannot be done. Moreover, although dynamic changes in the individual shapes of spines can be detected, resolution is not sufficient to count numbers or identify exactly where synapses occur. Recent studies have suggested that the cranial window itself can cause substantial glial activation and influence spine dynamics. An alternative approach involves viewing neurons through a thinned skull preparation. Results from this method have shown naturally occurring changes in dendritic spine number and shape but of a less extensive nature than that observed with a cranial window. However, examining deep structures such as the hippocampus with this method is difficult.

Using these techniques, it has been shown in cultured neurons, fixed and live brain slices, as well as in the intact brain that dendritic spines are remarkably dynamic structures. In the adult brain, their numbers are subject to change as a result of spine formation and elimination. Dendritic spines are also highly motile undergoing changes in size and shape over a timescale of seconds to minutes. Because small, thin dendritic spines are most likely to undergo these structural changes, whereas large, so-called ‘mushroom’ spines tend to maintain their form, it has been suggested that mushroom spines are more stable memory spines whereas the more plastic thin spines are learning spines.

Spines contain a postsynaptic density (PSD), the electron-dense thickening on the spine head that consists of receptors, ion channels, and signaling systems involved in synaptic transmission. The size of the spine head is proportional to the area of the PSD, to the number of postsynaptic glutamate receptors, and to the number of presynaptic docked vesicles. Thus, large spines are sites of strong synapses and, accordingly, the growth of the spine head likely correlates with a strengthening of synaptic transmission. In addition to changes in dendritic spine number and morphology, structural plasticity might also reflect modifications in a number of other measures including, but not limited to, changes in the size and configuration of the PSD.

Hormones

Estrogen

Numerous lines of evidence implicate the ovarian hormone estrogen as one of the most potent modulators of dendritic spines. Rapid fluctuations in spine density of CA1 pyramidal neurons of the hippocampus occur over the 5-day estrous cycle of the rat; during proestrus, when estrogen levels are at their highest, spine density is maximal. Between proestrus and estrus, when estrogen levels

decline, a 30% reduction in spine density occurs. Similarly, elimination of estrogen in young adult rats by ovariectomy is accompanied by decreased hippocampal dendritic spine density – an effect that can be reversed by estrogen treatment. The regulation of dendritic spines by estrogen also occurs in nonhuman primates as well as in cultured hippocampal neurons *in vitro*.

Serial reconstruction electron microscopic studies have shown that spine density changes associated with exposure to estrogen, either exogenously or endogenously across the estrous cycle, reflect a change in the density of synapses. Moreover, the increase in spine synapses with estrogen treatment results from the addition of new dendritic spines to preexisting boutons, which in turn make connections to multiple different postsynaptic cells. Thus, not only does estrogen increase the density of excitatory synaptic input to individual CA1 pyramidal cells, it also alters the pattern of synaptic connections. Additionally, it has been reported that estrogen modifies spine morphology by increasing the number of spines with mushroom shapes.

How do these estrogen-induced alterations in synaptic structure influence behaviors associated with the hippocampus? Although there are some exceptions, several studies have reported a beneficial effect of estrogen on cognitive ability. For example, classical eyeblink conditioning is enhanced during proestrus, when spine density is at its peak, relative to other stages of estrus. A correlation between spatial working memory and the estrogen-induced increase in spine density has also been demonstrated – working memory is improved when female rats are tested at a time after estrogen treatment when spine density is elevated but not before spines are increased or after spine density has returned to low levels. The influence of ovarian hormones on dendritic spine/synapse density and cognitive function extend beyond the hippocampus. For example, in rodents and monkeys, estrogen improves performance on tasks that depend on the medial prefrontal cortex and produces a parallel increase in dendritic spine/synapse density. Overall, these studies indicate that hormonally regulated changes in synaptic structure may have an important impact on certain forms of learning and memory.

Testosterone

Much of what is known about gonadal steroid-induced neuroplasticity has focused on the effects of estrogen. However, Recent work has shown that androgens also exert important effects on hippocampal structure. Specifically, castration leads to a loss of dendritic spines indicating that androgens maintain spine number in males. Although the loss of dendritic spines following castration can be reversed by treatment with testosterone, estrogen is without effect. Thus, the hormonal regulation

of hippocampal spine density differs in males versus females. As with estrogen, there are data to suggest that testosterone-mediated changes in spine density may be associated with cognitive ability. Testosterone depletion reduces cognitive performance – an effect that can be prevented by testosterone replacement.

Experience

Stress

The effects of aversive stressful experience on dendritic spines are influenced by the type, intensity, and duration of the stressor, the brain region examined, as well as numerous other factors. For example, acute stress alters the number of dendritic spines on hippocampal CA1 pyramidal cells of adult rats, but this is dependent on the sex of the animal and in the case of females on the stage of estrus. Exposure to brief, intermittent tailshocks increases the number of dendritic spines on pyramidal cells of males. In contrast, females show a decrease in the number of dendritic spines on pyramidal cells when exposed to the stressor during diestrus but not estrus. These sex differences in the effects of stress on dendritic spines are paralleled by stress-induced alterations in classical eyeblink conditioning – stress enhances learning in males but decreases learning in females. Thus, exposure to an acute stressful experience has a similar effect on spine density as it does on new learning, at least of this particular learned response.

In the CA3 region of the hippocampus as well as in the medial prefrontal cortex, acute and chronic stressors induce spine loss and dendritic remodeling of pyramidal neurons. These structural changes have been suggested to underlie stress-induced deficits in spatial navigation learning and attention – cognitive abilities that depend on these respective brain regions. By contrast, aversive experience increases spine density in the amygdala – a locus of storage for fear memories.

Enrichment

Beginning with the seminal studies of Rosenzweig and colleagues, it has been repeatedly shown that exposure to complex or enriched settings either during development or adulthood enhance multiple measures of neuronal morphology in the hippocampus and cortex of rodents and primates. Compared to laboratory control animals, animals living an enriched environment have more dendritic spines and synapses as well as increased dendritic branching. Other studies have shown that environmental enrichment has a beneficial impact on the ability to perform a variety of spatial, nonspatial, hippocampal dependent, and independent tasks. Two points are worth mentioning when considering the effects of

enrichment on brain structure and function. First, enriched environments typically include enhanced opportunities for social interactions, exercise, and learning. The extent to which one or more of these factors contribute to the consequences of environmental enrichment remains unknown. However, some studies have suggested that physical activity may play an important role. For instance, exercise and living in enriched environments similarly enhance dendritic spine density on granule cells of the dentate gyrus and improve performance on some learning tasks. Another point to consider is that under standard laboratory housing conditions, dendritic architecture, spines, and synapses may atrophy from disuse. Enrichment may restore these measures to a baseline closer to that of wild animals. Thus, it's possible that experimental enrichment represents a reversal of the impoverishment generally found in the laboratory setting rather than enrichment over a natural setting.

Long-Term Potentiation

Numerous studies have attempted to link morphological changes with long-term potentiation (LTP) – an enduring enhancement of synaptic transmission widely assumed to be a cellular model of learning and memory. Although early studies failed to find evidence for alterations in overall spine number, more recent work indicates that the induction of LTP is associated with the growth of new spines, some of which become mature synaptic contacts. For example, live-imaging with two-photon microscopy has shown that LTP in hippocampal slice cultures leads to the formation of new spines, and that inhibition of LTP with an N-methyl-D-aspartic acid (NMDA) receptor antagonist prevents this structural change. One possible reason for the contradictory findings may relate to the stimulation methods used in older studies which were not localized and could have reduced the chances of detecting a change.

Modifications in spine morphology, including an enlargement of the spine head as well as a widening and shortening of the spine neck, have also been reported to follow LTP. These effects can occur rapidly – within minutes after stimulation – and can be persistent, lasting up to 24 h. The growth-inducing effects of LTP at one spine can even lead to spine enlargement at neighboring spines. Other structural changes have been associated with LTP, including alterations in the size of the PSD, spine splitting, the formation of perforated synapses, changes in spine curvature, and the formation of multiple spine synapses. Taken together, these data demonstrate that the formation of new spines and modulations in synaptic structure correlate with LTP. Whether these alterations contribute to enduring changes in synaptic strength remains to be determined.

Long-term depression (LTD) is also thought to play an integral role in learning and memory processes but, in contrast to LTP, is a long-lasting reduction in synaptic transmission. Although the morphological correlates of this form of synaptic plasticity have received less attention than those accompanying LTP induction, evidence indicates that LTD produces morphological changes that are seemingly the converse of those associated with LTP. Specifically, induction of LTD results in a shrinkage and retraction of dendritic spines. The functional significance of these observations is presently unknown, but they, nonetheless, provide further evidence that alterations in dendritic spines are dynamically regulated by activity.

Learning and Memory

Invertebrates

There are several examples in the invertebrate world of synaptic reorganization following learning. For instance, spines are altered during learning in honeybees such that their first orientation flight leads to spine-stem shortening. In the marine snail *Aplysia*, nonassociative modification of the gill-siphon withdrawal reflex is accompanied by structural transformations in the network mediating the behavior. Following long-term habituation, in which the snail learns to ignore a neural stimulus, the number of synapses between sensory and motor neurons decreases. Following sensitization, in which the snail reacts more strongly to a noxious stimulus with repeated presentations, synapses between these neurons exhibit changes in size, shape, and number.

Birds

There is also evidence that learning can induce spine changes in vertebrate species such as birds. For example, one-trial passive-avoidance conditioning in chicks is associated with an increase in spine density on neurons of two forebrain regions important for memory storage. These changes appear specific to memory formation since chicks rendered amnesic for the experience by subconvulsive shock shortly after training do not exhibit an increase in spine density. Morphological correlates of learning have also been shown to occur in songbirds. Those songbirds that learn large song repertoires have a greater number of dendritic spines in a specific forebrain nucleus of the song system than birds that learn small repertoires. These studies suggest that neurons of the bird brain are also sensitive to learning-related changes in dendritic spines.

Mammals

In mammals, increases in hippocampal spine number have been reported following training on a variety of tasks, including passive-avoidance conditioning, olfactory

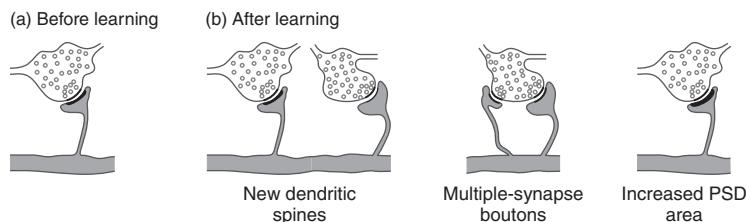


Figure 2 Pyramidal neurons in area CA1 of the hippocampus undergo alterations in synaptic structure following classical eyeblink conditioning. (a) An axospinous synapse prior to learning. (b) Following learning, new dendritic spines appear, multiple-synapse boutons are formed, and the area of the PSD (depicted as the darker area in the dendritic spine) is increased.

discrimination, spatial navigation, and classical eyeblink conditioning, although there are some differences in the magnitude, time course, and location of the effects across studies. For example, eyeblink conditioning increases hippocampal dendritic spine density 24 h later (Figure 2), an effect that is prevented by blocking learning with an NMDA-receptor antagonist. However, the learning-induced changes in spine density are localized to CA1 pyramidal neurons and do not occur on granule cells of the dentate gyrus. Moreover, changes in spine number are found on basal dendrites of stratum oriens but not on apical dendrites of stratum radiatum. It is possible that changes in spine density could occur in other cell regions at earlier or later time points and at different stages in the learning process. However, the observation that classical conditioning does not affect spine density on apical dendrites is consistent with other work reporting that synapse density in CA1 stratum radiatum is unaltered by learning the trace version of the eyeblink conditioning task. These data suggest that structural modifications following learning may occur without an overall change in synapse number. Indeed, trace eyeblink conditioning has been shown to increase the number of boutons that synapse with multiple dendritic spines and to enlarge the area of the PSD (Figure 2). That learning may be accompanied by a remodeling of existing synapses rather than a lasting increase in their number may also explain other results demonstrating transient changes in spine density/synapse number after training in the water maze or on a passive-avoidance task. In addition, finding no overall change in synapse density does not exclude the possibility for increased density of certain spines at the expense of others. Since it appears that changes in spine formation are activity driven, it is likely that strengthened synapses may be preserved, whereas spines that are activated but not strengthened are reduced. Indeed, some type of homeostatic process is probably necessary to maintain a balance between structure and stability and so learning could involve changes in spine morphology in addition to, or instead of, alterations in spine number.

Learning also influences structural plasticity in brain regions other than the hippocampus. For example, a large

body of work by Greenough and colleagues has shown that synaptogenesis occurs in the cerebellar and motor cortices after motor skill learning. Increased synaptic number in the cerebellum has also been associated with eyeblink conditioning and in the piriform cortex following olfactory learning. In the lateral amygdala, fear learning leads to increased numbers of dendritic spines as well as an enlargement of the PSD.

Human Brain Disorders

Accumulating evidence indicates that structural synaptic plasticity plays a vital role not only in normal brain functions, but also in the development of neurological and mental disorders. In humans, abnormalities in dendritic spine number and structure have been associated with deficient cognitive capabilities. There are reports indicating an abnormality of dendritic spines in numerous mental retardation disorders such as Down's and Fragile X syndromes. In persons with Down's syndrome, the density of dendritic spines is decreased in the neocortex and hippocampus while the incidence of abnormally long and short spines is increased. Fragile X syndrome is characterized by abnormal spine morphology and an increased density of dendritic spines – possibly as a result of deficits in spine maturation and pruning. Similar findings have been observed in a mouse model of Fragile X syndrome, although the developmental regulation of the spine changes differs from that in the human. Schizophrenia is another disorder characterized by poor performance on cognitive tasks, particularly those in which the dorsolateral prefrontal cortex is important. Spine density in this region, as well as the temporal cortex, is decreased in schizophrenic subjects. Finally, dendritic spine abnormalities have been observed on cortical pyramidal neurons of patients with Alzheimer's disease and senile dementia as well as in animal models of Alzheimer's. Taken together, these data implicate dendritic spines as an anatomical substrate important for normal cognitive processes and suggest that dendritic spine alterations may contribute to the cognitive dysfunction characteristic of these disorders. However, other

interpretations are feasible and the possibility remains that structural changes are a consequence and not a cause of the disorder.

Functional Implications

Some fundamental questions with regard to the relationship between synapse formation and cognitive function have yet to be elucidated. For example, is learning-induced spine/synapse formation an anatomical substrate for information storage or does it represent a more general property of the engaged parts of the brain circuitry underlying these behaviors? In the case of eyeblink conditioning, the latter possibility may be more likely. Notably, the hippocampus is necessary for acquiring the learned response but it is not the site of memory storage. In addition, the increase in dendritic spine number occurs following hippocampal-dependent and -independent types of eyeblink conditioning, both of which are known to enhance excitability in the CA1 pyramidal cell region during, and for some time after, training. Thus, excitability and spine density tend to correlate. Whether the increase in excitability results in the formation of new spines or vice versa is debatable but, in either case, this increased network excitability could thereby enhance its processing capability. This idea proposes a rather broad and nonspecific effect of training on spine formation and is thus inconsistent with the effects of LTP on synapse formation and, more generally, with traditional ideas of how spines may be involved in cognitive processes. It is assumed that the changes occurring at the synaptic level are relatively specific to just a few synapses necessary to encode an association; learning would not influence most or even a substantial number of cells in the network. However, hormones and experience, including learning, lead to 20–30% increases in spine density – substantial changes which are not likely relegated to just a few neurons in a network.

Along similar lines, it is unknown whether the structural changes are temporary or long lasting and representative of memory storage. If temporary, it remains to be determined how changes in spine number would alter processes involved in learning. One might propose that the presence of spines enhances synaptic efficacy and thereby enhances the excitability of the network involved in the learning process. In this particular scenario, learning is not necessarily dependent on spine density changes; rather changes in spine density indirectly enhance the support for information processing; that is, they provide anatomical structure for processes that are either occurring or will occur in the future. In the case of estrogen, for example, an increase in spine density would alter the presence of spines during proestrus – a time in the female cycle when she is most exploratory and when

mate selection would generate offspring. In the case of stress, the increase in spine density in males precedes the enhancement in learning ability; that is, within 24 h of the stressful event, increased spine density is evident and persists for several days thereafter. This is a time period when the chance of stressful encounters (e.g., with predators) remains relatively high. The presence of spines may thereby provide anatomical support for forming associations at a more rapid rate during that time period.

Concluding Remarks

Although the work reviewed here supports the long-held belief that synapse formation and remodeling may be important substrates underlying memory formation, other mechanisms are also likely to play a role. For example, neurogenesis occurs in the adult hippocampus and has been linked to some types of learning and memory. Understanding how these mechanisms interact or whether they have a unique contribution to cognitive function is a challenge for future work. It is also important to keep in mind that despite a number of advances in the field, much of the current data is correlative and no causal relationship between cognitive processes and synapse formation has been established. Advances in imaging technologies, allowing for the observation of structural changes in behaving animals, combined with molecular approaches enabling the manipulation of dendritic spines *in vivo* should help to resolve this issue and bring us closer to elucidating the role of this form of structural plasticity in brain function.

Acknowledgments

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See also: Aging and Cognition; Analysis of Learning in Invertebrates; Animal Models of Learning and Memory; Brain Aging: Structural Imaging Biomarkers of Risk of Alzheimer's Disease; Cognition: Attention and Impulsivity; Cognition: Learning and Memory: Pavlovian; Cognition: Learning and Memory: Spatial; Effects of Stress on Learning and Memory; Environmental Influences on Adult Neurogenesis; Eyelid Classical Conditioning; Fear Conditioning; Hormones and Memory; Memory and Aging, Neural Basis of; Memory Consolidation; Memory in the Honeybee; Neural Basis of Classical Conditioning; Neurogenesis and Memory; Neuron Excitability and Learning; Protein Synthesis and Memory; Role of Gene

Transcription in Long-Term Memory Storage; Stress and Brain Morphology; Synaptic Mechanisms for Encoding Memory.

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Hormones and Female Sexual Behavior

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Glossary

Conditioned place preference – A behavioral technique in which the rewarding aspects of a particular situation can be assessed. In the study of sexual behavior, the rewarding aspects of particular mating situations can be studied.

Copulatory behaviors – Those behaviors which result in successful transfer of sperm from the male to the female; used instead of receptivity, because it connotes an active participation by the female, and because the same term is applied to the active behaviors shown by males. Lordosis is a sexually receptive behavior.

Estradiol – The predominant steroid hormones (usually 17β -estradiol, but the 17α -estradiol isomer is also active in some systems) belonging to the class of hormones – estrogens – circulating in vertebrates. Although estradiol is sometimes incorrectly called estrogen, the two terms should not be confused. Estradiol is a specific hormone; estrogens are a class of hormone.

Estrogen receptor – A protein that binds estradiol and other estrogenic compounds. Although there are two genes synthesizing distinct estrogen receptors – estrogen receptor (ER) α and ER β – the indispensable function of ER α has been best characterized. There are other forms of ERs; however, the classic ER acts as a transcription factor.

Estrogens – A class of steroid hormones defined either by structure, by binding properties, or by their influence on particular responses.

Genitosensory stimulation – The mating stimulation received by a female from a male or, in some cases, from manual palpation by the experimenter or a mechanical probe.

Lordosis – The prototypical, reflexive element of sexual behavior in many species, characterized by immobility and dorsiflexion of the spine to accommodate intromissions by a male.

Paracopulatory behaviors – Species-typical behaviors displayed by females which arouse males and stimulate them to mount. These behaviors have sometimes been termed proceptive, precopulatory, or solicitational behaviors.

Progestative behaviors – Those behaviors that occur throughout mating, which maximize the likelihood that pregnancy will occur.

Progesterone – The predominant steroid hormone of the class progestins or gestagens, circulating in vertebrates.

Progestin receptor – A protein that binds progesterone and other progestin-like compounds. Although there are other types of progestin receptors, the classic progestin receptor exists in two forms, A and B, and acts as a transcription factor.

Progestins – A class of steroid hormones defined either by structure, by binding properties, or by their influence on particular responses (also called gestagens or progestagens).

Pseudopregnancy (also called the progestational state) – A physiological condition that resembles pregnancy, which is initiated by twice-daily surges of prolactin, resulting in rescue of the corpus luteum. It can result from primarily intromissive stimulation received during copulation or by experimenter-applied vaginal cervical stimulation.

Vaginocervical stimulation – A reproductively relevant genitosensory stimulus that is an important component of mating stimulation received by females. It can be provided by males during intromissions or ejaculations, or it can be provided by an experimenter with a glass or plastic rod. It should be noted that the experimenter-applied stimulation is primarily stimulation to the uterine cervix.

Introduction

The study of female sexual behavior has been an active, perhaps the most active, area of investigation within the field of Behavioral Endocrinology for over 75 years for a variety of reasons. Female sexual behavior is robust, reliable, and, at some levels, relatively easy to study. Furthermore, it lends itself to both behavioral analysis and mechanistic analysis. Sexual behavior is undoubtedly biologically important, and no inference is necessary to understand its relevance to the animal. Sexual behavior is also the prototypical model for sexual differentiation of the brain, since in most species the behavior is sexually dichotomous. Finally, although this was not clear in earlier years, female sexual behavior in some rodent species

provides a useful model for understanding the role of hormones in certain aspects of human sexuality.

Female sexual behavior has also been used extensively as a model behavior for understanding the mechanisms by which hormonal and afferent neural signals from the social environment interact at the cellular level to modulate behavior. Some of the most interesting aspects of this model are the many ways in which behavioral response is shaped by environmental stimuli, as well as the ways in which behavioral output changes physiology. Although various components of these complex interactions can be studied in a reductionistic manner, they also lend themselves to analysis at all levels from organismic to cellular and molecular.

Model Species

Because of its obvious biological importance, the sexual behaviors of many species have been studied, and much is known about the female sexual behaviors of all species from primates to the most primitive sexual organisms. Although there are many interesting differences among species in the behaviors and their regulation, a great deal of research on hormones and female sexual behavior has used rats, guinea pigs, hamsters, and mice as model species. These species have much in common with each other, and considerable similarities to regulation of sexual desire in humans. This article focuses primarily on rats with some discussion of commonalities with work involving other species. Although the behaviors themselves differ widely among species, the hormones involved are quite consistent, and the mechanisms and brain areas involved are quite conserved.

Classifications of Sexual Behaviors

Sexual behaviors in female rodents include postural stances and adjustments required for vaginal intromission by males and other behaviors that attract the male and modulate the timing of sexual interactions. Some of these

behaviors facilitate the transfer of sperm from the male to the female reproductive tract and increase the probability of successful pregnancy. Some other behaviors are relatively stereotyped and reflexive; other, more complex patterning of behavioral events during copulation are necessary for successful pregnancy.

Numerous metrics have been used to study sexual behavior, and each has its appropriate place in the armamentarium available to the researcher. The appropriate measure chosen for a particular experiment depends upon the specific questions being asked. If the question focuses on whether a particular treatment induces the expression of estrous behavior, a simple measure of lordosis is sufficient. Alternatively, if the question is one of sexual readiness and the female's motivation to mate, then other behaviors might be more relevant, as well as tests for sexual motivation. When examining the effects of the female's sexual behavior on neuroendocrine function and reproductive success, behaviors involved in the induction of pregnancy and facilitation of sperm transport are studied.

Historically, most studies of rodent female sexual behavior relied on the lordosis reflex as the sole measure of sexual behavior, and this continues to be quite useful to answer questions relating to the neuroendocrine bases of a relatively simple behavior. The study of the lordosis response allows for reliable assessment of the female's responsiveness to the mating stimuli provided by the male. Likewise, it allows the study of the cellular and molecular underpinnings of a relatively simple behavior and how these factors are integrated in the moment-to-moment changes in behavioral expression.

Generally, female sexual behavior is considered to comprise three basic elements as proposed by Frank Beach (**Table 1**): receptivity, proceptivity, and attractivity. Beach acknowledged the multiple components of female sexual behavior, and his terms provide a framework for studying female sexual behavior. More recently, however, recognition of the additional behaviors involved, and the recognition that females are active rather than passive participants, in sexual interactions called for a more appropriate set of terms. Blaustein and

Table 1 Classifications for female sexual behavior

Category	Example in rats
<i>Beach's characteristics of female mammals in estrus (1976)</i>	
Attractivity	stimulus value in evoking sexual responses by male
Receptivity	lordosis
Proceptivity	ear-wiggling, darting and hopping
<i>Blaustein and Erskine's classification of female sexual behaviors (2002)</i>	
Copulatory	lordosis
Paracopulatory	ear-wiggling, darting and hopping
Progestative	Pacing

Erskine described three functional components of female sexual behavior, which are useful in studying the neural and endocrine regulation of female sexual responsiveness: copulatory, paracopulatory, and progestative behaviors, and this article will be organized along these components.

Copulatory Behaviors

Copulatory behaviors refer to behaviors that result in transfer of sperm from the male to the female. It is used instead of Beach's term, 'receptivity,' because it emphasizes the female's active role. Females of many species, including rats, display the lordosis posture, which positions the vagina to allow penile intromission in response to flank and perineal somatosensory stimulation typically received from males during mounts. The reflexive dorsiflexion of her spine, lordosis, involves her becoming immobile, extending her rear legs, elevating her head, and deflecting her tail (**Figure 1**).

Lordosis has been used as the dependent measure in the majority of mechanistic and neuroanatomical studies of female sexual behavior. The percent lordosis responses observed in response to mounts from the male (lordosis quotient) is used as an index of the basic level of sexual receptivity. The lordosis rating of the intensity of each spinal dorsiflexion is used as an index of the magnitude of the lordosis response. Unlike rats, in which the display of lordosis occurs briefly ($\approx 0.5\text{--}1.5$ s), in some species the duration that the posture is held is used as an index of strength of response, since it can last for minutes in hamsters and 10–20 s in guinea pigs.

Hormones

Hormones are generally thought of as increasing the probability of a particular behavior occurring in a specific



Figure 1 Drawing illustrating the immobile lordosis posture adopted by a sexually receptive female rat in response to copulatory stimulation by a male rat. Note the deflection of the tail and dorsiflexion of the spine.

condition. That is to say, appropriate hormonal priming increases the probability that a female will express particular sexual behaviors when confronted with a sexually vigorous male of the same species. The frequency and rate with which these behaviors are expressed are influenced by ovarian steroids, by the rate at which a male copulates with a female, and by the specific experimental conditions under which mating is observed.

A prominent characteristic of the estrous cycle of rats, guinea pigs, hamsters, and mice is that the secretion of estradiol followed by progesterone induces a period of sexual behavior (heat or behavioral estrus) that is linked to the time of ovulation (**Figure 2**). After heat terminates, sexual receptivity is not expressed until the same stage of the next estrous cycle (**Table 2**). Most experiments on the neuroendocrinology of female sexual behavior have assessed influences only on receptivity. Removal of the ovaries causes the cessation of the expression of female copulatory behaviors due to the loss of ovarian hormones. While there is a good deal of commonality with other sexual behaviors, caution in generalization is warranted.

Although female copulatory behavior is generally considered to be hormone dependent, like most behaviors, it is regulated by, but is not entirely dependent on, hormones. First, consider the copulatory behavior – lordosis – which is the most reflexive of the sexual behaviors. During the estrous cycle, estradiol and progesterone are secreted sequentially, and both are essential for the expression of lordosis. Although estradiol alone, particularly when administered for prolonged periods or at high dosages, can induce lordosis, under typical physiological conditions, both estradiol and progesterone are required. However, in the apparent absence of both hormones, the reflex can be induced by an extraordinary stimulus (e.g., experimenter-applied, vaginocervical stimulation (VCS) with flank stimulation).

Often, after exposure to progesterone, rats, hamsters, guinea pigs, and mice become refractory to further facilitation of copulatory behavior by either progesterone alone or, in some cases, estradiol and progesterone. Progesterone desensitizes the response to itself, which has been suggested as a mechanism by which progesterone-facilitated sexual behavior terminates (i.e., heat termination).

The time course of action of estradiol on copulatory behavior differs markedly from that of progesterone. While estradiol priming typically takes about a day, progesterone when administered intravenously can facilitate the expression of lordosis much more quickly, even within less than an hour of injection. This is seen in ovariectomized rats, as well as during the estrous cycle, in which estradiol secretion precedes progesterone by about a day, and sexual behavior commences soon after the start of the rise in progesterone levels.

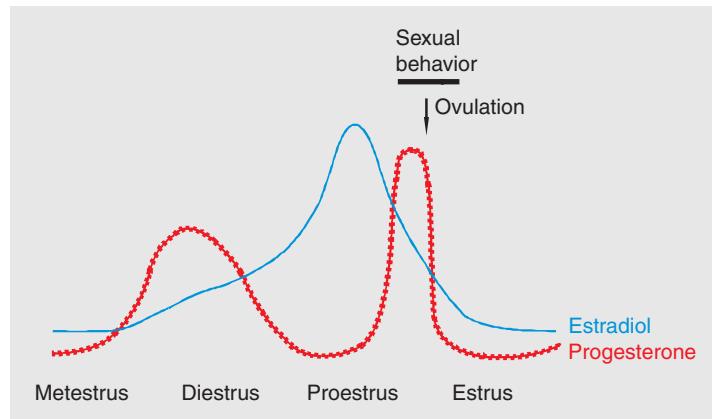


Figure 2 Schematic illustrating the relative changes in estradiol and progesterone levels during the estrous cycle of female rats. It is the peak of progesterone following the rise in estradiol levels that results in the expression of sexual behaviors overlapping the time that ovulation occurs. The second increase in progesterone levels during metestrus/diestrus is a result of secretion from the short-lived corpus luteum in species with short estrous cycles.

Table 2 Facilitation and subsequent desensitization of female sexual behavior by progesterone in estradiol-treated rats and some other rodent species

Day 1 injection	Day 3 injection	Day 3 test	Day 4 injection	Day 4 test
Estradiol	Oil	No		
Estradiol	Prog	Yes		
Estradiol	Oil	No	Prog	Yes
Estradiol	Prog	Yes	Prog	No

Prog = progesterone.

No = absence or low levels of sexual behavior.

Estradiol and progesterone are not the only steroid hormones involved in the regulation of female copulatory behaviors. In fact, dihydrotestosterone is inhibitory to the induction of sexual receptivity, and it has been suggested as one of the factors that determines the duration of the period of sexual receptivity during the estrous cycle. Furthermore, corticosterone, secreted from the adrenal gland, typically inhibits the expression of female sexual behavior.

Paracopulatory Behaviors

Paracopulatory behaviors, which have also been called proceptive, precopulatory, or solicitational behaviors, are species-typical behaviors displayed by females which arouse the male and stimulate him to mount. These behaviors differ markedly among species. Paracopulatory behaviors exhibited by estrous female rats during mating include hopping, darting, and ear wiggling, a presenting posture, a rapid sequence of approach to, and withdrawal from a sexually active male, and production of ultrasonic vocalizations. These behaviors are expressed repeatedly before and between mounts.

Hormones

Paracopulatory behaviors are generally considered to be dependent on progesterone as well as estradiol. While lordosis can be seen within an hour of progesterone treatment, maximum levels of paracopulatory behaviors are generally seen with a slightly longer latency. Although estradiol alone can induce copulatory behaviors in ovariectomized rats, in most cases the expression of paracopulatory behaviors requires progesterone either administered exogenously or secreted by the adrenal gland. It should be noted that paracopulatory behaviors have sometimes been observed even in ovariectomized–adrenalectomized rats administered only estradiol, suggesting that paracopulatory behaviors are not absolutely dependent on progesterone.

Progestative Behaviors

Female rats receive multiple, spaced mounts and mounts with intromissions prior to an ejaculation. Progestative behaviors include pacing of the rate of stimulation through intermittent approaches to and withdrawal from the male, the selection by the females of males that are ready to ejaculate, and the females' postejaculatory

interval, which enhances sperm transport by preventing rapid displacement of the copulatory plug deposited by the male. These short-term behavioral adjustments in the patterning of copulatory stimulation increase the likelihood of optimal reproductive success.

Given an opportunity, by using a two-chamber testing apparatus in which the male is restricted to one chamber, but the female can move freely, or a bi-level testing apparatus, female rats actively regulate the types and amounts of VCS received during mating by pacing their interactions with the male. The females return to the male with shortest latency after a mount. The latency to return to the male is longer after an intromission and even longer after an ejaculation. Therefore, the rate of receipt of mounts is greatest, followed by intromissions and then ejaculations. Females mated in undivided test arenas are unable to avoid contact with the male and thus receive nonpaced coital stimulation. Typically, paced mating, in which the female determines the rate of stimulation, occurs at a slower rate than nonpaced mating, in which the male determines the rate. Mating stimulation by a male is reinforcing when the female is allowed to pace the sexual interaction, which is consistent with the idea that paced mating, besides being a progestative behavior, is a rewarding aspect of sexual behavior.

The temporal patterning of VCS that females receive during mating is critical in determining whether the neuroendocrine responses required for pregnancy occur, including secretion of sufficient progesterone from the corpus luteum to promote successful implantation (i.e., induction of a progestational state necessary for pregnancy). Intromissions and ejaculations are effective, whereas mounts-without-intromission typically are not. The postejaculatory interval imposed by the female is also progestative, because this interval may ensure that maximum sperm transport has occurred.

Hormones

Pacing behavior is dependent on estradiol and progesterone, as seen both in estrous-cycling rats and ovariectomized rats injected with estradiol and progesterone. Although pacing appears to be dose-dependent on progesterone, the influence of estradiol seems to be all-or-none. Much of the work on hormones and sexual behavior has focused on the action of hormones in the brain. However, the effects of hormones on peripheral tissue must also be considered. For example, systemic injection of ICI-182,780 – an anti-estrogen, which does not cross the blood–brain barrier – is without effect on the expression of lordosis in ovariectomized rats. However, in a paced mating situation, the anti-estrogen caused a lengthening of the return latencies after intromission and ejaculations. It has been suggested that estradiol decreases the aversive component of genitosensory stimulation via a

peripheral site of action. This is consistent with earlier findings that estradiol induces robust changes in the sensory field of the pudendal nerve, which innervates the perineal region, and the report that transection of this nerve disrupts pacing behavior in estradiol-treated rats.

Motivation

The rate of display of paracopulatory behaviors, such as darting, hopping, and ear-wiggling (actually rapid head oscillations giving the ears the appearance of wiggling or quivering) is often used as an index of a female rat's motivation. However, other tests that are more direct tests of motivation to mate have been developed. These include tests of partner preference in which a female is given an opportunity to seek proximity to a sexually active male over another stimulus animal, the use of a runway to assess the female's motivation to approach a male, and conditioned place preference in which rewarding aspects of a particular mating situation can be assessed. Unfortunately, the extensive studies to understand the role of particular hormones that have been undertaken for copulatory behavior have not been performed for tests of motivation.

Partner Preference

In general, rats in the proestrous/estrous stage of the estrous cycle – when estradiol and progesterone levels are elevated – or ovariectomized rats injected with estradiol, an androgen, or estradiol and progesterone are more likely to approach sexually active males, suggesting, not surprisingly, that ovarian hormones increase the female's sexual motivation. The female's motivation to mate is also influenced by the ability of the male to copulate with her, with them typically showing a stronger preference for males that are not allowed to copulate with them than with males that are. However, if sexual interaction or genitosensory stimulation by the males is restricted (for instance, by keeping the males in a wire cage or by preventing intromissions), females prefer a sexually active male. These results suggest that females initially seek out contact with sexually active males, but that the mating stimulation results in subsequent avoidance of this stimulation. This is consistent with the idea that there is an aversive component to genitosensory stimulation.

Runway Method

Similar influences are seen when a runway is used to assess the female's motivation for a male. Ovariectomized rats treated with estradiol or estradiol and progesterone increase speed and time spent in proximity to a male – as

is seen during the proestrous–estrous stages of the estrous cycle when ovarian hormone levels are highest.

Conditioned Place Preference

The use of conditioned place preference to assess the sexual motivation of female rats has demonstrated that ovariectomized female rats primed with estradiol and progesterone prefer a compartment which has previously been associated with sexual interaction. Interestingly, similar to partner-preference experiments, females prefer places associated with males which are not allowed to copulate.

Generally, females develop a conditioned place preference for compartments in which they have been allowed to pace the mating, but not places where rate of copulation has been determined by the male. Progesterone is essential for the development of a conditioned place preference in female rats that pace their rate of sexual interaction. Interestingly, recent work suggests that nonpaced mating also induces a conditioned place preference if a single male provides all of the intromissions, but not if multiple males are used. So while the conditioned place preference technique is a powerful one for assessing female's preference, it is also very sensitive to relatively small differences in the stimulus animal.

Summary of Hormonal Influences on Female Sexual Behaviors

It is clear that the prototypical copulatory behavior – lordosis – requires the sequential action of estradiol and progesterone during the estrous cycle. However, under some conditions, in ovariectomized rats, it can be induced with estradiol alone. The situation with paracopulatory behaviors is a bit different. Although most reports suggest estradiol injection must be followed by progesterone, there are some cases of high levels of paracopulatory behavior with estradiol alone. The progestative behavior – pacing – requires both estradiol and progesterone, perhaps because pacing is dependent upon motivation for sexual behavior. To the extent that it has been studied, female sexual motivation seems to be dependent on both progesterone and estradiol.

Mechanisms of Action of Steroid Hormones

Many studies on the cellular mechanisms by which estradiol and progesterone act in the brain have relied on the female sexual behavior model. Although steroid hormones act through a wide array of mechanisms from membranes to genes, the most well-characterized mechanism for feminine sexual behavior involves

intracellular steroid receptors acting as transcriptional regulators. Although an understanding of the cellular mechanisms involved in the effects of estradiol and progesterone on the motivational aspects of sexual behavior and pacing is of tremendous importance, most work has focused on lordosis.

Estradiol's Mechanisms of Action

The discovery of estrogen receptors (ERs) in the brain 40 years ago led to studies to determine if binding of estradiol to those receptors was causally related to the induction of female sexual behavior by estradiol and other estrogenic compounds. The necessity of ERs for the actions of estradiol on sexual receptivity has been assessed by use of a variety of techniques: estrogen antagonists – which block the binding of estradiol to ERs; ER gene-disrupted mice (ER knockouts (ERKOs)) – in which the gene for ERs has been disrupted; and by techniques to block the synthesis of ERs either globally or in specific brain regions. Although other mechanisms are undoubtedly involved, the results of each approach are consistent with the conclusion that ERs are essential for the effects of estradiol on the expression of copulatory behavior.

There are two different genes for transcription-factor ERs that are independent but have a great deal of homology. Although it is clear that ER α is indispensable for female sexual behavior, the role of ER β is less defined, perhaps acting to modulate response to ER α .

Progesterone's Mechanisms of Action

Progesterin receptors (PRs) were first characterized in the brain close to 30 years ago. The discovery of neural PRs led to the hypothesis that the facilitation of sexual behavior by progesterone requires interaction of progesterone with those receptors in critical neurons. As was the case with ERs, a variety of techniques have been used to demonstrate that PRs are necessary for the facilitation of sexual behavior by progesterone: injection of progestin antagonists, antisense oligonucleotides to PR mRNA, and PR-knockout strains of mice. It follows that sensitivity to progesterone is determined by the concentration of PRs available in those neurons, and behavioral response requires an adequate concentration of PRs activated by progesterone in those neurons. Estradiol induces response to progesterone, presumably by increasing the concentration of PRs in relevant cells. The decrease in behavioral response seen following heat termination subsequent to progesterone treatment, and indeed, heat termination itself, is referable to the downregulation of PRs by progesterone in some neurons.

Role of the Social Environment and Nonhormonal Influences on Sexual Behavior

One of the most interesting aspects of female sexual behavior is that it is an interactive behavior. As a social behavior, response is dependent not only on the presence of proper hormones in the proper sequence, but on the appropriate stimulation as well, typically by a sexually active male.

Tactile stimulation provided by a male of the same species or by the experimenter influences both the moment-to-moment response of the female as well as the longer-term response. For example, female rats during the appropriate stage of the estrous cycle or ovariectomized rats primed with estradiol and progesterone respond to tactile stimulation of the perineal and/or back or flank region with the lordosis response.

In addition to its regulation by ovarian hormones, the microregulation of copulatory behavior is influenced by afferent input from the social environment, that is, from stimulation received from mating. For example, the postural changes which accompany lordosis – developed in response to stimulation of the receptive field of the pudendal nerve by stimulation of the flanks and perineum – facilitate intromission. Ovarian hormones influence the receptive field of the pudendal nerve, and changes in the response of that nerve in turn influence the female's response to mounting stimulation by the male.

Genitosensory stimulation from social interactions can also have dramatic effects on longer-term changes in behavioral response. Stimulation of the vagina and/or cervix (VCS) either by a male rat or artificially by a mechanical probe may cause abbreviation of the period of sexual receptivity (i.e., early heat termination). On the other hand, nonintromissive, genitosensory stimulation can both prolong heat duration in rats and even induce sexual behavior in the absence of progesterone in estradiol-primed, ovariectomized rats.

As discussed earlier, the expression of sexual behaviors requires interaction of estradiol and progesterone with their respective intracellular receptors. However, as has been shown in many other tissues, a variety of messengers – including neurotransmitters – can act on neurons to substitute for progesterone in its effects on sexual behaviors. While some of these may activate downstream consequences of PR activation (e.g., modulation of neurotransmitter synthesis or release), others facilitate the expression of sexual behavior by acting in neurons containing PRs to activate those receptors. This ligand-independent activation does not occur by binding of the neurochemical to the receptor in the way that progesterone acts; rather, some neurotransmitters, acting through their own membrane receptors, activate second-messenger

systems that, in turn, activate PRs. Likewise, stimulation received from mating can substitute for progesterone, presumably by tapping into this mechanism; that is, mating stimulation induces the release of some of these neurotransmitters onto PR-containing neurons. This, in turn, induces intracellular changes that activate those receptors in the absence of progesterone, resulting in the expression of copulatory and paracopulatory behaviors.

Effects of Mating Stimulation on Reproductive Physiology

In discussing female sexual behavior, it is important to keep in mind the outcomes of the expression of sexual behavior. On one level, it is obvious that the appropriate expression of sexual responses ultimately leads to the opportunity for successful fertilization. On another level, this stimulation results in a variety of physiological responses, some which promote pregnancy, and others which influence the expression of the luteinizing hormone (LH) surge, which in turn results in ovulation.

Prolactin Release and Pseudopregnancy

Vaginocervical stimulation in rats, typically derived from mating stimulation by a male rat, results in pseudopregnancy (also called the progestational state) – a pregnancy-like physiological state characterized by twice-daily surges of prolactin and resultant high levels of progesterone secreted from the corpus luteum. In rats and other species with short (4–5 day) estrous cycles, the corpus luteum is short-lived. VCS, through its actions on the brain, induces surges of prolactin from the pituitary gland twice per day, and these in turn rescue the corpus luteum, causing it to be a functional, secretory tissue for 10–12 days. In the case of a fertile mating, pregnancy continues beyond this period.

Although pseudopregnancy can be induced by cervical probing or mating stimulation during the estrous stage of the estrous cycle, the optimal stimulation is that of paced mating. A particular number of intromissions is more effective if they are spaced in a pattern characteristic of the species.

Prolactin levels also rise in unmated females in conjunction with the preovulatory LH surge stimulated during each estrous cycle by rising estradiol levels. However, intromissive stimuli received during copulation in estrous females also induce an acute prolactin response within 5 min of mating that quickly terminates. This early, VCS-induced prolactin response is not influenced by the female having the opportunity to pace the rate of VCS or by the number of intromissions. Therefore, there are multiple mechanisms by which genitosensory stimulation can influence reproductive physiology.

Oxytocin

Genitosensory stimuli, including VCS, induce the synthesis and release of neural oxytocin in some mammalian species. Mating can induce an increase in oxytocin that may act on the lactotrophs of the pituitary gland to induce the prolactin response to intromissive stimulation; that is, oxytocin is integral to the initiation of the prolactin surges that are a prerequisite for pseudopregnancy.

LH Release and Induced Ovulators

The best examples of species in which mating influences the secretion of LH and ovulation are reflex (or induced) ovulators – that is, species that do not ovulate without appropriate genitosensory stimulation (e.g., cats, rabbits, ferrets, and camels). Although rats, like humans, are spontaneous ovulators, ovulating spontaneously without external stimulation, rats can become induced ovulators (expressing an LH surge in response to VCS) during aging or by maintaining them in an environment of constant light. There is some evidence that humans, like many other spontaneously ovulating species, can become induced ovulators under certain conditions, but this is quite controversial.

In addition to the induction of the LH surge by VCS in reflex ovulators, LH release can be increased in response to mating or VCS in rats undergoing normal estrous cycles. This is consistent with the idea that, even in spontaneous ovulators, genitosensory stimulation influences reproductive physiology.

Relevance of Animal Models to Understanding Human Sexual Desire

For many years, the study of hormones and female sexual behavior in rodents was considered a strong model for studying hormone–brain–behavior relationships within a biological context, but it was generally not thought of as an animal model for understanding hormones and human sexual behavior. For a variety of experimental design reasons, early studies failed to demonstrate a relationship between hormones and sexual desire in humans that might parallel the relationship between hormones and sexual motivation or sexual behavior in rodents. However, more recently, it has become clear that if studies are designed to consider the sexual desire of the females, rather than merely incidence of sexual

intercourse, the study of hormones and rodent sexual behavior can inform an understanding of hormones and sexual desire in humans.

See also: Infant Bonding and Attachment; Male Sexual Behavior; Neural Basis of Gender; Parental Behavior; Perinatal Influences on Behavior and Neuroendocrine Functions; Sexual Motivation; Social Bonding and Attachment.

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Hormones and Memory

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Glossary

Dual-solution task – A maze that can be solved equally well using either place or response learning strategies.

Estrogen receptors (ERs) – A set of receptors that bind the hormone estradiol. The receptors have several forms including: (1) intracellular receptors which, when activated by estrogen, translocate to the nucleus to influence gene transcription and (2) G-protein-coupled membrane receptors which, when activated, initiate intracellular signaling cascades. Both types of receptors include both α - and β -receptor forms, encoded by separate genes.

Memory consolidation – Processes initiated at the time of an experience that provide the mechanisms underlying long-lasting memory.

Memory modulation – Processes initiated at the time of an experience that regulate the mechanisms of memory formation.

Memory retrieval – Recall of previously learned information on later test trials.

Place learning – Acquisition of correct performance on a maze with an optimal solution based on integration of extramaze cues.

Response learning – Acquisition of correct performance on a maze with an optimal solution based on egocentric selection of turns.

of coherent information without a need for long-lasting change. Included in this category are events that are perceived but are not committed to memory. We discuss evidence that specific hormonal responses to some experiences will set the neural stage for memory formation, up-regulating those substrate mechanisms that form memories. To illustrate this feature of hormones and memory, this article discusses stress-related hormones that modulate memory formation. Second, it is also clear that the same information impinging on an animal may be processed, learned, and remembered differently depending on the internal state of the animal. The internal state, in this case, might be considered a background state instead of one that is initiated by the experience. Hormones important to stress, reproductive status, and motivational status appear to create conditions that favor learning certain aspects of a situation more than others. In particular, hormones differentially regulate the balance among memory systems, resulting in an animal using one or another strategy to learn a task and resulting in the animal remembering better one dimension or another of that task. In this article, we use estrogens to provide the principal example of hormones that regulate strategy selection.

While this article deals with memory, hormonal effects on memory should be placed into the broader context of the behavioral actions of the hormones – preparing animals for fight-or-flight, for mating behaviors, or for feeding and drinking behaviors. Regulation of these behaviors, including memory, as well as the effects on physiological processes, represents the functions of the hormones. This article extracts only a small portion of the functions of those hormones we describe.

Introduction

The general field of neurobiology of learning and memory focuses on the mechanisms by which neurons and neural systems change their input–output functions. At a cellular and molecular level, the issue is how particular forms of cell–cell communication can initiate plasticity like long-lasting changes in dendritic spines, in receptor number or binding properties, or in the dynamics of neurotransmitter release. Classes of neural plasticity such as these are believed to characterize the major substrate mechanisms of memory formation.

However, there are layers of additional richness that overlie and interact with these processes. First, it is clear that not all neural activity results in long-lasting changes that might represent memory. Most communication between neurons is likely to be responsible for passage

Arousal and Memory

Imagine that you are waiting at a red light before crossing a busy street from east to west on the way to work. Cars are passing in front of you and pedestrians crossing in the direction on your right walk toward and away from you. The light changes to green, your turn to cross arrives, and you walk across the intersection and continue on to work. A few hours later, your co-worker walks to your desk and asks about your morning. “Did you have trouble with the traffic this morning? It took me 30 min longer than usual to get here.” “It sure was cold this morning but the sun shining made it feel warmer than it was.” But now, the

co-worker gets specific. "What color jacket was the woman wearing who crossed the street when you did?" "What make of car passed in front of you just before you crossed the street?" The blank look on your face says that you have no idea how to answer those last questions.

Now imagine a different scenario. You are again waiting at a light before crossing a busy street as before. As you are waiting for your light to change, there is an automobile accident. A woman runs across the street to see if she can help. A car swerves into oncoming traffic to avoid a pile-up. A police officer is at the same corner and you see her call for help as she approaches the accident. The light changes and, since everything is now under control, you walk across the intersection and continue on to work. A few hours later, your co-worker arrives with the same questions. This time questions like "what color jacket" and "what make of car" elicit long and intricate answers.

The amount of information impinging on your nervous system in the two episodes is about the same. Why would you remember the details of one episode and not the other? One answer is that the accident resulted in a set of physiological responses that included up-regulation of memory formation processes. In extreme form, these responses may make memories more indelible, resulting in flashbulb memories or, in more extreme form, recurring and intrusive memories of posttraumatic stress disorder. As an aside, flashbulb memories while subjectively quite strong can be inaccurate. That is an interesting area of study but not one that is considered here. The important point for this article is that the memories are intense and are maintained with great (even if sometimes erroneous) detail for a very long time. In part, it appears that hormonal components of arousal and stress are important in engaging the mechanisms of memory formation.

Epinephrine Enhancement of Memory

One of the best-studied examples of hormonal enhancement of memory formation comes from experiments that examine the effects of epinephrine on memory. Epinephrine is released into blood from the adrenal medulla in response to arousal and stress and is a classic flight-or-fight hormone. The hormone is released in graded manner across levels of arousal and stress. In rats, epinephrine levels in the blood can increase by 10–15 times across increasing levels of arousal.

Initial studies to show that epinephrine enhances memory involved training rats in a one-trial inhibitory (passive) avoidance task and administering epinephrine near the time of training. Memory is tested at 24 h or later. A standard version of this task is a straight alley divided by a door into a well-lit start (safe) compartment and a

dark shock compartment. Because rats prefer the dark, they will quickly cross from the start compartment to the dark compartment. Upon entering that compartment, the rats receive an electric shock to the feet delivered through metal flooring. If the shock level is sufficiently high, the rat will remain in the start compartment on the memory test trial. If the shock level is lower, the rat might hesitate but still cross into the dark compartment. Thus, the latency to cross into the dark compartment is the operational definition of memory; high latencies indicate strong memory while low latencies indicate weak memory. Because the higher and lower shock levels differ in how aversive they are, it is unremarkable that the rats' performance on memory tests reveals better memory after strong versus weak shock.

The question here, however, is: What is the contribution of epinephrine release to making memories strong? To address this question, rats were trained with a low shock intensity and received epinephrine injections immediately after training, that is, a posttraining design (**Figure 1**). The results show that epinephrine injections after low shock intensity resulted in strong memory – as might come from a higher shock intensity – on later test trials. The effects of epinephrine on memory follow an inverted-U dose-response curve; high doses can cause amnesia instead of hypermnesia. The optimal dose of epinephrine resulted in blood epinephrine levels comparable to those obtained by increasing the shock intensity. Therefore, the findings suggest that epinephrine is released in response to an experience in a manner that modulates the formation of memory for that experience.

The posttraining design is of interest in two main respects. First, because the hormone is administered after training is complete, epinephrine effects on memory cannot readily be attributed to nonmnemonic actions such as sensory, motor, or motivational differences. Second, injections of epinephrine delayed by an hour or more after training have no effect on later memory scores, indicating that epinephrine has retrograde and not anterograde effects on later memory and is therefore exerting its effects on the processes responsible for the formation of memory. The retrograde actions fit the endogenous pattern of epinephrine release. In many experiences, the information to be remembered may well precede the release of the hormone by a short time duration.

Since these early experiments, additional research has shown that epinephrine enhances memory for a wide range of tasks, including both aversive and appetitive tasks, and also enhances memory in a wide range of species including the humans. In addition, epinephrine enhances long-term potentiation (LTP) in both acute and chronic preparations, in the latter case especially delaying the rate of decay of LTP. Therefore, epinephrine appears to modulate a form of neural plasticity that may participate in memory formation.

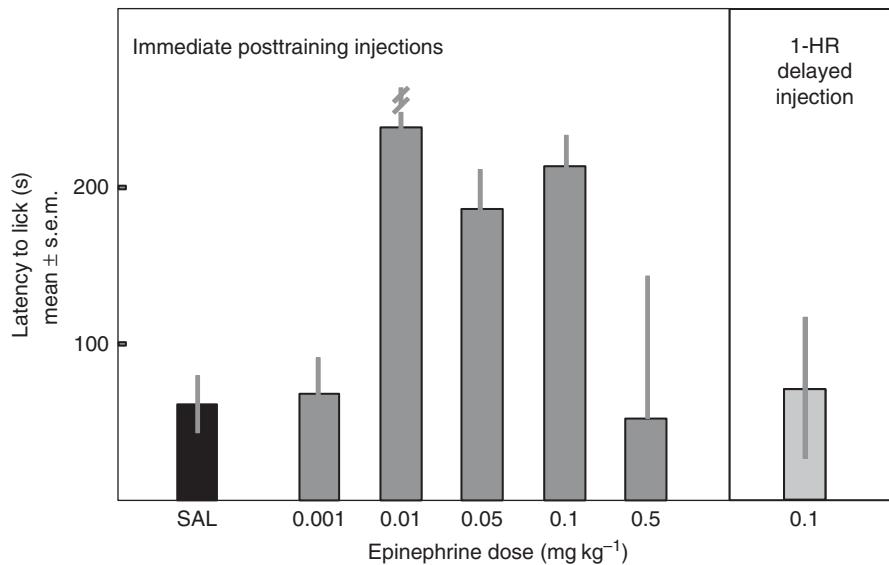


Figure 1 Epinephrine enhancement of memory. Rats received a single injection of epinephrine immediately or 1 h after training on a one-trial inhibitory avoidance task. Memory was assessed 24 h later. Rats that received epinephrine immediately after training exhibited enhanced 24-h memory in an inverted-U dose-dependent manner. The retrograde effects of epinephrine on memory had dissipated by 1 h following training. From Gold PE and van Buskirk RB (1975) Facilitation of time dependent memory processes with posttrial epinephrine injections. *Behavioral Biology* 13: 145–153.

The generality of epinephrine effects extends beyond task and species. Epinephrine may also contribute to both enhancement and impairment of memory by many treatments. The main evidence for this statement is that peripherally administered adrenergic receptor antagonists, blocking the peripheral effects of epinephrine, attenuate the effects on memory not only of epinephrine but also of treatments such as subseizure and supraseizure electrical stimulation of the brain, analeptic drugs, neurotransmitter synthesis inhibitors, and protein synthesis inhibitors. Thus, epinephrine may be part of a common pathway for the effects of multiple treatments on memory. Results suggest that impairments of memory by many treatments – for example, those that produce seizures or inhibit protein synthesis – may reflect extreme physiological stress responses to those treatments, releasing epinephrine and other modulators of memory at levels that reach the high end of inverted-U functions to cause amnesia.

Mechanisms of Epinephrine Enhancement of Memory

Although there is a relatively small epinephrine neurotransmitter system within the brain, epinephrine does not enter the brain from the blood under normal physiological conditions. Therefore, there must be intermediate peripheral mechanisms that are sensitive to epinephrine and then act on the brain to modulate memory. A major contributor to the effects of epinephrine on memory

appears to be glucose. One of the classic effects of epinephrine is to liberate glucose from hepatic stores upon activation of β -adrenergic receptors. There is considerable evidence showing that the increase in blood glucose levels contributes importantly to epinephrine effects on memory. One example is that glucose itself, injected or ingested by laboratory rodents and by humans, enhances memory. In rats, memory enhancement by glucose follows an inverted-U dose-response, as seen earlier for epinephrine. The doses of epinephrine and glucose that are optimal for enhancing memory result in comparable blood glucose levels. However, there are three circumstances in which epinephrine modulation of memory is attenuated while glucose modulation of memory is retained. These nonparallel results each provide good evidence that epinephrine modulation of memory is mediated by increases in blood glucose levels. First, as noted above, adrenergic receptor antagonists block the effects on memory of epinephrine and many other treatments. For epinephrine, there is direct evidence that the receptor antagonist also blocks release of glucose after epinephrine administration, consistent with the idea that epinephrine acts through glucose. In addition, even in the presence of adrenergic receptor blockade, glucose retains the ability to enhance memory, apparently acting downstream from the function of epinephrine. Second, food-deprived rats lose the glucose stores in the liver, making epinephrine ineffective at releasing glucose into blood. Food deprivation results in decreased efficacy of epinephrine enhancement of memory but robust enhancement of memory with glucose. Third, senescent

Fischer-344 rats exhibit poor memory, evident as rapid forgetfulness of new information. During aging, the ability of epinephrine to release glucose and to enhance memory is diminished. However, glucose remains as potent an enhancer of memory formation in aged as in young rats. Thus, these several dissociations of epinephrine and glucose effects on memory each suggest that increases in glucose represent a mechanism downstream from epinephrine in modulating memory.

In contrast to the exclusion of circulating epinephrine from the brain, glucose itself enters the brain via a facilitated uptake mechanism. Still, it remains possible that glucose also acts on peripheral physiology to enhance memory by an additional mechanism outside the brain. Evidence that glucose acts directly on the brain to enhance memory takes two forms. First, direct microinjections of glucose into specific brain targets such as the hippocampus and amygdala enhance memory. Second, measurements of extracellular glucose levels in the hippocampus before and during memory tests indicate that glucose is depleted by these tests and is repleted by peripheral injections of glucose. Interestingly, the depletion of glucose in the hippocampus is far greater in aged rats, which do not exhibit increases in blood glucose levels during training, and memory is restored to the values seen in young rats by either central or peripheral injections of glucose that block the depletion of brain glucose during memory testing.

Understanding the mechanisms by which glucose acts to modulate memory within the brain is an active area of investigation but the story is not complete. One mechanism with some support is that glucose closes potassium-adenosine triphosphate (K-ATP) channels on neurons, thereby increasing excitability and neurotransmitter release. This mechanism is based on the manner in which glucose regulates release of insulin in the β -cells of the pancreas, modulating stimulus-secretion coupling by availability of ATP derived from glucose. Drugs that close and open K-ATP channels enhance and impair memory, respectively, when microinjected directly into the brain. In addition, glucose enhancement of memory is accompanied by increases both in acetylcholine and norepinephrine release at the time of behavioral testing. Both of these are neurochemical modulators of memory processing in the brain. Whether these or other neurotransmitters mediate enhancement of memory by glucose is unclear.

Corticosterone Enhancement of Memory

In addition to the adrenomedullary hormone epinephrine, the adrenal cortical hormone corticosterone also has potent effects on memory. Like epinephrine, corticosterone enhances memory for many tasks, also generally

following an inverted-U dose-response curve and also exhibiting time-dependent enhancement of memory. Unlike epinephrine, corticosterone can enter the brain directly and act on brain steroid receptors. Corticosterone is effective at enhancing memory if direct brain injections are administered into the hippocampus or amygdala. The amygdala is especially important to the effects of corticosterone on memory formation. Lesions of the basolateral nucleus of the amygdala block the effects of corticosteroids on memory. In addition, lesions of the stria terminalis – an output path from the amygdala – also block enhancement of memory by corticosterone. Furthermore, corticosterone does not enhance memory in rats pretreated with injections of β -noradrenergic antagonists into the amygdala. The results obtained with intra-amygdala infusions of β -noradrenergic receptor antagonists are consistent with ideas that noradrenergic mechanisms in the amygdala integrate modulators of memory and regulate memory formation in many brain areas.

Although corticosterone treatments generally enhance memory formation when administered near the time of training, the same corticosterone treatments impair retrieval of memory when administered shortly before test trials. In contrast to modulation of memory formation, impairments of memory retrieval by corticosterone injections do not appear to involve the amygdala as a primary target of the hormone but, instead, the amygdala seems to be permissive for these impairments. Infusions of corticosterone into the amygdala near the time of test trials do not impair retrieval of memory for a swim task. However, lesions of the basolateral nucleus of the amygdala or pretreatment of the basolateral nucleus of the amygdala with β -adrenergic receptor antagonists block impairment of memory at retrieval resulting from intrahippocampal injections of glucocorticoids.

Estrogenic Enhancement of Memory

Estrogens, including estradiol (the predominant form of estrogen produced by the ovary in humans and other mammals), modulate memory formation. In this regard, the basic information is similar to that for epinephrine and corticosterone. Both pre- and posttraining injections of estradiol near the time of training result in enhancement of learning and memory for several tasks. For example, injections of cyclodextrin-encapsulated estradiol, a rapidly metabolized estrogen, given to ovariectomized rats immediately after training in a swim task enhanced memory when tested 24 h later. Similar results have been obtained with other estrogenic compounds administered after inhibitory avoidance, object recognition and place recognition training. In each case, injections delayed by 1–2 h after training had no effect on the later memory

tests. Direct injections of estrogens into the hippocampus also enhance memory. Thus, estrogens are effective retrograde enhancers of memory formation after training in several tasks, providing results similar to those obtained with epinephrine and corticosterone. However, the available information about the dose-response properties, the mechanisms of action on memory, and the brain loci, beyond the hippocampus, for time-dependent modulation of memory is scant at the present time.

Estrogens and Learning Strategies

While estrogens are better known for their enhancing effects on memory, there is growing evidence that performance on some cognitive tasks is impaired by estrogen treatment. The direction of estrogenic effects depends upon several factors including the stress or age status of the animal and the learning strategy required for optimal task performance. Most notably, acute treatments of

estradiol to young adult rats deprived of ovarian hormones through ovariectomy produce impairments in sensorimotor learning that requires rats to make a body turn – that is, right or left – to find food in a maze or to use a cued target to escape water. In these instances, rats with negligible-to-low levels of estrogens perform quite well. In analogous tasks that require rats to use place- or spatial-based strategies in which rats use extramaze cues to guide navigation, the same treatments of estradiol produce enhancements in learning (**Figure 2**). Moreover, when given an option between response and place strategies in dual solution tasks, rats with high estrogen profiles tend to use place strategies while rats with low levels use response strategies. Therefore, both high and low levels of estrogens regulate learning strategy. Learning speed in these dual-solution tasks is similar across rats with different hormonal profiles, further supporting the idea that ovarian hormones regulate not only how much, but also *what* information is acquired. Stress also biases the

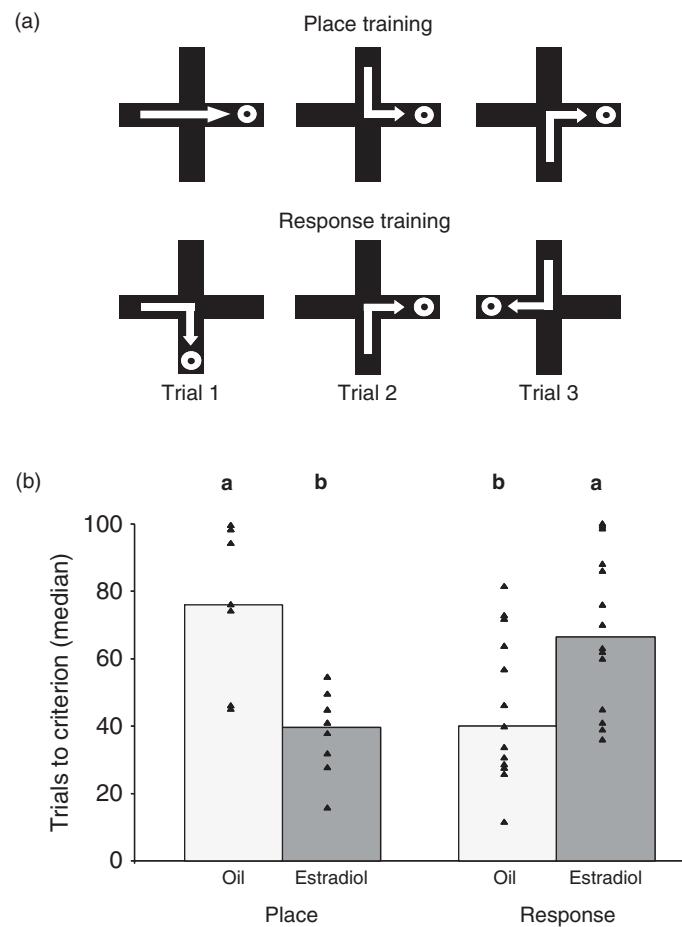


Figure 2 Effects of systemic estradiol on place and response learning in ovariectomized rats. (a) Rats were trained to find food in a plus-shaped maze using either a place strategy, which the goal was always in the same place in the room, or by using a response strategy in which the goal was reached by turning right (or left). (b) Estradiol-treated rats exhibited enhanced learning in the place task relative to oil-treated rats, while the converse was true for response learning. Triangles indicate number of trials for individual rats. Groups marked with different letters are significantly different from each other. From Korol DL and Kolo LL (2002) Estrogen-induced changes in place and response learning in young adult female rats. *Behavioral Neuroscience* 116: 411–420.

relative use of different cognitive strategies, providing possible evidence of convergence between reproductive and stress hormones and highlighting the possibility that a broad array of hormones may regulate learning strategy.

Mechanisms of Estrogenic Effects on Memory

Anatomical substrates and loci of action for estrogens

From findings in male rats, it is well established that response and cued learning is impaired by structural or chemical lesions to the dorsolateral striatum, while place learning is impaired by lesions to the hippocampus. Thus, the opposing effects of estrogens are likely due to differential modulation of these, and perhaps other, memory systems, that is, the specific neural structures and related networks upon which optimal task performance relies. In fact, estradiol infused directly into the hippocampus enhances place learning with no apparent effect on response learning, whereas infusions into the striatum produce response-learning impairments without having an effect on place learning. Estradiol administered to the hippocampus and prefrontal cortex enhances working memory, but with different effective time courses for the two neural loci. From results such as these, it is becoming clear that estrogens potently regulate learning, memory processing, memory formation, and long-term retention through actions across many diverse brain regions.

Some of the earliest reports examining ‘nonreproductive’ regions of the brain pointed to the hippocampus as a particularly estrogen-sensitive tissue. It has been known for decades that high estrogen levels render the hippocampus more seizure prone due to increased neuronal excitability. Increased activity may result from or even lead to other forms of plasticity such as changes in neuronal structure and chemistry. A landmark set of studies in the 1990s demonstrated that the density of dendritic spines and excitatory synapses in the hippocampus fluctuated in synchrony with circulating levels of ovarian steroids either naturally across the rat 4–5-day estrous cycle or following treatments of estradiol in ovariectomized rats. Perhaps the most remarkable characteristics of these synaptic changes are their magnitude and timing. As large as a 40% increase in synapse density can be observed within 2–3 days of estradiol treatment. Equally impressive is that synapse loss with declining levels of hormone occurs over the course of about 1–2 days. Thus, the synaptic content of the brain is in constant flux, with the dynamic regulated by ovarian hormones. Glucocorticoids have also been shown to restructure dendrites in the hippocampus. In this way, estrogens and other neuroactive hormones may orchestrate information processing and subsequent cognitive function.

The focus on the hippocampus as a primary site for estrogenic modulation is warranted given the robust

responsiveness of the hippocampus to estrogen treatment. However, several other brain regions that are implicated in learning and memory, such as the prefrontal cortex, basal forebrain nuclei, striatum, and amygdala, also demonstrate structural, chemical, and functional plasticity following estrogen exposure and most likely play important roles in estrogen modulation of specific aspects of learning and memory. For example, estradiol treatments facilitate acetylcholine synthesis in the striatum and augment amphetamine- or behavior-induced nigrostriatal dopamine release. It is thought that these neurochemical systems are important for reward and stimulus-response based learning. Interestingly, estradiol reduces glutamate signaling through N-methyl-D-aspartate (NMDA) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors in the striatum and thus may impair cortical-striatal plasticity and related behaviors such as response learning.

Neurochemical actions of estrogens

One striking feature of estradiol is its robust effects on neuromodulator systems. Estrogens may act through the modulation of other neuromodulators to produce effects on cognition and underlying neural mechanisms. Several reports show alterations in the synthesis, release, and metabolism of acetylcholine derived from the basal forebrain. Moreover, acute treatments of estradiol facilitate potassium-stimulated and training-induced increases in acetylcholine. In addition, estrogen-induced synaptic change in the hippocampus depends upon the presence of acetylcholine. When selective lesions are made to the cholinergic afferents to the hippocampus, estradiol treatment no longer increases spine density or facilitates place learning, indicating that estrogens may act through acetylcholine modulation of structure and function. Other neurotransmitters such as dopamine, norepinephrine, and serotonin have also been implicated in estrogen-induced changes in brain and cognition. Thus, estrogens may act as ‘metamodulators’ to regulate cognition indirectly by modulating cognitive modulators.

Estrogen receptors

Like other steroid hormones, estrogens up- and down-regulate gene transcription by binding to cognate receptors, translocation to the nucleus of receptor–hormone complexes, and binding to specific response elements on a variety of genes. Estradiol effects on learning may be mediated through the activation of two classical subtypes of estrogen receptors (ERs), ER α and ER β , differentially distributed throughout the central nervous system (CNS). Intrahippocampal ER blockade with an estrogen-receptor blocker can reverse the enhancing effects of systemic estradiol on place and object-recognition learning while intrastratial ER blockade attenuates the impairing effects

of systemic estradiol on response learning, suggesting that estradiol likely acts through ERs in specific brain regions to modulate cognition.

Not all brain regions express classical ERs, not all neurons within a brain region are ER-positive, and not all ER-positive neurons express both subtypes of ERs. The complexity of ER expression may lay the foundation for the complexity of estrogen effects on cognition, in that estradiol activation of ER α produces a different profile of gene activation than does ER β activation. Through a variety of cell biological methods, ERs have been localized to the hippocampus, amygdala, and prefrontal cortex. While the prefrontal cortex is enriched in ER β , both ER α and ER β can be found in the hippocampus and the amygdala; in some cases, both receptor subtypes are expressed in the same neurons. Interestingly, assessments of the mRNA and protein for both ER subtypes suggest little-to-no nuclear distribution of ERs in the striatum. It may thus be surprising that estradiol dramatically alters striatal dopamine release and calcium signaling in dissociated striatal neurons, functions that may also play a role

in estrogen regulation of striatum-sensitive learning. However, recent evidence demonstrates that estrogens can initiate cell-signaling pathways leading to rapid activation of transcription factors, precluding the need for classical, nuclear ERs to produce emergent effects on brain. Importantly, extranuclear ERs have recently been found in dopaminergic neurons in the substantia nigra that project to the striatum and also in cholinergic interneurons in the striatum. It is very likely then that estrogens modulate striatal function and striatum-mediated learning through nonclassical receptor-mediated events. Similar rapid effects of estrogens on hippocampal and cortical neurons have been described, suggesting that parallel rapid and slower pathways are active in neurons in these brain regions (Figure 3).

Estrogens may also produce very different effects on brain function and cognition when acting through ER α , ER β , or both subtypes, irrespective of the mode of ER activation, that is, by rapid or slow actions. Based primarily on early behavioral reports and on ER distribution patterns in different brain regions, for example,

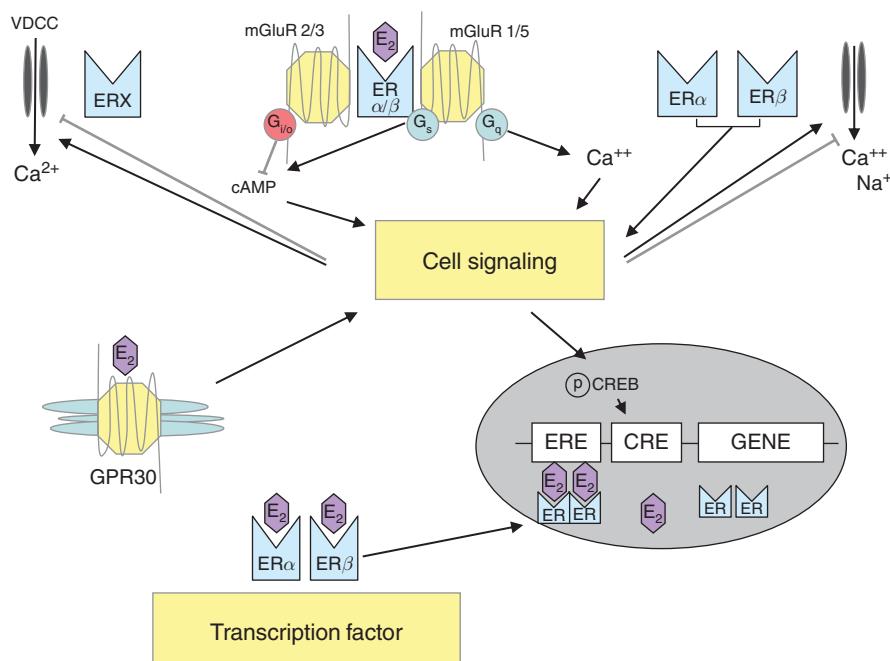


Figure 3 Schematic depicting cellular mechanisms of estradiol (E₂) signaling. Shown in this graphic is a subset of possible pathways through which estrogens can influence neuronal cell function and thus learning and memory through several different mechanisms. The classic mode of estrogen signaling is through estrogen receptors (ERs) that act as transcription factors. Both ER α and ER β subtypes of ERs translocate to the nucleus upon ligand binding, dimerize, and bind to estrogen-response elements (EREs) on genes either to activate or to repress transcription. Estrogens can also act through nonnuclear means to activate or inhibit signaling cascades, some of which may act on L-type or voltage-dependent calcium channels (VDCC) or neurotransmitter channels (NMDA/AMPA) while other cascades activate, by phosphorylation, the transcription factor, cAMP response-element-binding protein (CREB). In both hippocampal and striatal neurons it has been suggested that membrane-associated ER α and ER β interact with metabotropic glutamate G-protein-coupled receptors (GPCR) to increase and decrease phospho-CREB (pCREB) depending upon the glutamate receptor subtype and the activation status of the cells. A new GPCR, GPR30, that binds estradiol has been found in endoplasmic reticulum of neurons. These nonclassical forms of signaling can be quite rapid in onset, despite the possibility for longer more durable effects through pCREB-related gene transcription. Grey lines indicate inhibition, black arrows activation.

hypothalamic versus other, it has been suggested that estrogenic regulation of sexual behavior relies on ER α activation but regulation of cognition relies on ER β activation. Direct tests of the involvement of different ER subtypes in estrogenic modulation of cognition have been made by using compounds that bind preferentially to one ER subtype, including phytoestrogens, such as genistein derived from soy, that selectively bind ER β and selective ER agonists or modulators (SERMs) targeted toward ER α or ER β . ER β -selective agonists enhance memory in a variety of contexts, for example, in the spatial version of an escape swim task and in a passive avoidance task. Interestingly, ER α agonists are also effective memory enhancers for passive avoidance, suggesting a role both for ER α and ER β activation in learning and memory. The evidence pointing to the cognitive role of one ER subtype over another is clearly mixed. However, while it is tempting to sort estrogenic effects solely by ER subtype, it is perhaps more parsimonious to sort effects by neural systems that are engaged during behavior, and thus, by association, the pattern of ER subtypes across these different neural regions.

Temporal aspects of estrogen-induced modulation

As alluded to above, estrogens may act on cognition through mechanisms that are slower in onset, through rapid-signaling events, or both. The induction of dendritic spines in the hippocampus, shown to occur across the estrous cycle and to peak after 48 h of estrogen exposure, can occur rapidly within 30 min of estradiol exposure, most likely through nongenomic signaling processes involving mitogen-activated protein kinases. Many of the cognitive effects of estradiol can be also seen within 1–2 h of treatment. For example, estradiol infused into the striatum 2 h prior to training impairs response learning to the same degree as 2 days of exposure. Immediate treatments of estradiol, but not those delayed by 2 h, either into the hippocampus or systemically, enhance spatial learning and memory. Together, these data suggest that estrogen actions are rapid in onset and may modulate both learning processes and memory-formation mechanisms. The activating effects of steroids may promote or facilitate the genomic response. Equally likely, and not mutually exclusive, the slower, more trophic actions of steroids may provide an activated neural state or platform upon which the faster, activating effects may take place. In this way, hormones may put the brain into a state ready for plasticity.

Acknowledgments

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See also: Amnesia; Animal Models of Learning and Memory; Basal Ganglia; Cholinergic Systems in Aging and Alzheimer's Disease: Neurotrophic Molecular Analysis; Cognition: Learning and Memory: Spatial; Cognitive Decline in Laboratory Animals: Models, Measures, and Validity; Effects of Stress on Learning and Memory; Emotion–Cognition Interactions; Hormonal Contributions to Arousal and Motivation; Hormones and Memory; Memory and Aging, Neural Basis of; Memory Consolidation; Neurotransmitters and Neuromodulators Regulating Sleep and Wakefulness; Psychoneuroendocrinology of Stress; Sex Hormones, Mood, and Cognition.

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Male Sexual Behavior

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Glossary

Corpora cavernosa – A pair of sponge-like tubes along the length of the penis that contain most of the blood during erection; they merge at the head of the penis.

Corpus spongiosum – A sponge-like tube along the bottom of the penis that contains the urethra and enlarges at the distal end to form the glans penis.

Postejaculatory interval – The time after an ejaculation until the next vaginal intromission.

Spinal nucleus of the bulbocavernosus (SNB) – A nucleus in the lumbosacral spinal cord that exerts somatomotor control of genital reflexes through the pudendal nerve, which splits into motor and sensory branches, both of which also carry sympathetic efferents.

Urethrogenital reflex – A model of ejaculation and orgasm elicited in anesthetized, spinally transected male rats by distending the urethra with saline and then releasing the pressure; it is characterized by clonic contractions of the perineal muscles, rhythmic firing of the cavernous nerve, erection, and ejaculation.

Vomeronasal organ – A pair of tunnels along the sides of the nasal cavity that contain receptors for species-specific chemosensory stimuli; it is the first processing stage of the accessory olfactory system.

Most, but not all, male mammals ejaculate after multiple intromissions. Ejaculation in rodents includes a deeper, longer thrust during which semen is ejected. The male dismounts and begins genital grooming. Male canids begin to ejaculate soon after penile insertion; a swelling at the base of the penis results in a lock of the male to the female. Male ungulates ejaculate immediately upon intromitting. In humans, rhythmic contractions of skeletal and striated perineal muscles accompany ejaculation and are associated with orgasm.

Ejaculation is followed by sexual quiescence, the post-ejaculatory interval (PEI). In rats, this lasts 5–10 min and is accompanied by 22 kHz ultrasonic vocalizations during the first 75%, called the absolute refractory period, because the male will not copulate in response to any stimulus. During the remaining PEI, the relative refractory period, the male may resume mating in response to nonspecific arousal or a novel female partner. After the PEI, male rats copulate until they reach satiety, often after seven to nine ejaculations. Copulation to satiety increases dopamine (DA) metabolites in the medial preoptic area (MPOA) and nucleus accumbens (NAcc) and enkephalins in the hypothalamus. Androgen receptor density decreases in the MPOA, NAcc, and ventromedial hypothalamic nucleus (VMH).

Sexual Experience

Sexual experience decreases latencies to copulate and to ejaculate. It also may decrease the disruptive effects of a novel environment, various lesions, and castration. Experienced rats have higher levels of nitric oxide synthase (NOS) in the MPOA, greater androgen secretion, and more cells in the MPOA and NAcc activated by ejaculation.

Puberty and Aging

Male rats begin mounting between 40 and 50 days of age, intromitting between 44 and 75 days, and may show the behavioral pattern of ejaculation between 48 and 75 days.

Patterns of Male Sexual Behavior

Description of Behavioral Elements

Most male mammals mount females dorsally from the rear. The female may assume a reflexive dorsiflexion of the spine, called lordosis. The male then begins pelvic thrusts, which may or may not result in vaginal insertion. During a nonintromissive mount, the male dismounts slowly. Intromission is characterized by a deeper thrust, followed by a springing dismount and genital grooming.

Prepubertal castration prevents copulation, while exogenous testosterone (T) or estrogen (E) can hasten its onset. However, exogenous hormones cannot hasten copulation in male Syrian hamsters. In male rats, T levels start to rise about day 40, with a surge occurring around day 50. However, the T surge occurs after the onset of copulatory behaviors and *ex copula* reflexes. Copulation in male hamsters begins after the increase in T begins, but before the T surge; the lack of pubertal T impairs T-induced mating in adulthood.

Aging in humans, monkeys, and rodents decreases the probability of mating and increases latencies to mount, intromit, and ejaculate. In male rats and humans T levels decline, but exogenous T may only partially restore sexual ability. A decline in estrogen receptors (ER), but not androgen receptors (AR), may mediate the ejaculatory deficit of old male rats. Behavioral deficits in middle-aged rats (18–19 months) may be associated with decreased DA and norepinephrine (NE), and increased serotonin (5-HT), in the NAcc and MPOA.

Sexual Reflexes

Observations during Copulation

In many species, the penis is visible during copulation; however, erections in rodents are very brief and hidden from view. Erection, intromission, and ejaculation are usually inferred from characteristic behaviors, and the female's vagina can be examined for the presence of sperm. Penile pressure and electrical activity in the striated perineal muscles can be measured during copulation; however, these techniques are technically difficult. Therefore, *ex copula* measures of sexual reflexes are often used. However, *in copula* and *ex copula* erections may differ in their hormonal, physiological, and neurochemical regulation.

Ex-Copula Sexual Reflexes

Male rodents occasionally have erections without any obvious sexual stimulus; such erections may be increased by certain drugs or the presence of an inaccessible receptive female. They consist of extension of the engorged glans from the penile sheath and are often accompanied by genital grooming. Reflexive erections can be elicited by manual stimulation in many species. However, such stimulation in rats inhibits erection. Genital reflexes can be elicited in rats or mice by restraining them on their backs and retracting the penile sheath. Pressure around the base of the penis elicits erections and anteroflexions (flips); seminal emission may also occur. The urethrogenital reflex in anesthetized, spinally transected male rats is a model for both erection and ejaculation. The urethra is distended with saline; when the pressure is released,

clonic contractions of the perineal muscles occur, with rhythmic firing of the cavernous nerve, erection, and ejaculation. A similar pattern is observed in human climax and rats' ejaculation.

Mechanisms of Erection

Erection of the vascular penes of humans, monkeys, dogs, cats, and rodents results primarily from vascular relaxation, coordinated with striated muscle contraction, whereas the fibroelastic penes of ungulates, such as sheep and goats, are extruded by the penile muscles. Most of the penile shaft comprises the paired corpora cavernosa; the corpus spongiosum surrounds the urethra and enlarges into the glans at the end of the penis. The corpora cavernosa are enclosed by a tough capsule, so when they fill with blood, pressure against the venous outflow traps blood in the penis. Contraction of perineal striated muscles enhances the erection.

Three major pathways control penile erection: the pelvic nerves (mostly parasympathetic, proerectile), the hypogastric nerves (sympathetic, antierectile), and the pudendal nerves (somatosensory and motor). The pelvic nerve exits the lumbosacral spinal cord and travels to the penile corpora and vasculature through the pelvic plexus and cavernous nerve. It also carries some sympathetic axons. The spinal nucleus of the bulbocavernosus (SNB) exerts somatomotor control through the pudendal nerve, which splits into motor and sensory branches, both of which also carry sympathetic efferents. Stimulation of the striated perineal muscles increases rigidity of an erection, but does not result in erection if the penis is flaccid. Sympathetic, primarily antierectile influence arises from two sources. The lumbar splanchnic nerves synapse in the hypogastric plexus, from which hypogastric nerves travel via the cavernous nerve to the penis. Axons from the paravertebral sympathetic chain travel via the pelvic nerve to the pelvic plexus, then through the cavernous nerve to the penis. Tonic sympathetic input keeps the penis flaccid. However, the sympathetic system contributes to erection, perhaps by constricting nonpenile vessels, thereby diverting blood to the penis.

Cellular Mediators of Erection

The main mediator of erection is nitric oxide (NO), a soluble gas produced by nitric oxide synthase (NOS), that acts as both a second messenger and a neurotransmitter. Parasympathetic nerves contain neuronal NOS (nNOS), and the endothelium contains endothelial NOS (eNOS). NO from parasympathetic nerves diffuses into smooth muscle cells and activates guanylyl cyclase, which produces cGMP, which then activates protein kinase G, and to some extent protein kinase A. These enzymes phosphorylate proteins that sequester Ca^{2+} , leaving less in

the cytoplasm and relaxing the smooth muscle. Phosphodiesterase 5 (PDE₅) terminates cGMP activity. Sildenafil citrate (Viagra), tadalafil (Cialis), and vardenafil (Levitra) treat erectile dysfunction by inhibiting PDE₅. The initial increase in blood flow induces shear stress in endothelial tissue, which activates eNOS and prolongs the erection. Erection can also be elicited by vasoactive intestinal peptide, calcitonin gene-related peptide, and prostaglandin E₁.

Ejaculation

Ejaculation depends on coordinated autonomic and somatic responses. Seminal emission includes autonomic activation of the prostate, and ejection involves rhythmic contraction of perineal and pelvic floor striated muscles. Friction on penile skin and intravaginal pressure stimulate seminal drain into the posterior urethra, and chemical and mechanical stimulation of the urethra by the semen triggers expulsion. A central pattern generator in the lumbosacral cord includes a group of galanin-containing neurons that integrate and relay genital sensory and motor signals related to ejaculation. Serotonin (5-HT) from the nucleus paragigantocellularis (nPGi) in the medulla tonically inhibits ejaculation, though intraspinal 5-HT may act through 5-HT_{1A} and 5-HT_{2C} receptors to elicit ejaculation. Dopamine (DA), norepinephrine (NE), acetylcholine (ACh), and oxytocin may also stimulate ejaculation.

Role of Gonadal Steroids

Testosterone (T) and its Metabolites

Male sexual behavior is heavily dependent on T and its metabolites estradiol (E₂) and dihydrotestosterone (DHT). Although steroids are essential for mating in most rodents, they play a more modulatory role in humans. T exerts organizational effects during sex differentiation and activational effects in adulthood. T has primarily slow, genetically mediated effects, although it can also have faster effects through membrane receptors. T levels are higher than necessary to activate sexual behavior; higher levels are needed for sperm production in the testes.

Castration and T Restoration

Plasma levels of T become immeasurable by 24 h after castration, though male rats may continue to copulate for days or weeks. However, intromission latency increases within days, and the number of intromissions before ejaculation actually decreases; thus, T may increase intromissions preceding ejaculation, thereby increasing sperm in the ejaculate and triggering a progestational state in the female. Half to two-thirds of men who were

castrated as treatment for sexual offenses lost sexual interest rapidly, while others reported gradual decreases. Long-term castrated rats require 5–10 days of T to restore mating; 5–7 weeks are required for hamsters. However, in rats, T affected MPOA firing within minutes, and rats and mice started mounting in 35 and 60 min, respectively. Thus, steroids activate brain areas within minutes but require slower genomic effects to restore copulation fully. Compared to copulation, *ex copula* reflexes are lost more quickly after castration and restored more rapidly after T replacement. In spinal transected rats, reflexive erections were decreased 24 h after castration and restored by 24 h of T replacement. Spinally intact males required an additional day of T, probably to reduce supraspinal inhibition.

Role of T Metabolites

T is primarily a prohormone, being converted in target organs to either E₂ or DHT. There are at least two E receptors, ER α and ER β . Both T and DHT bind to the AR, but DHT binds with greater affinity. Some target cells produce both E₂ and DHT and have both ERs and ARs. The relative importance of estrogenic and androgenic stimulation is species specific. In castrated rats and mice, E₂ can reinstate most aspects of copulation. DHT, which cannot be aromatized to E₂, cannot. However, E is usually insufficient to fully maintain or restore copulation. Thus, stimulation of both ER and AR is necessary to fully restore mating. In addition, DHT is both necessary and sufficient to maintain and restore *ex copula* reflexes in rats. Gonadally intact males that lack ER α (ER α knockout mice, ER α KO) or aromatase (ArKO) have almost no ejaculations. However, treatment with T and a dopamine agonist in ER α KO mice restored copulation. Aromatization is not required in other species, including rabbits, guinea pigs, hamsters, deer mice, and monkeys. However, males normally produce both classes of hormone, which together promote all aspects of mating. Men with erectile dysfunction have relatively normal T levels. However, T or DHT treatment of hypogonadal men or aging men with moderate decreases in T can improve erectile function.

Systemically and Intraventricularly Injected Drugs

Dopamine (DA)

In the late 1960s, the DA precursor L-Dopa was found to increase libido and sexual potency in Parkinsonian patients. In rats, mice, and men, systemically administered DA agonists facilitate, and DA antagonists impair, copulation and sexual motivation. There are two families of DA receptors: the D₁-like family activate adenylyl cyclase

and comprise the D₁ and D₅ subtypes; the D₂-like family consists of D₂, D₃, and D₄ subtypes that inhibit adenylyl cyclase. D₁-like agonists increase sexual motivation in rats and mice and facilitate sexual behavior across taxa, including whiptail lizards, geckos, quail, and starlings. The effects of DA agonists are dose dependent; low doses facilitate, and high doses inhibit copulation, perhaps by inducing stereotypic behavior. There are contradictory effects of D₁- and D₂-like agonists on *ex copula* reflexes, due perhaps to lack of selectivity of the agonists.

Norepinephrine (NE)

NE can either facilitate or inhibit male sexual behavior, depending on the dose and receptor subtype activated. Sympathetic axons to the penis promote detumescence. However, increased NE activity, either by blockade of α₂ autoreceptors or stimulation of α₁ adrenoceptors, can increase sexual arousal. The influence of β adrenoceptors is not clear.

Serotonin (5-HT)

5-HT generally inhibits male sexual behavior. Selective serotonin reuptake inhibitors (SSRIs) increase 5-HT in the synapse and impair sexual function in humans and rats. However, 5-HT_{1A} receptor stimulation markedly facilitates male rat ejaculation. 5-HT's inhibitory actions may be mediated by 5-HT_{1B} and 5-HT₂ receptors in rats. In mice, in contrast to rats, both the 5-HT_{1B} and the 5-HT_{1A} subtypes inhibit mating. Stimulation of 5-HT_{2C} receptors can increase erections and inhibit ejaculation in monkeys and facilitate erection in rats.

The fact that the facilitative effects of 5-HT_{1A} agonists on ejaculation are opposite to those of 5-HT itself and that somatodendritic autoreceptors are the 5-HT_{1A} subtype, suggest that 5-HT_{1A} agonists act at those auto-receptors to reduce 5-HT release. However, facilitative effects of 5-HT_{1A} agonists are obtained after infusion into the sites where only postsynaptic receptors are found. Therefore, postsynaptic receptors may mediate the facilitative effects.

Glutamate

Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS). Low doses of kainic acid, an agonist at α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate glutamate receptors, enhance copulatory behavior in sexually sluggish male rats, but not in good copulators. Systemic injection of an N-methyl-D-aspartate (NMDA) antagonist impairs mating in experienced and naïve male rats and blocks the improvement due to noncopulatory exposures to estrous females.

Nitric Oxide (NO)

NO facilitates erection (parasympathetic) but inhibits ejaculation (sympathetic). Neuronal nitric oxide synthase knock-out (nNOS KO) mice have normal penile function due to a compensatory increase in endothelial NOS (eNOS) and ejaculate with fewer mounts and intromissions. Intraperitoneal injection of NO's precursor, L-arginine, enhances copulation in naïve and experienced rats, whereas intracerebroventricular (icv) injection of a NOS inhibitor impairs mating in naïve rats.

Endocannabinoids

Endocannabinoids are retrograde neuromodulators. The cannabinoid CB1 receptor is located almost exclusively at axon terminals, where it inhibits neurotransmitter release. Delta-9-tetrahydrocannabinol (THC) and other endogenous and exogenous CB1 agonists impair copulation in mice and rats, while a CB1 antagonist accelerates ejaculation.

Endogenous Opioids

There are three major classes of endogenous opioid peptides: endorphins, enkephalins, and dynorphins. Exogenous opiates, such as morphine and heroin, impair sexuality in male addicts and in various other species. Furthermore, sexually inactive male rats have increased basal concentrations of several endogenous opioids in the hypothalamus. Endogenous opioids act on μ, δ, and κ receptors, with μ and δ receptors mediating most of the inhibitory effects. Opioid antagonists facilitate sexual behavior in sexually inactive or naïve rats, reverse sexual satiation, decrease ejaculatory threshold, and increase the percentage of ejaculating animals.

However, opioids may also have facilitative effects, depending on dose, brain site, time of the day, and sexual-activity level of the animals. Opioid peptides are apparently released during mating, since physiological mechanisms of analgesia and reward are activated during sexual behavior, and naloxone blocks both. Systemic morphine inhibits reflexive erections and seminal emission; naloxone antagonizes those effects, but a low dose, administered alone, also inhibits erection, suggesting that some opioid activity can facilitate reflexes.

Oxytocin (OT)

OT is released from the posterior pituitary and in numerous brain and spinal sites. Systemic or icv injections of OT facilitate copulation in male rats, and icv injections of an OT antagonist impair or abolish copulation. Systemic OT can reverse the inhibitory effects of chronic fluoxetine, an SSRI. Furthermore, OT in cerebrospinal fluid markedly increases after copulation.

Gonadotropin-Releasing Hormone (GnRH)

GnRH stimulates the anterior pituitary to release gonadotropins. GnRH is released naturally when male rodents encounter female vaginal fluid. It facilitates motivation or copulation in several rodent species and exogenous GnRH restores fertility and sexual activity in hypogonadal men. However, adverse effects of GnRH can result from continuous high doses, which inhibit LH release and gonadal function; endogenous GnRH is released in a pulsatile fashion.

Orexin/Hypocretin

Orexin/hypocretin (orx/hcrt) is produced in neurons of the perifornical lateral hypothalamus that project to monoaminergic nuclei in the midbrain and brainstem and to basal forebrain areas, including the medial preoptic area (MPOA). Orx/hcrt regulates feeding and wakefulness and also enhances male sexual behavior. Orx/hcrt expression is decreased after castration and restored by E₂, and systemic administration of an orx/hcrt antagonist impairs copulation.

Other Neurotransmitters

GABA is the main inhibitory neurotransmitter in the mammalian brain. Systemic administration of GABAergic drugs inhibits male rat sexual behavior, and cerebrospinal fluid levels of GABA increase dramatically during the postejaculatory interval (PEI). Prolactin (PRL), secreted by the anterior pituitary, promotes

lactation but has numerous other functions. Hyperprolactinemic patients often have erectile problems and low sexual desire. Plasma PRL levels increase markedly after ejaculation in men and may contribute to postejaculatory refractoriness. Chronically elevated PRL impairs male rat sexual behavior, but short-term PRL has no effect or facilitates copulation. Early studies using high doses of acetylcholine agonists and antagonists produced contradictory results. However, lower doses of nicotine and muscarinic agonists have produced facilitative effects.

Brain Areas and Circuitry

See **Table 1** for a summary of neurotransmitter effects in specific brain areas.

Sensory Inputs

Olfactory bulbs

Volatile odors are transduced by receptors in the nasal mucosa, whose axons project through the cribriform plate to the main olfactory bulb (MOB). Both nonvolatile and volatile species-specific cues are detected in the vomeronasal organ (VNO), located at the base of the nasal cavity and projecting to the accessory olfactory bulb (AOB). Vomeronasal cues are important in rodents, but the VNO and AOB are regressed in humans. Olfactory bulbectomy abolishes mating by male Syrian hamsters, though the relative importance of the main and accessory systems is not clear. Male rats are less dependent on chemosensory

Table 1 Brain areas and neurotransmitter effects on male sexual behavior

	MeA	MPOA	PVN	Mesolimbic Tract	LH	nPGi	Spinal Cord
Dopamine		↑ cop. D1 ↑ erec. D2 ↑ ejac.	↑ erec., ejac	↑ cop.			↑ erec., ejac.
Norepinephrine							↑ erec., ejac.
Serotonin	1A ↑ cop.	1A ↑ cop. 1B ↓ cop.	1A ↑ cop.		↓ cop.	↓ cop.	1A, 2C ↑ cop
Acetylcholine		↑ cop.					↑ erec., ejac.
Oxytocin		↑ cop.	↑ erec., ejac.				↑ erec., ejac.
Glutamate		↑ cop.	↑ erec., ejac.				
GABA		↓ cop.					↓ erec., ejac.
Opioids		Lo dose ↑ Hi dose ↓ cop.	↓ erec.	↑ DA			
Nitric oxide		↑ cop.	↑ erec., ejac.				
hypocretin/orxin		↑ cop.					
Galanin/Cholecystokinin/Neurokinin					↑ cop.		↑ ejac.

MeA, medial amygdala; MPOA, medial preoptic area, PVN, paraventricular nucleus, LH, lateral hypothalamus; nPGi, nucleus paragigantocellularis; GABA, gamma amino butyric acid; cop., copulation; erec., erection; ejac., ejaculation

stimuli, though bilateral bulbectomy severely compromises mating in some males. In rats and mice, the main olfactory system is more critical than the vomeronasal system. Odor cues result in neurons immunoreactive for the immediate early gene product c-Fos (Fos-ir) in the MOB and AOB of male hamsters, rats, and mice, whether they can mate or not; however, only experienced copulators show Fos-ir in all downstream structures of the VNO.

Amygdala

The amygdala is a collection of nuclei that contribute to learning, motivation, and fear (central nucleus and basolateral division) and chemosensory processing and social behaviors (corticomedial division). The medial amygdala is larger in males than in females, and the corticomedial region is critical for integration of chemosensory, genito-sensory, and hormonal stimuli. Corticomedial lesions impair copulation, with the severity dependent on the specific location and species. The posterodorsal medial amygdala (MeApd) is one site at which serotonin 5-HT_{1A} agonists facilitate copulation. A subregion of the MeApd is linked to sexual satiety. Fos-ir in the MeA, but not the MPOA, of male rats correlates with the length of the PEI, suggesting that some neurons there contribute to postejaculatory quiescence. T or E, but not DHT, implants in the MeA of castrated rats and hamsters delay the loss of, or partially restore, sexual behavior. However, DHT implants in male rats treated with subthreshold systemic E can restore mating. Thus, both ARs and ERs in MeA contribute to mating.

A major output of the MeA is to the MPOA. Unilateral lesions of the MPOA impair, but do not abolish copulation in male rats or gerbils; however, contralateral lesions of the MeA and MPOA severely disrupt mating. Chemical stimulation of the MeA increases MPOA DA release similar to that during copulation, and microinjection of a DA agonist into the MPOA restores copulation abolished by large lesions of the MeA. Small radiofrequency MeA lesions do not affect basal MPOA DA but do eliminate the DA response to a female and also impair copulation. Thus, MeA activity increases MPOA DA in anticipation of and during mating. The MeA of rats contains no dopaminergic neurons, but MeA efferents may directly or indirectly activate MPOA DA cell bodies or terminals.

Bed nucleus of the stria terminalis (BST)

Axons from the MeA either travel directly to the MPOA or synapse in the BST, which then relays information to the MPOA and other sites. However, the BST does more than simply relay input. The posteromedial BST has abundant steroid receptors and is important for male sexual behavior. Males also have more BST neurons that contain arginine vasopressin, galanin, and the aromatase enzyme, which converts testosterone to estradiol. In

male rats, hamsters, and gerbils, copulation, or to a lesser extent, exposure to female odors, elicits Fos-ir in the BST. However, mating decreases Fos-ir in male macaques.

Central tegmental field and subparafascicular nucleus of the thalamus (CTF/SPF)

The midbrain tegmentum, MPOA, MeA, and anterior hypothalamus are reciprocally connected. Subregions of the tegmentum have been called the central tegmental field (CTF) or dorsolateral tegmentum (DLT). The CTF/DLT is dorsal to the lateral half of the substantia nigra and may include the adjacent subparafascicular nucleus (SPF) and several other nuclei. Bilateral lesions of the CTF in rats impair mating but not sexual motivation. The medial parvocellular division of the SPF (SPFp) relays somatosensory input from the genitals to the MPOA and MeA. In male rats, the SPFp receives projections from lumbar spinothalamic neurons that are essential for ejaculation. Fos-ir is increased in the CTF or SPFp only after ejaculation in rats, gerbils, hamsters, and musk shrews. In men, ejaculation stimulates blood flow in the SPFp. In addition, electrical stimulation of the CTF facilitates mating in rats. SPFp neurons contain ARs, and many AR-ir neurons that project to the MPOA express ejaculation-induced Fos-ir. Thus, the SPFp and CTF convey ejaculation-related somatosensory input to higher brain areas.

Major Integrative Sites

Medial preoptic area (MPOA)

The MPOA is a critical integrative site for male sexual behavior. It receives indirect input from all sensory modalities and sends reciprocal connections to modify processing of that input. Steroid receptors in the MPOA and its afferents bias input to favor sexually relevant stimuli. Efferents to hypothalamic, midbrain, and brainstem nuclei regulate somatomotor or autonomic patterns and motivational states. A medial periventricular zone regulates neuroendocrine function, and a medial zone, including the medial preoptic nucleus (MPN) and posterodorsal preoptic nucleus (PdPN), controls male sexual behavior and maternal behavior. Large MPOA lesions abolish copulation in numerous species. More severe deficits occur with more caudal lesions that include part of the anterior hypothalamus. MPOA lesions also diminish, but do not eliminate sexual motivation. Stimulation of the MPOA facilitates sexual behavior in numerous species, but does not reverse sexual satiety. Repeated electrical stimulation of the MPOA in noncopulating male rats can lead to mating on subsequent stimulation-free tests. In anesthetized rats, MPOA stimulation increases intracavernous pressure and can elicit the urethrogenital reflex, even without urethral stimulation. Axons from the MPOA do not project directly to the lumbosacral cord, but

stimulate downstream sites that then control the reflexes. However, the MPOA is not necessary for genital reflexes.

Steroid implants in the MPOA facilitate sexual behavior in castrated rats, ferrets, birds, and lizards, but do not completely restore mating. Aromatization of T in the MPOA is important for T's facilitative effects. T implants in castrated quail facilitated copulation correlated with induction of aromatase immunoreactivity (ARO-ir) in the preoptic area. Sexual preference in rams is also related to aromatization in the MPOA. Rams that prefer to mate with other males have lower levels of serum T and E and decreased MPOA aromatase activity, compared with those that prefer to mate with females. In contrast to E, DHT in the MPOA of castrated male rats is relatively ineffective, unless accompanied by subthreshold systemic E or DHT. However, an antiandrogen in the anterior MPOA impairs copulation, but not partner preference; in the posterior MPOA it impairs motivation, but not performance.

Neurons of the periventricular DA system, along the third ventricle, project laterally into the MPOA and anterior hypothalamus. MPOA microinjections of classic D₁/D₂ agonists and antagonists facilitate and inhibit, respectively, copulation, *ex copula* reflexes, and sexual motivation. A DA agonist in the MPOA can restore copulation in males with large amygdala lesions. D₁-like agonists facilitate parasympathetically mediated erections and the early phase of copulation, and D₂-like drugs produce dose-dependent effects. Low doses disinhibit reflexes (decrease latencies), but high doses of D₂-like agonists, or of D₁ antagonists, shift autonomic balance toward sympathetically mediated ejaculation. It is not clear whether these dose-dependent effects are mediated by different receptor subtypes or populations of neurons with different levels of tonic inhibition.

Large doses of serotonin (5-HT) microinjected into the MPOA inhibit copulation, in part through 5-HT_{1B} receptors. However, reverse-dialysis of a 5-HT_{1A} agonist into the MPOA facilitates mating and increases both DA and 5-HT levels. Some of these facilitative effects are mediated by increased extracellular DA, stimulating D₂-like receptors. There are numerous GABAergic neurons in the MPOA of male rats, and some mating-activated neurons in male gerbils contain GABA. Enhancing MPOA GABAergic transmission impairs copulation in rats and blocking either GABA synthesis or GABA_A receptors enhances mating. However, a GABA_A antagonist did not reverse sexual satiation. Microinjection of low doses of a μ or a κ opioid agonist into the MPOA can facilitate copulation, and a μ antagonist prevents induction of sexual reinforcement. However, high doses of μ agonists impair copulation. Microinjection of NE facilitates sexual behavior, and decreasing NE levels by stimulating autoreceptors impairs copulation.

Reverse-dialysis of the NO precursor L-arginine into the MPOA facilitates copulation, and a NOS inhibitor impairs it, but increases the number of *ex copula* seminal emissions. NO also mediates the facilitative effects of repeated exposures to an inaccessible estrous female. Castration in both rats and hamsters decreases NOS-ir in the MPN; there is also less NOS-ir in ER α KO mice. nNOS is co-localized with both ER α and AR in the MPOA of rats, mice, and hamsters. Reverse-dialysis of an NO donor into the MPOA of castrated rats maintained on systemic DHT (to maintain genital and sensory structures) fully restored copulation in half the animals. NO's effects are at least partially mediated by cGMP.

Glutamate microinjections into the MPOA of anesthetized male rats elicit erectile responses and also the urethrogenital reflex without genital stimulation. Conversely, an NMDA antagonist inhibits mating in male rats. Nearly all neurons in the MPOA that show mating-induced Fos-ir contain NMDA receptors, and an NMDA antagonist decreases mating-induced Fos-ir. Thus, glutamate in the MPOA facilitates mating, at least in part through NMDA receptors.

Orx/hcrt, acetylcholine, and prostaglandin E₂ in the MPOA also exert facilitative effects. Some MPOA neurons increase their firing rates only before male rats or monkeys copulate; others increase only during mating. DA levels in male rats rise in the presence of an inaccessible female and increase further during mating; the DA response shows both anatomical and behavioral specificity. Both basal and female-stimulated DA levels are hormone dependent, with E mediating most, but not all, of the facilitative effects of T on DA levels and behavior. Tissue (stored) DA levels are actually higher in castrates than in intact males, suggesting that castration impairs DA release, but not synthesis. Release is controlled by NO, and T and E positively regulate MPOA NOS-ir. MeA lesions block the DA response to a female but do not affect basal levels; those males show suboptimal mating ability. Similarly, olfactory bulbectomy in hamsters impairs both copulation and MPOA DA release.

Copulation increases Fos-ir in the MPOA of male rats, gerbils, hamsters, and mice. At least some of the Fos-ir is in AR-containing neurons. Previous sexual experience enhances Fos-ir to sexual stimuli in rats and hamsters. Efferents from the MPOA project to other areas of the hypothalamus, midbrain motivation and somatomotor regions, and midbrain and brainstem areas that project to the spinal cord. These connections are mostly reciprocal, allowing downstream sites to influence their own input. Output to the nPGi may disinhibit genital reflexes, while other efferents may activate autonomic areas that regulate erection and ejaculation.

Mesocorticolimbic and Nigrostriatal DA Tracts

DA cell bodies in the VTA send axons to the NAc and mPFC; this mesocorticolimbic tract is critical for motivated behaviors. DA is released in the NAc both before and during copulation. Sexual behavior activates both DA and non-DA neurons in the VTA, apparently mediated by endogenous opioids, which inhibit GABAergic interneurons, thereby releasing DA cells from tonic inhibition. The mPFC sends largely glutamatergic axons back to VTA, providing positive feedback. mPFC axons also contact the NAc, MPOA, BST and subparafascicular nucleus (SPF). Lesions of the mPFC, VTA, and NAc impair sexual arousal. Stimulation of the dorsal VTA facilitates, but ventral stimulation inhibits, copulation. Mating induces Fos-ir in the NAc and VTA, and sexual experience enhances Fos-ir in response to estrous females. Decreasing DA activity, by blocking NAc DA receptors or stimulating VTA autoreceptors, slows motor behavior and may decrease sexual motivation.

The nigrostriatal DA tract originates in the substantia nigra (SN) and projects to the dorsal striatum. DA is released only after copulation begins, suggesting greater importance for motor activation than motivation. Bilateral SN lesions slow copulation and decrease ejaculations.

Paraventricular nucleus of the hypothalamus (PVN)

The PVN integrates endocrine and autonomic functions. Parvocellular neurons project to several brain areas and the spinal cord, and magnocellular neurons release OT and vasopressin from the posterior pituitary. Axons projecting to the spinal cord release several transmitters, including OT, vasopressin, and DA. Input to parvocellular PVN includes DA from periventricular neurons and NE and 5-HT from the brainstem. The PVN is important for noncontact erections and seminal emission, but is less critical for reflexive erections and copulation.

Microinjection of DA (especially D₄) agonists, OT, NO donors, or NMDA elicits drug-induced erections, increases reflexive erections and seminal emissions, and increases intracavernous pressure in anesthetized rats. Both noncontact erections and copulation are accompanied by increases in DA and NO. Intra-PVN morphine inhibits both noncontact erections and the NO increase. Sexually competent male rats have more OT mRNA and less opioid mRNA in the PVN than do impotent males, and NOS and OT are co-localized. The PVN projects to the hippocampus, lumbosacral spinal cord, and other areas, including the nPGi, where terminals form close appositions to serotonergic neurons that inhibit genital reflexes.

Lateral hypothalamus (LH)

The LH contributes to autonomic, endocrine, and emotional responses. Electrical stimulation induces copulation in male rats, and lesions impair mating. 5-HT in the anterior LH (aLH) delays and slows copulation and decreases basal and female-elicited DA release in the NAc. 5-HT levels are increased during the PEI. The aLH contains orx/hcrt neurons, which contribute to arousal and reward and are activated by copulation. 5-HT in the aLH may inhibit copulation by inhibiting orx/hcrt neurons, which would eliminate their facilitation of VTA DA cell firing.

Ventromedial hypothalamus (VMH)

The VMH, known primarily for its role in female lordosis, may also influence males. It has numerous ERs and ARs and receives genital and chemosensory input. Mating induces Fos-ir in the VMH of rats and gerbils, but not in musk shrews, hamsters, mice, ferrets, or macaques.

Major Motor Outputs

Midbrain periaqueductal gray (PAG)

PAG lesions blocked elicitation of the urethrogenital reflex by MPOA electrical stimulation. There are numerous ERs and ARs in the PAG, and afferents from the MPOA end near ER- and AR-ir neurons, some of which project to the nPGi. Thus, hormones can affect control of the nPGi by the MPOA through the PAG.

Nucleus paragigantocellularis of the medulla (nPGi)

Much of the supraspinal inhibition of genital reflexes arises from the nPGi. Lesions facilitate copulation and reflexive erections and allow the urethrogenital reflex to be elicited without spinal transection. They also increase the number of ejaculations preceding satiety. Electrical stimulation activates sympathetic fibers in the pudendal nerve. Most nPGi axons to the lumbosacral cord contain 5-HT, which suppresses the urethrogenital reflex.

Spinal cord

Erection is elicited by inhibiting the thoracolumbar sympathetic antierectile pathway and stimulating the proerectile parasympathetic sacral and pudendal pathways. A central pattern generator for ejaculation in the lumbosacral cord includes neurons that contain galanin, cholecystokinin, and neurokinin receptors. Stimulation of these neurons elicits seminal emission followed by expulsion. Although 5-HT is primarily inhibitory, 5-HT_{1A} and 5-HT_{2C} agonists facilitate the urethrogenital reflex. Cerebrospinal fluid levels of GABA, and to a lesser extent glutamate and aspartate, increase markedly after ejaculation. GABA may inhibit, and glutamate, DA, NE, and ACh facilitate, sexual reflexes at the spinal level.

Sexual Behavior in the Context of Mammalian Social Behavior

Brain areas that control male sexual behavior influence other social behaviors as well, including female sexual behavior, maternal behavior, aggression, and territorial marking. Most of those areas, except the midbrain, contain abundant steroid receptors, and all influence more than one behavior. Perinatal, adolescent, and adult hormones can provide a bias toward sexually dimorphic responses to social stimuli. It is not clear whether the same neurons within a structure contribute to more than one behavior, or whether neurons specific for one behavior lie among those specific for other behaviors. However, there are common themes underlying the various social behaviors and the neural mechanisms that control them.

See also: Animal Models of Sexual Function; Hormonal Contributions to Arousal and Motivation; Mating Behavior; Sexual Motivation.

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Measuring Stress

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Glossary

Allostatic load – The physiological costs of chronic exposure to the neural or neuroendocrine stress response. The allostatic load index is conceptualized as a summary measure capturing the cumulative physiological burden exacted on the body to adapt to life's demands. It is thought to reflect the tax on the body and brain resulting either from chronic overactivity or inactivity of physiological systems that are involved in adaptation to environmental challenge.

Cortisol – A corticosteroid hormone that is produced by the adrenal gland (in the zona fasciculata and the zona reticularis of the adrenal cortex) in response to a wide range of stressors. It has a wide range of physiological effects, that is, increases blood pressure and blood sugar, and reduces immune responses; virtually every single nucleated cell in the body is a potential target for cortisol. Only 5–10% of the total plasma cortisol is unbound and biologically active (c. 90% of the fraction is silenced by cortisol binding protein).

Hypothalamic–pituitary–adrenal (HPA) axis – This axis is constituted by a complex set of direct influences and feedback interactions among the hypothalamus, the pituitary gland, and the adrenal glands. It is a major part of the endocrine system that controls responses to stress and regulates many physiological processes, including the immune system, energy storage and expenditure, and a broad range of psychological as well as biological mediators of adaptation to stressful events. The HPA axis mediates stress adaptation. Under stress, the hypothalamus secretes corticotropin-releasing factor and arginine vasopressin that provoke the release of adrenocorticotropic hormone (ACTH) from the pituitary, which targets the adrenals and triggers the secretion of cortisol. The functioning of the axis is controlled by a negative-feedback loop, with cortisol feeding back not only on the pituitary to downregulate ACTH but also on the hypothalamus and the hippocampus, where it acts as a brake of the HPA-axis response to stress.

Introduction

Background

Stressors can take many forms, for example, loud noise, extreme temperature, physical exertion, pathogens, social disturbance, and emotional arousal. Social and psychological stressors are especially prevalent in primates and humans because of their complex social environments and advanced capacity for emotional responding. The perception of social hierarchy and interpersonal exchanges, the capacity for worry and rumination, the pain of self-doubt and low self-esteem, and the preoccupation with possible future events are among the common sources of chronic stress. The organism's repertoire of responses is not well suited to dissipate the impact of these sources of chronic stress. Indeed, the organism's response as it extends over time creates a state of pathology that is characterized by psychological disturbance and physiological states that can progress into illness.

During the mid-twentieth century, Hans Selye, an endocrinologist born in the Austrian–Hungarian Empire and later naturalized Canadian, pioneered the scientific investigation of stress and developed the concept of the 'general adaptation syndrome' to describe the common biological responses to stress. Specifically, he noted the progression from alarm, to adaptation, and then to exhaustion. The initial alarm response is associated with a marked increase of attention to the stimulus and physiological readiness to engage in the fight-or-flight response, which was first described by Walter Cannon at the beginning of the century. The brain triggers an organism-wide response through activation of the sympathetic nervous system and the hypothalamic–pituitary–adrenal (HPA) axis. These systems allow optimal adaptation for the organism to flee a threat or to repel a threat through increased respiratory and cardiac output, release of glucose, blood, and other nutrients to supply fully engaged muscles, constriction of blood to the extremities, and a variety of additional reactions. Selye argued that these neurophysiological responses cannot be indefinitely sustained, and the organism will enter a state of

exhaustion. In fact, subsequent research has demonstrated that the responses do cease, but transform into a sustained and altered state that are damaging.

While Selye believed that these response patterns are nonspecific and account for any type of stressor, Mason, an American endocrinologist, challenged this position in the late 1960s by demonstrating that the HPA response clearly varies with the emotional reaction to the stressor. The exact emotion that is triggered by the stressor and, therefore, the intensity and dynamics of the HPA response depend on factors such as perception of uncertainty, anticipation, novelty, unpredictability, uncontrollability, and ego involvement. These observations have been confirmed in numerous studies in the past 40 years. In addition, it has been found that the HPA response habituates under repeated exposure to stressors in most subjects, once the situation loses the characteristics described above, while the sympathetic response remains largely unchanged under such conditions. This article provides a brief overview of psychological and biological measures of acute and chronic stress.

Definition of Stress

This brief history illustrates the complexities of the definition of stress in showing that there are three distinctive components comprising it. Researchers have focused on different aspects of the definition, often to the exclusion of other parts of the definition, which has created some confusion of the meaning, indeed, even the utility of the term stress. The first approach has been to define stress as an environmental. Records of the number of recent life events or of single, major event, such as death of a loved one, are examples of this approach. The second approach is to measure people's reactions to the environment, regardless of what has actually happened to them. This allows for individual differences such that a given environment could engender little perceived stress in one individual, yet high levels of stress in another (presumably because of differential life histories or constitutional makeup). The third approach is to measure the physiological response of the individual to the environment, which also allows for individual differences in response patterns. We shall discuss measurement strategies for each of these conceptualizations of stress with extra emphasis on physiological measures of stress. A book on stress measurement was published several years ago, and the organization of this article is very similar to the one used by the authors of this book.

Distinction and Importance of Acute versus Chronic Measures

An important distinction in stress research is that between acute stress and chronic stress. While most species are highly adapted to cope with an acute stressor, chronic

stress poses a much more challenging situation. The response to acute stress is designed to provide the organism with the alertness, energy, physiological regulation, and immunological activation that are necessary to counterbalance the effects of the stressor in order to survive. The removal of an acute stressor allows the organism to return to baseline. In contrast, chronic stress presents an unrelenting challenge that can exhaust the organism's response and results in a chronic state of dysregulation.

While the impact of acute and chronic stress may be distinctive, the measurement of the two is less well defined. Measurement of acute and chronic marital discord shows the association between the two measurements. A single marital argument could be considered an acute stressor; in fact, laboratory study of arguments between spouses has shown considerable evidence of brief, but intense, physiological activation. Chronic marital stress can be defined as frequent arguments over some reasonable period of time. In this case, the measurement strategy for acute and chronic stress is distinguished only by duration. However, another strategy for measuring marital stress is to obtain a single rating about marital stressors pertaining to a period of time (e.g., child rearing, finances, etc.). Both measurement strategies are valid, but they probably measure somewhat different aspects of the relationship between spouses. We will note both acute and chronic measures throughout the article.

Environmental Measures of Stress

Self-report questionnaires are the most common method of measuring stress. Several approaches have been developed reflecting different theoretical frameworks for conceptualizing stress. Some of the earliest environmental approaches focused on the occurrence of life events as a means of quantifying the amount of stress the individual had been exposed to. Initially, only major events were measured, while later minor events or hassles and the person's perception of stress caused by the event were incorporated in the measures. Another, more recent approach is real-time assessment of events, mood, and coping responses. In contrast to the traditional retrospective questionnaire approach, real-time assessment involves multiple prospective assessments of the respondent's experience at the moment. (The real-time approach is applicable to the measurement of both environmental and perceived stress.)

The earliest measures of stress focused on the occurrence of life events. The Holmes and Rahe Social Readjustment Scale, published in 1967, includes 43 weighted items of important life changes – both positive and negative, such as marriage or death of family member – that might have happened to the respondent in the last 12 months. The cumulative sum of events was viewed as an

index of the degree of stress experienced by the individual. Ten years later, Sarason and colleagues developed the Life Experiences Survey that asks the respondent to indicate if any 30–57 life events (depending upon instrument version) occurred in the last 12 months and to rate the impact of the event on a 5-point scale that yields life change units. Negative and positive event scores can be summed separately or together for a total score. Bruce Dohrenwend developed the Psychiatric Epidemiology Research Interview (PERI) life events scale in the 1980s, and more recently, Stamm and colleagues developed a 20-item questionnaire, the Stressful Life Experiences Screening, that focuses on traumatic life events and asks the respondent to indicate the extent to which they have experienced each, and the degree of stress at the time of the event and now.

Scales assessing only major life events were criticized by some for failing to include typical sources of chronic stress for most people. Richard Lazarus and colleagues asserted that small daily difficulties or hassles should be the focus of stress measurement to capture the extent to which an individual is psychologically burdened by chronic stressors. They developed the 117-item Hassles Scale including items relating to difficulties with finances, time pressure, employment, health, and family. While earlier research supported the association of major life events and subsequent health, subsequent work documented the independent association of stress induced by daily hassles in predicting health outcomes.

Another approach is a change of measurement method more than item content. Assessment of stress can be done for much shorter time periods, thus avoiding the bias of retrospective reporting. Stone and colleagues were among the first to measure stress on a daily basis prospectively in the early 1980s. This approach has been able to capture the relationship between elevations in stress preceding onset of a respiratory infection and concurrent changes in immunological function. In addition, it has raised doubt about the accuracy of measures of coping that generalize across time and situations.

This was taken to an even finer level of detail with the development of the strategies termed the Experience Sampling Method (ESM) and Ecological Momentary Assessment (EMA). These measurement strategies involve sampling peoples' momentary experiences as they occur using hand-held recording devices, which yield a profile of the experiences throughout the day. These self-reports of both environmental occurrences (small events) and inner feelings (stressfulness) can be associated with ambulatory measurements of physiological indicators, enabling a dynamic view of environmental, perceived, and physiological approaches to stress measurement. A good example of this research is that of Thomas Kamarck, who is studying psychosocial stress exposure during the course of the day. In this work, an individual could be characterized as experiencing work

stress if they felt work experiences were very demanding, yet that they also had little control over them (as opposed to providing the same information based on recall judgments of the same).

Perceptual Measures of Stress

The second type of stress measurement has generated measures that focus on the subjective appraisal of stress in one's life and on associated emotions. With the recognition of posttraumatic stress disorder in the 1980 Diagnostic and Statistical Manual of Mental Disorders, questionnaires and structured interviews began to incorporate the designated psychiatric symptomatology. These measures all involve self-reports from individuals about their feelings and appraisals of their psychosocial environment; thus, they are all questionnaire-based assessments.

Several questionnaires have been designed to assess the degree of chronic stress an individual perceives. The Perceived Stress Scale (PSS) published by Sheldon Cohen and colleagues in 1983 is a typical example. Currently, three versions are available: 4-items, 10-items, and 14-items; all use a reporting period of the last month, and yield a total score. The scale's items are designed to reflect the degree to which the respondent experiences life as unpredictable, uncontrollable, and overloaded, and that the resources necessary to respond to the event are insufficient. Scores from a normative sample of over 2300 adults have been published. Cohen has shown that the PSS not only is associated with psychopathology, but it is also independently associated with health outcomes, such as development of a cold when a rhinovirus is introduced, likelihood of being a cigarette smoker, lower physical activity, and impaired immune function.

The Derogatis Stress Scale, first published in 1980, takes an even more complex approach. It is based on the interactional stress model of Lazarus and Folkman that incorporates measurement of three domains: environmental events (vocational, interpersonal, and health), personality characteristics (time pressure, excessive achievement drive, and relaxation potential), and emotional response (hostility, anxiety, and depression). The scale uses 77 items to assess the three domains and yields scale scores for each, a global score, as well as a score for the respondent's subjective level of stress. Normative data on 1000 working adults is published. Few studies have examined associations with biological or health outcomes.

The Impact of Events Scale, reported by Horowitz and colleagues in 1979, is an example of instruments designed to measure posttraumatic psychiatric symptomatology. This 15–22-item instrument asks respondents to indicate the frequency of intrusive thoughts, avoidance and numbing, and physiological hyperarousal in the last 7 days. It is most frequently used to document traumatic stress in

different populations and as an outcome measure in trials designed to reduce posttraumatic stress.

Physiological Measures of Stress

During the past decades many tests for laboratory research on acute stress in humans have been developed. Probably the most prominent is the Trier Social Stress Test (TSST). In front of an audience and monitored by a camera and tape recorder, subjects are asked to deliver a motivated performance task.

In thousands of TSSTs performed in many different laboratories worldwide, much data have been collected showing a robust rise of stress hormones, autonomic measures, immune parameters, and perceived stress in the majority of the tested subjects. Interestingly, physiological and psychological responses do not or only poorly correlate, indicating that both psychological and biological measures need to be applied for a reasonable assessment of the stress response.

For the TSST, broad intra-and inter-individual differences in the stress response have been observed. Age, gender, dietary and drug consumption, medical conditions and interventions, personality factors, social support and social hierarchy, menstrual cycle, pregnancy and lactation in women, time of testing, habituation, early-life experience, and genetic factors, among others, are known to influence the acute stress reaction. These factors and the complex interaction among them in a given individual finally explain such differences.

The TSST has been shown to exert robust effects on several psychobiological measures:

- Psychological measures: anxiety, negative mood, and perceived stress.
- Autonomic measures: blood pressure, heart rate, heart rate variability, electrodermal activity, perspiration, body temperature, epinephrine, and norepinephrine.
- Endocrine and metabolic measures: adrenocorticotrophic hormone (ACTH), plasma and saliva cortisol, prolactin, growth hormone, and glucose.
- Hematological measures: hematocrit, hemoglobin, and plasma volume.
- Coagulation measures: fibrinogen, von Willebrand factor antigen, D-dimer, and clotting factors.
- Immune measures: neutrophils, eosinophils, basophils, lymphocytes, interleukin-6, and tumor necrosis factor alpha (TNF α).
- Genetic measures: repression/induction profiles of genes in target tissues.
- Psychomotor measures: muscle activity (electromyogram), voice (spectral analyses), limb movements, and dexterity (**Figure 1**).

This broad spectrum of response measures has turned out to be useful in both basic and clinical research. For example, it has been shown that specific pharmacological and psychological interventions change single profiles, predicting comparable effects in clinical practice.

As mentioned above, measures of perceived stress are poorly associated with biological measures of stress. This is also true for the assessment of chronic stress. For example, both elevated and dampened cortisol levels have been reported to be associated with depression, posttraumatic stress disorders, irritable bowel disease, burnout, chronic fatigue, fibromyalgia, etc. Thus, an endocrine status does not necessarily predict a psychological status or specific stress-related disorders. Rather, it seems that the HPA axis can adapt to chronic stress by first becoming up-and later down-regulated. In both cases, however, corticotropin-releasing factor (CRF)/ arginine vasopressin (AVP) neurons of the hypothalamus may be overactivated, first triggering HPA axis hyperactivity, and later becoming disinhibited due to low cortisol levels.

While a hypercortisolemic state may promote the metabolic syndrome and disorders of the immune system, hypocortisolism seems rather to facilitate pain, fatigue, and irritability, probably by disinhibition of proinflammatory cytokines, prostaglandin synthesis, and noradrenergic neurons in the central nervous system. Effects of chronic stress on the sympathetic nervous system seem to be mainly observed in panic disorder and essential hypertension.

Yet another approach to use biomarkers as measures of chronic stress is the concept of ‘allostasis’ and ‘allostatic load.’ The basic assumption is that allostasis allows adaptation to chronic stress by maintaining (homeostatic) stability through change. There are four conditions under which this kind of adaptation occurs: (1) failure to habituate to repeated stressors of the same kind; (2) failure to turn off each stress response in a timely manner due to delayed shut down; (3) repeated frequency of stress responses to multiple novel stressors; and (4) inadequate response that leads to compensatory hyperactivity of other mediators. Allostatic overload refers to dysregulations of multiple physiological systems, which exert cumulative strain on multiple organs and tissues. Measures of allostatic load predict some variance of diverse health outcomes, including cognitive and physical functioning, cardiovascular and inflammatory disease, and even mortality. Allostatic load is assessed as a composite index by the number of biomarkers for which an individual is at risk.

However, the physiological pathways linking chronic stress to health outcomes are affected by the interplay of multiple variables: genetic and epigenetic determinants, brain maturation during pre- and postnatal development, duration, quality, and intensity of life events, and resilience; socioeconomic conditions; coping skills, organ

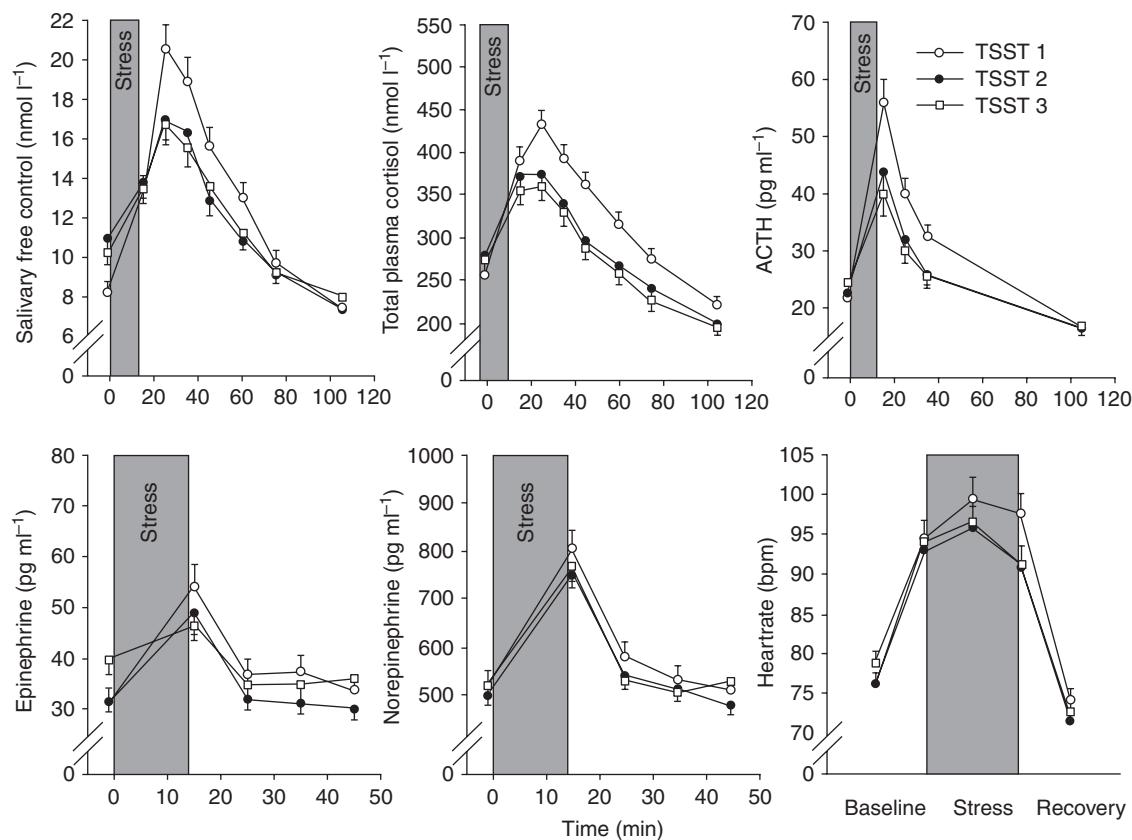


Figure 1 Endocrine and heart rate responses in 88 healthy volunteers under three repeated TSST exposures (\pm SEM; 4-week intervals). Reproduced with permission from Schommer NC, Hellhammer DH, and Kirschbaum C (2003) Dissociation between reactivity of the hypothalamus–pituitary–adrenal axis and the sympathetic–adrenal–medullary system to repeated psychosocial stress. *Psychosomatic Medicine* 65(3): 450–460.

function, etc. Thus, chronic stress affects subjects quite differently, and the individual outcomes of stress are very heterogeneous. Clearly, a meaningful interpretation of stress effects on health needs to consider such individual constellations.

From such a viewpoint, measures of chronic stress have to be defined differently, particularly if they should serve a diagnostic purpose. Hypothetically, one could define neuroendophenotypes, which describe discrete brain systems that participate in the stress response. For each of these systems, one could develop psychological, biological, and symptomatic outcome measures, which reflect the activity or reactivity of each system. Furthermore, one could describe how gene–environment interactions affect these systems. A diagnostic assessment of chronic stress effects would then comprehend a number of measures which could probably tell, which of these neuroendophenotypes participate in stress-related disorders of a given patient. If so, individualized pharmacotherapeutic and psychotherapeutic treatments could be assigned.

A first approach of this kind has been termed Neuropattern. To reduce complexity and heterogeneity

as well as to avoid the missing covariance of the psychological and biological stress response, this approach solely focuses on the interfaces, that participate in the cross talk between the brain and the rest of the body. Endophenotypes for the activity and reactivity of these interfaces were defined and are assessed by measures of concomitant psychological, biological, and symptomatic events. Practically, each physician can apply Neuropattern to explore if and how stress affects his/her patient's health. The Neuropattern kit contains questionnaires, a small electrophysiological device, and tubes for the collection of saliva. In his/her office, the physician provides master file data, a brief medical history, and takes several measures, such as blood pressure, waist-to-hip ratio, body mass index, etc. At home, the patient fills questionnaires, collects saliva samples before and after a low-dose dexamethasone test, and uses a portable electrocardiogram. Once all data have been collected, the patient sends the kit to a company, which performs the laboratory analyses of all the data and performs a comprehensive medical report for the physician. This strategy allows to transport expert knowledge to the practitioner across medical disciplines,

and without implying specific education or expertise from the respective physician.

Perspectives

Both our knowledge and methods in the assessment of stress rapidly proceed, and, obviously, new biotechnologies and psychological measure will enable us to assess neuroendophenotypes of stress. In the future, it should be possible to refer such neuroendophenotypes to discrete neurobiological systems and functions, which can be assessed with respect to their genetic, epigenetic, physiological, and psychological features, so as to assess their relevance to the development of stress-related psychological and physical disorders.

See also: Animal Tests for Anxiety; Circadian and Ultradian Clocks/Rhythms; Depression; Effects of Stress on Learning and Memory; Maternal Deprivation; Offensive and Defensive Aggression; Psychosocial Influences on Immunity; Regulation of the HPA Axis by Acute and Chronic Stress; Social Bonding and Attachment; Stress and Social Behavior.

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Neuropeptides and Regulation of Water Intake

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Glossary

Angiotensin II – An octapeptide that plays a prominent role in the regulation of cardiovascular and body fluid homeostasis.

Blood-brain barrier – A property of capillaries throughout most of the brain that makes them less permeable to blood-borne substances, thus restricting movement from blood to brain.

Circumventricular organ – A brain structure lacking a blood-brain barrier.

Dipsogen – A substance that produces thirst.

Extracellular fluid – One of the major fluid compartments of the body comprising all fluid residing outside of cells.

Ghrelin – A peptide produced predominantly not only within the stomach and small intestine, but also within the brain. Ghrelin plays a role in the regulation of growth hormone secretion and food intake.

Intracellular fluid – One of the major fluid compartments of the body comprising all fluid within cells.

Neuropeptides – The peptides that are produced by neurons.

Opioid peptides – The peptides that bind to one or more members of the opioid receptor family, including μ , κ , and δ receptors.

Oxytocin – A peptide produced almost exclusively within the hypothalamus that is released from the posterior pituitary and from neural projections to numerous intra- and extrahypothalamic brain sites.

Vasopressin – A peptide produced by magnocellular hypothalamic neurons and by centrally projecting neurons within the hypothalamus and amygdala.

Venticle – A cavity within the brain that is filled with cerebrospinal fluid. The cerebroventricular system comprises four ventricles: two lateral ventricles, the third ventricle, and the fourth ventricle. Cerebrospinal fluid flows from the lateral ventricles, to the third ventricle, and then to the fourth ventricle before leaving the brain and entering the central canal of the spinal cord or into the subarachnoid space.

Introduction

Maintenance of body fluid homeostasis is critical for survival. The deleterious effects of dehydration are well recognized by the scientific and lay communities; however, perturbations in the opposite direction and overconsumption of water are equally problematic and potentially fatal. Drinking large volumes of water has led to fatalities during fraternity hazing rituals, radio station contests, and after strenuous physical activity. Given the importance of maintaining a proper balance of fluid, it is not surprising that numerous physiological factors act in a coordinated effort to maintain body fluid homeostasis. A key component of this regulation involves behavioral changes leading to increased or decreased intake of water and salt. The mechanisms underlying these behavioral changes often hinge on the action of neuropeptides. The information that follows focuses on these actions and extends the discussion beyond neuropeptides to include information about peptides that are neither produced nor secreted by neurons, but that act on the central nervous system (CNS) to regulate water intake.

Vasopressin

From a historical perspective, one of the earliest associations between a peptide and fluid balance is the relationship between dehydration and arginine vasopressin (AVP). These classic studies, mostly the work of E. B. Verney carried out in the 1940s, used dogs that had been preloaded with water such that baseline urination was elevated. Using this preparation, Verney measured urine output after intravenous or intracarotid infusions of various hyperosmotic solutes. Using measures of urine output as a bioassay, he discovered that intracarotid infusions of hyperosmotic solutions that were excluded from cells (e.g., NaCl, sucrose, and mannitol), therefore drawing water from the cells by osmosis and stimulating a form of dehydration referred to as ‘osmotic’ or ‘intracellular’ dehydration, reduced urine output. In contrast, solutes

such as urea that raised extracellular osmolality, but could equilibrate across cell membranes, did not alter urine flow. Moreover, intracarotid infusions of solutes were significantly more effective than intravenous administration, suggesting the brain as a site of action. Although these experiments did not provide direct evidence for the involvement of a peptide in the observed responses, Verney postulated the involvement of a circulating factor that we now know as AVP. Indeed, the subsequent development of a radioimmunoassay for AVP provided confirmation of all of Verney's original findings. The role of AVP in water restriction by the kidney is quite clear, but a role for a peptide as a stimulus for drinking, rather than as a parallel response to dehydration, would not be demonstrated for another 20 years.

Angiotensin

With respect to a direct role of a peptide on fluid intake, the most notable example is that found in the octapeptide angiotensin II (AngII). Angiotensin production starts with the rate-limiting step of renin release from the kidney (**Figure 1**) that occurs under situations of decreased blood volume referred to as 'hypovolemia' or 'extracellular

dehydration.' Renin acts on a plasma α -globulin, angiotensinogen, which is abundantly available in the circulation. The cleavage of angiotensinogen by renin produces a smaller, biologically inert peptide referred to as 'angiotensin I.' Conversion of angiotensin I into the bioactive AngII is performed by angiotensin converting enzyme, which not only is expressed by cells of the lung vasculature, but is also found in other tissues. The bioactive AngII has a diverse array of effects on a variety of tissues, each of which insures the proper behavioral and physiological response to a condition of decreased volume of the extracellular compartment. The discovery of the dipsogenic effect of AngII stemmed from a handful of early experiments demonstrating that renal extracts stimulated drinking in animals that should not otherwise have been thirsty. Initially, the focus of these experiments was on a relatively large AngII precursor, renin, but knowing that the biological effects of renin occur largely, if not exclusively, through AngII, scientists quickly turned their attention to AngII. There had been hints of the dipsogenic potency of AngII in the literature. In the 1960s, studies by David Booth, which focused on hypothalamic injections of norepinephrine, also included experiments using AngII. In this case, however, AngII was included largely as a control for specific aspects of the

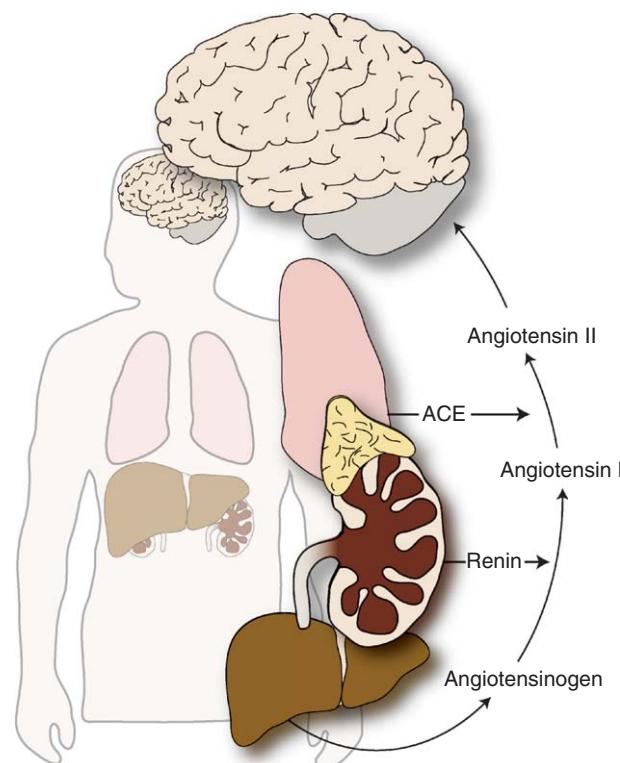


Figure 1 The renin–angiotensin system. The dipsogenic/natriorexigenic peptide angiotensin is produced by a biochemical cascade that begins with the rate-limiting step of renin release from the kidney. Renin cleaves circulating angiotensinogen, which is produced by the liver, to produce angiotensin I. Angiotensin I is biologically inert until it is further cleaved by angiotensin converting enzyme, predominantly in the lungs, to generate angiotensin II, which acts at a variety of tissues including the brain.

study and received very little attention in the report. Shortly thereafter, the studies of Anne Daniels (no relation to the present author), who would later become Daniels-Severs, also demonstrated the efficacy of brain injections of AngII. These studies, first published in abstract form and later as a brief communication, were particularly focused on the pituitary responses to AngII injected into the lateral cerebral ventricle, but included the demonstration that these injections reliably stimulated drinking. The experiments of Booth and of Daniels and coworkers, however, used rather large doses of AngII, making it difficult to draw conclusions about a specific site of action. At the same time these experiments were underway, James Fitzsimons and his colleagues were performing a series of experiments that would clearly demonstrate the dipsogenic actions of AngII. These experiments included intravenous injections and direct injection of small amounts of AngII into several central sites. Interestingly, the efficacy of AngII injected into the lateral ventricle of the brain (intracerebroventricularly, ICV) remained unclear. In fact, Epstein and his coworkers reported that AngII produced drinking only in one of the two rats given ICV injection of AngII, but note that the report of Daniels and his coworkers "leaves the question of ventricular sensitivity unsettled." Of course it is now quite clear that the cerebral ventricles are a very sensitive route of delivery for dipsogenic responses to AngII. Indeed, the responses to AngII are so reliable that numerous laboratories use injections of AngII to verify proper forebrain ventricular cannula placement in laboratory rats and mice, even for experiments unrelated to fluid homeostasis.

The source of the variability in the earlier experiments using AngII remains unclear, but the issue of ventricular sensitivity was decidedly settled by the studies of Johnson and Epstein. These studies, originally designed to investigate parenchymal sites that are sensitive to AngII application, noted that most of the responsive locations in the earlier studies of Epstein and his coworkers were either in close proximity to the cerebral ventricles or were in regions where the cannula shaft traversed a ventricle en route to the intended site. Unsatisfied with the conclusions that could be drawn from the behavioral experiments, the authors used radiolabeled AngII to monitor the diffusion of peptide from the cannula tip and clearly showed that the peptide effluxed up the shaft of the cannulae, thereby gaining access to the ventricles. Indeed, when cannulae targeted a structure shown to be responsive, but were implanted using an angle that avoided the ventricle, the animals failed to drink. These studies also showed that the doses needed to generate a drinking response after ventricular application were actually lower than those needed for the earlier parenchymal injections, clearly implicating the ventricles, and perhaps

the ependymal tissue surrounding them, in the behavioral response.

Although the dipsogenic potency of the exogenous AngII was quite compelling and it was clear that the peptide had a central site of action, the results were initially troublesome. Specifically, it was not clear how a circulating peptide could penetrate the blood-brain barrier to gain access to structures in the CNS involved in the arousal of thirst. Although the answer seems obvious now, the implication of the circumventricular organs was a major breakthrough for the study of the regulation of behavior by hormones, especially peptide hormones. In fact, while Johnson and Epstein's analysis of AngII-sensitive sites progressed, Simpson and Routtenberg were focusing on the subfornical organ (SFO), a relatively small brain structure located just ventral to the hippocampal commissure (also referred to as the 'ventral fornical commissure'). These studies demonstrated that doses of AngII, which were mostly ineffective when given to the ventricle, produced marked increases in drinking when applied directly to the SFO. Moreover, destruction of the SFO blocked the dipsogenic effect of AngII given systemically or into other brain areas. Furthermore, direct SFO application of an AngII receptor antagonist blocked the drinking response to systemic AngII. Importantly, these studies provided an interesting dissociation by showing that lesions of the SFO attenuated drinking after challenges modeling hypovolemic thirst, but had no effect on stimulation of intracellular (osmotic) thirst, helping to establish the role of AngII as a signal of extracellular (hypovolemic), but not intracellular thirst.

In addition to the increases in water intake as discussed above, AngII also stimulates ingestion of saline solutions. The stimulatory effect of AngII on salt ingestion was first suggested by two independent reports that brief infusions of large doses of AngII into the cerebral ventricles or surrounding tissue of the anterior forebrain increased the intake of relatively dilute, yet hypertonic, saline solutions. In subsequent work, Epstein's and Fitzsimons's groups simultaneously demonstrated that more prolonged elevations of AngII in the brain elicited substantial intake of strong salt solutions that were steadfastly avoided by animals not receiving AngII. These studies were useful in pointing to a role of AngII in water and saline intake, but determining the physiological relevance was difficult. The difficulty arose mainly from the doses required to stimulate the salt ingestion and the potential confound of the resultant natriuresis, likely pressor induced, which was directly related to the temporal pattern and magnitude of salt intake. These problems, however, were solved when it became appreciated that the experimental animals used were not in the normal physiological setting under which AngII is normally secreted. Indeed, elevated AngII normally occurs in concert with other responses to hypovolemia, including

increases in circulating levels of the steroid hormone aldosterone. These discussions and debates led to the development of the synergy hypothesis, which suggests that the intake stimulated by the combination of AngII and aldosterone is greater than the addition of the intake stimulated by each in isolation. In support of this hypothesis, exogenous mineralocorticoid and AngII, each at doses that were insufficient to elicit salt intake alone, produced a robust salt appetite when given together. This effect was much greater than additive over a broad range of doses of each hormone, it was specific for saline intake, and it was not secondary to excessive renal sodium excretion. It was further demonstrated that blockade of AngII or aldosterone action alone resulted in a partial reduction in the appetite elicited by dietary deprivation, whereas the simultaneous inhibition of both hormones in the brain, but not in the periphery, abolished it. Collectively, these experiments with agonists and antagonists appeared to confirm the validity of the synergy hypothesis in situations where the appetite was associated with elevated endogenous levels of AngII and aldosterone.

The multiple forms of ingestive behavior stimulated by AngII (water and saline solutions), each coordinated to defend body fluid homeostasis, make this peptide a useful tool for illustrating the diversity of peptide regulation of behavior. Given that AngII is an important factor in the response to hypovolemia, the finding that it stimulates intake of saline, in addition to intake of water, is not surprising. Considering the distribution and composition of body fluids is key to understanding why the stimulation of both forms of ingestive behavior is illustrative. The typical adult human contains approximately 42 l of water, which is nearly 60% of total body mass. The majority of this water, approximately two-thirds of body fluid, is found within cells and is therefore referred to as 'intracellular fluid (ICF).' The ICF contains potassium and bicarbonate ions as well as glucose and several amino acids. The presence of these and other molecules in the ICF results in an osmolality of approximately 300 mOsm. The generation of this osmotic pressure is the key to understanding the dynamic balance between the ICF and the other main compartment of body water, the extracellular fluid (ECF). ECF represents approximately one-third of total body water and comprises several sub-compartments, including circulating fluid (blood plasma) and fluid surrounding cells (but not contained in blood vessels). As is true for the ICF, the ECF also contains several solutes, principally sodium and chloride ions. Thus, when an animal experiences extracellular dehydration, the critical remediating behavioral response must include the consumption of water, but the animal must also restore the lost sodium and chloride ions. On the other hand, depletion of the ICF generally occurs only when the concentration of the extracellular solution increases, thereby drawing water from the cells. Thus,

the increases in both water and saline after AngII injection are consistent with a role of this peptide in the response to hypovolemic, but not osmotic thirst. This hypothesis has been given recent support by studies using mice that are lacking a critical enzyme in the synthesis of AngII. These mice, which are unable to synthesize AngII, respond normally to stimuli inducing osmotic thirst, but they fail to drink in response to hypovolemia.

The example of AngII is also useful for discussing the diversity with which the body can use a single hormone. In this respect, it is unfair to define AngII as a peptide hormone or as a neuropeptide because it actually plays both roles. Indeed, the discussion so far has focused on AngII as a circulating hormone with a central site of action. Even the studies using direct injections of AngII into the brain were performed and discussed as models for peripherally derived AngII and used as evidence that this circulating AngII has a central site of action. It is nevertheless important to recognize that the brain appears to have an endogenous system of synthesizing AngII and may use this AngII as a transmitter within neural circuits. In fact, there is considerable evidence that all of the components illustrated in **Figure 1** can be found within the brain cells. Interestingly, some of the very same circuits that respond to circulating AngII appear to use centrally derived AngII as a peptide transmitter. Accordingly, studies using brain injection of AngII may have more relevance to AngII in its actions as a transmitter than its actions as a circulating hormone. Nevertheless, the circuits engaged by each source of AngII seem to be partially overlapping, if not identical, and the behavioral responses to central or peripheral AngII are markedly similar; most, if not all, differences have been attributed to the confounding actions of AngII on the vasculature directly. Indeed, the strong vasoconstrictive and hypertensive responses to AngII lead to the reduction of thirst through arterial baroreceptors. Thus, after a peripheral injection, AngII acts on the brain to stimulate intake at the very same time its hypertensive effects generate signals from the vasculature that decrease intake. Accordingly, dissociating behaviors solely mediated by AngII synthesized within the periphery or CNS has proven difficult, but it is clear that there is, at the very least, a great deal of overlap between the actions of AngII generated in either compartment.

AngII has also been an interesting illustration of the diversity of intracellular-signaling cascades stimulated by the so-called G protein coupled receptors. Although the traditionally ascribed mechanism of these receptors, including the receptors for AngII, involves the action of G proteins, it is becoming quite clear that members of this superfamily of receptors are capable of acting without their normally associated G proteins. Accordingly, it may be more appropriate to call this family of receptors the seven-transmembrane domain or heptahelical receptors. In the case of AngII receptors, it has become clear

that stimulation of several intracellular cascades, including the activation of p44/42 mitogen-activated protein (MAP) kinase (ERK1/2) occurs in the absence of G protein stimulation. Moreover, the behavioral relevance of each limb of intracellular signaling has also been illustrative. Recent evidence suggests that the G protein mediated limb of AngII receptor signaling is involved in AngII-induced water intake without playing a role in the NaCl intake that also results from AngII receptor stimulation. In addition, evidence is accumulating that MAP kinase family members, which can be activated without G protein stimulation, play a role in the NaCl intake stimulated by AngII, without being involved in the normally concomitant water intake. Although this demonstration of separate behavioral roles of divergent intracellular signals appears unique at the present time, further investigation of other transmitter systems may reveal similar mechanisms. Nevertheless, this recent advance in our understanding of the regulation of water and salt intake is simply an early step. Indeed, additional research is needed to fully understand the roles of the individual intracellular signaling pathways and the mechanism through which the behavioral dissociation may occur.

Oxytocin

In addition to AngII, other peptides are clearly involved in the regulation of water intake, although none has received the same level of attention as AngII (**Figure 2**). Oxytocin, for example, is commonly recognized for its endocrine role, and functions as a neuropeptide as well, being released from sites within the brain where it acts on neurons through a neurocrine (synaptic) mechanism or

through more diffuse paracrine actions. The majority of studies related to oxytocin and fluid balance have focused on its role in the consumption of saline solutions. Oxytocin decreases salt intake, but not water intake, in hypovolemic animals. Moreover, treatments that inhibit oxytocin action such as receptor antagonists or selective destruction of oxytocin-sensitive neurons enhance salt intake by hypovolemic animals, without having any effect on normovolemic animals. Although the relevant findings have been inconsistent, there is some evidence that oxytocin inhibits water intake as well as salt intake. This effect is especially notable in food-deprived rats, but has been observed in freely fed animals as well.

Atrial Natriuretic Peptide

Experiments focused on the role of atrial natriuretic peptide (ANP) in fluid balance reveal interesting commonalities with AngII and oxytocin. Similar to the studies using renal extracts that served as a precursor for the discovery of the renin–angiotensin system in drinking, a role for ANP in sodium balance arose from studies showing that atrial extracts had potent natriuretic effects. Also similar to AngII, which is synthesized in both the periphery and brain, ANP is secreted by brain cells as well as by the atria. Unlike AngII, which seems to have similar effects regardless of the site of production, there may be markedly different effects of centrally and peripherally acting ANP. Given the natriuretic effects of atrial extracts and peripherally administered ANP, one might expect a compensatory behavioral response that includes increased salt intake; however, the empirical findings have not fallen into line with this prediction. In

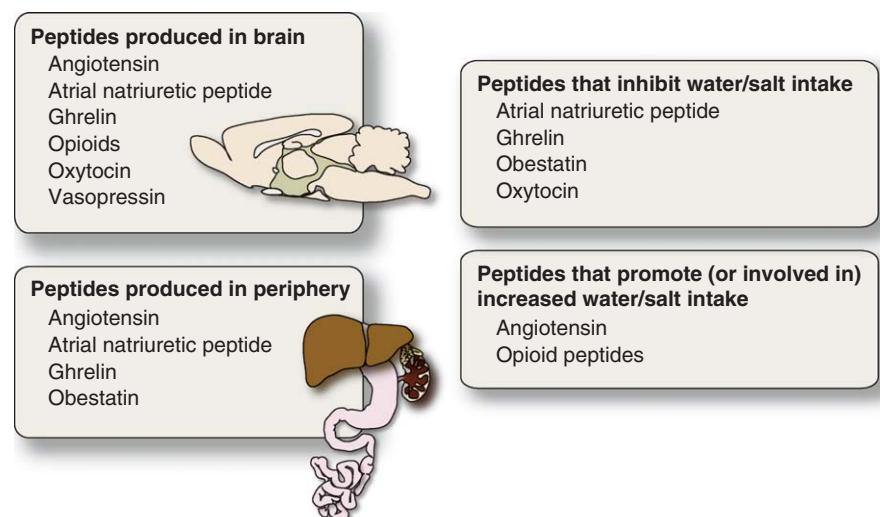


Figure 2 Summary of the peptides discussed in this article. Note that some peptides are produced in the brain and the periphery and that the site of production does not predict the direction of the effect on fluid intake.

fact, central administration of ANP reduces sodium intake in several experimental paradigms. Thus, it appears that the physiological role of ANP is to signal the need to reduce salt and this need is addressed by decreased salt intake and by increased natriuresis.

An inhibitory role of ANP in salt intake has been shown using a variety of techniques. Indeed, the paper described earlier that reported increased saline intake after selective destruction of oxytocin-sensitive cells, demonstrated a similar response after ANP-sensitive cells were destroyed using the same technique. These studies note an important difference between oxytocin and ANP. Specifically, when animals were made hypovolemic, which normally produces saline intake, but also injected with hypertonic saline or hypertonic mannitol, each of which normally counteract the natriorexigenic effects of the hypovolemia, animals without functioning ANP receptors increased salt intake. The comparison of hypertonic saline and hypertonic mannitol provides important mechanistic information because hypertonic saline increases both plasma osmolality and sodium concentration, whereas hypertonic mannitol increases plasma osmolality, without the addition of sodium. In fact, because it contains no sodium, injection of hypertonic mannitol dilutes the existing plasma sodium, effectively decreasing its concentration. Thus, the finding that destruction of ANP-sensitive cells removes the inhibitory effect of either hypertonic saline or hypertonic mannitol supports the hypothesis that ANP pathways are involved in both sodium- and osmolality-induced inhibition of salt intake. In contrast, destruction of oxytocin-sensitive cells had no effect on the inhibition of salt intake when hypovolemic animals were injected with hypertonic saline. Thus, it appears that ANP and oxytocin pathways are differentially involved in osmotically- and sodium-induced inhibition of salt intake under hypovolemic conditions.

Ghrelin and Obestatin

Ghrelin is a peptide produced predominantly in the stomach and small intestine that is best known for its role in food intake. Similar to oxytocin, ghrelin appears to be an example of a peptide that is most often associated with its endocrine actions, but there is increasing evidence that it is also produced within the brain where it functions as a neurotransmitter or neuromodulator. In addition to various anatomical investigations supporting the synthesis of ghrelin in the brain, there are subtle differences in the behavioral responses (specifically related to food intake) to centrally or peripherally administered ghrelin that argue for different roles of circulating and centrally released ghrelin. Although best known for its feeding

effects, a role for ghrelin in fluid balance is just beginning to be appreciated with several independent groups providing evidence for an antidipsogenic role of the peptide. One group found that ghrelin potently inhibited drinking by water-deprived rats. On the other hand, others have failed to observe an effect of ghrelin in water-deprived rats, but instead found that ghrelin reduced water intake stimulated by central AngII or by peripheral injection of hypertonic saline. In spite of these differences and the need for more research to reconcile these apparent discrepancies, it is becoming clear that ghrelin affects water intake and that the direction of the effect appears to be inhibitory.

The gene that encodes ghrelin also encodes another peptide named obestatin, which has been shown to reduce both water and food intakes; however, a careful analysis has suggested that the decreases are unrelated to each other. As is true for ghrelin, the mechanism underlying the behavioral effects of obestatin remains unclear, but the direction of the effects on food and water intake might suggest that ghrelin and obestatin, although encoded by the same gene, have markedly different actions in the CNS. Specifically, obestatin has similar actions on food and water intakes (suppressing each), whereas ghrelin has opposite effects on intakes of food (intake of which is increased by ghrelin) and water (drinking is decreased by ghrelin). The nature of these differences and the underlying mechanisms remain to be explored.

Opioid Peptides

Given the well-studied role of opioid peptides in motivated behavior, it should be no surprise that these peptides also modulate water intake. The involvement of opioids is not simple, however, and different effects have been attributed to different opioid receptor subtypes. Although additional research is needed to clarify some of the subtle issues involved, there is evidence that the involvement of specific receptor subtypes in drinking depends on the dipsogen causing the drinking. More specifically, deprivation-induced drinking appears to involve the μ_2 receptor, drinking after osmotic challenges such as injection of hypertonic saline seems to involve κ receptors, and drinking after AngII or isoproterenol (a β -adrenergic agonist) seems to involve μ_2 , δ , and κ receptor subtypes. These conclusions are based on a very small number of studies, however, and further work is needed to confirm and extend these findings. Nevertheless, given the different distribution of these receptor subtypes in the CNS, additional analysis of their involvement in drinking may reveal interesting information about the pathways involved in the responses to different dipsogens.

Conclusion

Similar to the discussion provided here, our understanding of the role of peptides in drinking behavior is far from complete. Other peptides not described here clearly influence drinking behavior and it is probable that future research will reveal new peptides that play a role in drinking or salt intake. The maintenance of body fluid homeostasis is of such importance, and also subject to so many perturbing factors, that it should come as no surprise that its regulation is complex with many redundancies. Even though a great deal of research has been devoted to understanding the regulation of fluid balance, a comprehensive picture of the neurochemical and neuroanatomical regulation continues to be elusive; however, understanding the peptides involved is an important step.

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See also: Cardiovascular Conditioning: Neural Substrates; Feeding; Genes and Behavior: Animal Models; History of Behavioral Neuroscience; Hormonal Contributions to Arousal and Motivation; Neural Systems of Motivation; Taste Perception and Behavior in Rodents and Flies; Thermoregulation.

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Psychoneuroendocrinology of Stress

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Glossary

Allostasis – The process of achieving stability through physiological or behavioral change. In allostasis, physiological variables are not kept constant; they vary according to what the organism needs to do.

Homeostasis – The process of achieving variability by counteracting variations in the internal milieu. In homeostasis, physiological variables are kept constant by processes that are activated by deviation from a set point and manage excess or insufficiency.

Stress – There are many definitions of stress. It usually refers to the response of the organism to challenges that tax its adaptive abilities.

Stressors – The internal or external factors that activate the stress response.

Psychoneuroimmunology is dealt with elsewhere in the encyclopedia.

Psychological Influences on Neuroendocrine Systems

The Early Pioneers

The study of psychological influences on bodily functions started in the early 1900s when Walter Cannon described the profound effects of rage and anger on the cardiovascular system and other organs innervated by the sympathetic nervous system. He pointed out the adaptive value of the alarm reaction for an organism exposed to situations that threaten its survival. He emphasized, in particular, the importance of this physiological response in helping the organism to meet the energy requirements of fleeing away from the danger, fighting it or hiding from it.

In the mid-1930s, Hans Selye made use of the same reasoning for his description of the response of the adrenal cortex to every stressor that threatens homeostasis. He proposed that the corticosteroids, which are released by the adrenal cortex in a nonspecific manner to all kinds of stressor, help in maintaining homeostasis and increasing the organism's resistance to the stressor. This formed the basis for his description of the General Adaptation Syndrome. Since Selye emphasized on the features of the response itself rather than on the circumstances in which it occurs, psychology was never an issue for him. This did not prevent Selye from wildly generalizing what he observed in response to catastrophic stressors (e.g., burn, hemorrhage, fractures, etc.) to what he called the stress of life, that is, the response to daily hassles.

From Stress to Psychoneuroendocrinology of Stress

Figure 1 summarizes the main differences between the physiological and the psychobiological models of stress. Cannon and Selye described the hard-wired systems that respond in a rigid manner to all kinds of threats. They both pointed to limits in the capacity of these systems to respond to stressors. These limits were attributed to the genetic makeup of the organism and/or its previous experience, and they accounted for the switch from physiology to pathology, in the form of the stress-related disorders.

Introduction

Psychoneuroendocrinology of stress is a very active field of research. Its success is not only due to curiosity but also due to the hope to understand how exposure to adverse events in life can ultimately culminate in the development of various pathologies gathered together under the label 'stress-related disorders.' These pathologies include both somatic disorders, such as cardiovascular diseases, and psychiatric disorders, such as depression and anxiety. In general, stressors by themselves do not trigger the pathological condition unless they are extreme but they modulate the rate of development of the disorder.

There are two intertwined facets in the psychoneuroendocrine studies of stress. One facet corresponds to the etymology of the word psychoneuroendocrinology. It corresponds to the study of psychological influences on the neuroendocrine systems that mediate the stress response. The second facet could be labeled 'neuroendocrinopsychology' since it actually corresponds to the study of the influences of neuroendocrine systems on behavior and cognition. Psychoneuroimmunology is a variant of psychoneuroendocrinology in which the variable of interest is the immune system instead of only the neuroendocrine systems that are activated by stressors.

This article focuses on the two facets of psychoneuroendocrinology of stress. However, how the stress response can translate into stress-related disorders is not discussed.

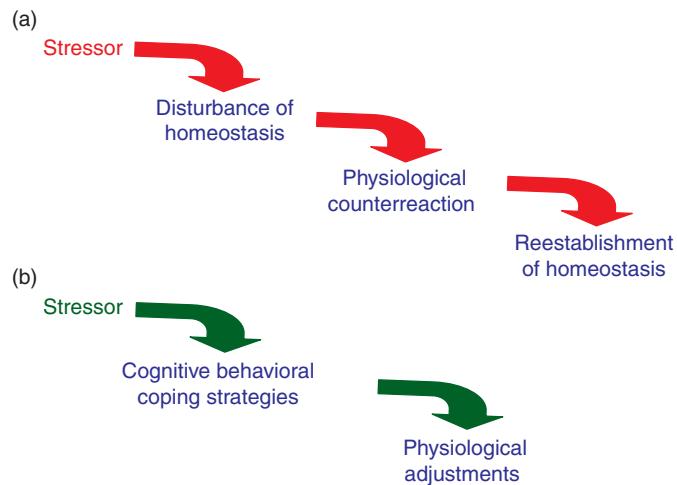


Figure 1 Physiological versus psychobiological perspectives on stress. (a) In the physiological model of stress, the stress response is totally determined by the nonspecific features of the stressor that disturbs homeostasis. (b) In the psychobiological model of stress, coping strategies that involve both cognitive and behavioral aspects determine the nature and intensity of the stress response.

Encouraged by the emphasis laid by Cannon and Selye on the sympathetic adrenomedullary and pituitary–adrenal responses to stressors, research on stress physiology progressed rapidly, thanks to the development of the relatively easily accessible biochemical methods for measurements of stress hormones. Despite these technological advances and the several studies that aimed at characterizing the hormonal stress response to various experimental and real-life situations, in both experimental animals and humans, those investigators who followed up on Selye's path had a difficult time trying to elucidate what exactly was responsible for activating stress hormones. John Mason, a neuroendocrinologist at the Walter Reed Institute, was the first researcher to propose a unified concept relating activation of the pituitary–adrenal system to the emotional arousal caused by the situation. He observed that the degree of change in circulating levels of cortisol in animals exposed to various stressors was more related to the excitation and behavioral agitation of the subjects under study than to the postulated derangement of homeostasis. Based on this observation, he reasoned that the cortisol response should decrease if excitation and behavioral agitation are minimized. Further, he demonstrated that habituating experimental animals to the experimental setup and masking the psychological aspects of the stressor, by making the subject unaware of its occurrence, annulled the cortisol response to various typical stressors such as cold, heat, or food deprivation.

Description of the stress response in terms of emotions was certainly a progress but not necessarily the end of the story since there was no general consensus on what exactly is an emotion and whether emotions could actually be studied in animals. Another important step forward was the demonstration by Seymour Levine

(or 'Gig' as he was affectionately called by his friends) of the importance of novelty, uncertainty, and lack of control in the modulation of the pituitary–adrenal response to stress in rats. An interesting example of the importance of controllability is conditioned taste aversion, which is a form of visceral conditioning in which the new taste of a drinking solution, for example, saccharin, is associated with the experience of sickness caused by nonlethal poisoning. One could think that reexposure to the taste solution previously associated with sickness should function as a stressor and activate the pituitary–adrenal axis. Levine showed that this was indeed the case. Rats that had recovered from poisoning and were inclined to sample the taste solution because they were thirsty displayed increased levels of plasma corticosterone. However, this response was observed only when the sole taste solution they had access to was the litigious taste. When previously poisoned rats were given the choice between the taste solution associated with sickness and water, they did not show any increase in plasma corticosterone levels. This experiment very well illustrates the potent pituitary–adrenal influence of being able to exert a choice, in this case avoiding the taste solution paired with sickness and going for the safe drinking solution.

Holger Ursin, in Bergen, Norway, went one step further and reinterpreted the data available on the importance of novelty, uncertainty, and lack of control in terms of cognition. He argued that the stress response typically occurs in cases of discrepancy of expectations, when the situation that is experienced by the subject differs from the expected one. This can take place in a situation in which the subject has learned to associate two stimuli in terms of the probability level of their association, or in a situation in which the subject has learned to expect certain consequences in response to his/her behavior.

The first situation corresponds to Pavlovian conditioning and the second one to instrumental or Skinnerian conditioning.

Stress and Coping

The shift from emotion to behavioral and/or cognitive elements of the situation is important since it sets up the scene for the convergence of biobehavioral stress research and psychological theories of stress. It also enables reconciling research on stress and psychological research on emotions. Coping theories have been built by psychologists independently of any reference to physiology to account for the factors that modulate the degree of emotional distress experienced during stress. The basic idea originally formulated by Folkman and Lazarus is that we constantly appraise the stream of events to which we are exposed for their threat value. During the first phase, or primary appraisal, we evaluate our possibilities to deal or cope with the situation. Then, during the second phase, or secondary appraisal, we evaluate the way our plan to deal with the situation is likely to succeed. This two-level appraisal process determines not only our level of emotional distress but also our physiological response to the situation. It is relatively easy to predict the direction and intensity of the physiological adjustments to the situation according to coping strategies. Active coping efforts are associated with activation of the sympathetic nervous system, as proposed by Marianne Frankenhausen, whereas the inability to control the situation is associated with activation of the pituitary–adrenal axis.

As pointed out by James P. Henry in his seminal review of the psychobiology of stress, the stress response can be best described over time in terms of a trajectory in the two-dimensional space delimited by the two orthogonal dimensions of coping attempts and coping failures. Another important feature of this representation is that each of these dimensions is truly bidirectional. Full mastery of the situation, together with social support, leads to deactivation of the pituitary–adrenal axis, whereas relaxation is associated with deactivation of the sympathoadrenomedullary response (Figure 2).

Allostasis versus Homeostasis

An important aspect of the stress response that has been left aside in this move from physiology to psychology is the biological meaning of this response. Selye considered the stress response as an attempt of the organism to restore homeostasis. The threats we are exposed to disturb homeostasis and the organism has no other alternative than to restore homeostasis to its original value as soon as possible. As pointed out by Henri Laborit, who promoted hibernation for the treatment of shock and whose seminal work decisively contributed to

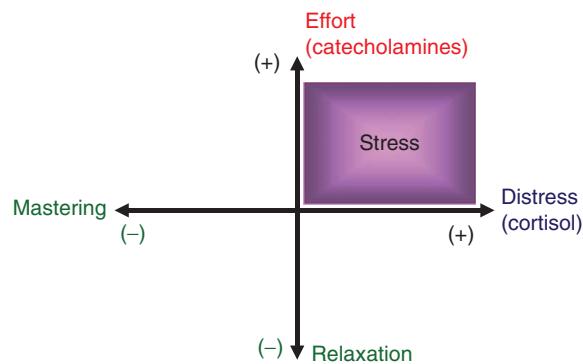


Figure 2 Influence of coping strategies on the stress hormonal response. Activation of the sympathetic nervous system is associated with effort to actively control the situation. Activation of the hypothalamic–pituitary–adrenal axis is associated with distress induced by uncontrollable and unpredictable challenges. Decreased sympathetic nervous system activation is associated with relaxation, whereas decreased hypothalamic–pituitary–adrenal-axis activity is associated with full mastering of the situation and high levels of social support. Note that stress in its classical acceptation corresponds to the upper right quadrant.

the development of neuroleptic drugs, the dictatorship of absolute homeostasis that was enthusiastically embraced by Selye was wrong. Homeostasis is not absolute but relative. The homeostasis of an organism exposed to a threat is different from that of the one at rest since everything – from behavior to physiology – must be attuned to the necessity of coping with the threat. We are constantly changing our homeostasis to adapt to the different situations we are facing throughout our daily life, and we do this in a controlled manner, in accordance with our expectations of what we will have to do.

The same concept was formalized by Peter Sterling and Joseph Eyer in the context of the neural regulation of blood pressure. The term ‘allostasis’ was coined to refer to the process of achieving stability through physiological or behavioral change. In homeostasis, physiological variables are kept constant because deviations from the set point feed back into a correction mechanism as soon as they are sensed. In allostasis, physiological variables are no longer constant but vary according to what the organism needs to do. Bruce McEwen applied the concept of allostasis to the neuroendocrinology of stress by proposing that secretion of glucocorticoids and activity of other mediators of stress, such as the autonomic nervous system and brain neurotransmitters, wax and wane with allostatic load. Adaptation to daily life events is dependent on these allostatic processes rather than on stress mechanisms that intervene only in extreme situations. The inability to turn on or off physiological and behavioral allostatic processes when needed in the face of repeated daily hassles leads to wear and tear or to an allostatic overload that is at the origin of stress disorders. In this acceptation, however, allostasis loses its original reference to the

influence of mental operations such as expectations on physiological variables. This makes allostasis differ from stress only by the inclusion of behavioral changes in addition to the physiological alterations induced by psychosocial stressors.

Social Stress

The study of influences of stress on physiology has benefited, during the last three decades, from an emphasis on psychosocial factors not only at the experimental level but also at the clinical level. Most mammalian species live in social groups that are kept cohesive by cooperation and social support and become disrupted in case of social conflict and competition. Social factors exert strong influences on the neuroendocrine system. The most striking illustration of this phenomenon is the inhibition of puberty and hormonal cycling that occurs in young female cotton-top tamarins (*Saguinus oedipus*) for the duration of their stay in the parental group. These animals start cycling as soon as they are separated from their parents. There is a tendency to believe that subordinate individuals are more stressed than dominant ones. However, this notion is wrong. In a dominance–subordination hierarchy, low-social-rank individuals do not always display heightened activity of the pituitary–adrenal axis compared to high-rank individuals. In fact, the hormonal pattern of subordinates depends on the behavioral strategies that dominants need to use in order to maintain their social rank, and the social support they receive from congeners. A dominant animal that always need to reaffirm its dominance because of repeated challenges by subdominant animals presents with a higher pituitary–adrenal activity than its congeners. Highly aggressive rodents that defend their home territory in an offensive manner display high sympathetic reactivity to stressors and behave in a relatively rigid manner in response to changes in their environment. In contrast, rodents with low aggressive tendencies have a high pituitary–adrenal reactivity and are more flexible in their cognitive strategies when confronted with a new environment. The existence of important individual variations in response to social factors makes the use of generic terms such as social stress ecologically nonrelevant. This does not prevent researchers from developing animal models of social stress or defeat to study the pathophysiology of psychiatric disorders and the mechanisms of action of neuroactive drugs.

Social Support and Other Stress-Buffering Factors

Psychosocial factors are not always negative. As mentioned above, cooperation and support are the rule rather than the exception in most social groups. Social

support refers to the physical and emotional comfort that we receive from others, especially in case of need, and it is dependent on the social network that is available to us and on the type of event we are exposed to. There is a general consensus that health is better in individuals who have high social support compared to individuals with limited or no social resources. It is important to note that the effect of social support can be due at least in part to only social proximity with familiar individuals. As an illustration of this, individuals who are exposed to stressors alone or in the face of an unfamiliar subject do less well than individuals who are exposed to stressors with individuals they already know. The emphasis by the American Psychology Association placed on positive psychology has favored the emergence of research on individual traits that buffer the impact of stress, such as hardiness, optimism, and resilience. However, these traits that are often valued by our social environment are not always beneficial. Optimism is a good example since its impact on another measure of the organism response to stress, immunity, depends not on just the tendency to have positive thoughts. As pointed out by Segerstrom, dispositional optimism that is defined by positive expectations about outcomes can be detrimental in the face of serious adversity that cannot be really mastered.

Neuroendocrine Influences on Psychological Functions

Influence of Stress Mediators on Emotion

Cannon had the intuition that the stress hormones that are released in response to stress are responsible not only for the somatic component of negative emotions but also for their psychological components, the feelings. However, his efforts toward demonstrating that the adrenalin, which is released during fear, can itself induce fear were not very successful. The psychologists who followed him in this enterprise did not achieve more success than he had. Injection of adrenalin to naive subjects so as to mimic circulating adrenalin concentrations during the emotion of anger and rage was unable to induce any emotion of this type. At the best, subjects reported feelings of a cold emotion in which they were not really engaged. Based on a limited set of experimental data collected from a series of experiments consisting of varying the socioemotional context in which subjects treated with adrenalin or a placebo were tested, Schachter and Singer proposed in 1962 that adrenalin increases arousal in a nonspecific manner. This nonspecific arousal sets up the stage for the occurrence of the specific emotional state that is triggered by the context in which the subject is immersed. In other words, specificity of the emotion caused by adrenalin is dependent on the context, not on the dose of adrenalin. Although widely accepted, the two-factor

theory of emotion of Schachter and Singer is plagued by many approximations in the analysis of the original data set and poor reproducibility of the experimental results.

Baseline versus Feedback Actions of Stress Mediators

Since these pioneering studies, many stress mediators produced not only by peripheral endocrine glands but also by the brain have been found to influence the neuronal circuits that underlie behavior. These actions are exerted both in a baseline manner and in a retroactive manner. The effects of corticosterone on submissiveness in mice submitted to repeated defeat by an experienced opponent provide a good example of the distinction between baseline versus feedback action of stress mediators on behavior. Administration of the natural adrenocortical hormone corticosterone to adrenalectomized mice facilitated the development of submissiveness, as measured by the number of attacks by the opponent that is necessary to trigger a submissive posture in the mice under study. However, this effect was more marked in the second confrontation with the opponent than in the first one. This difference indicates that an experience of defeat facilitates the effect of corticosterone on future submissiveness. This defines the feedback effect of hormones on behavior. The behavioral action of the hormone under consideration requires a prior critical experience. The best way to demonstrate a feedback effect is to modulate the intensity of the hormonal response that is triggered by the prior experience of defeat, which can be done by administering various doses of corticosterone immediately after the first defeat session to adrenalectomized mice and assess their submissiveness in a second test carried out 1 or 2 days later. The higher the corticosterone response to the experience of defeat in this experiment, the faster did the intruder mouse display submissive behavior. These results indicate that the hormonal response, the nature of which depends on the way the situation is perceived and represented and the individual behaves, influences by its feedback effect on the brain the way the individual will adapt to the same situation on further occasions.

Neuropeptides

Neuropeptides that are produced in the brain to regulate endocrine systems also have profound effects on the brain. The first example of such an effect was obtained by David de Wied. Working with Bela Bohus in Utrecht, the Netherlands, he observed that rats that had acquired an active avoidance response extinguished this response faster when treated with corticosterone but retained it longer when treated with adrenocorticotropic hormone (ACTH). The posterior pituitary neuropeptide

vasopressin was later found to have a similar effect to that of ACTH. Furthermore, peptides containing only part of the amino acid sequence of ACTH or vasopressin were found to have similar effects as the parent peptide, despite the loss of neuroendocrine effects. These results indicate that the amino acid sequence that codes for the behavioral activity of a neuropeptide is not necessarily the same as the one that codes for the endocrine activity of the peptide.

This field of research has seen a boom with the identification of a growing multitude of peptides in the brain, including peptides originally discovered in the gut. The search for behavioral activity of neuropeptides tends to be constrained by phrenology-like approaches that assign distinct roles to specific peptides, often in relation to their originally discovered activity. For instance, vasopressin was originally seen as a memory-enhancing peptide, in line with its enhancing effects on retention of passive avoidance. However, vasopressin has turned out to be involved in the formation of social memory and to control pair bonding in voles, commonly known as field mice. Prairie voles (*Microtus ochrogaster*) are highly social and monogamous, whereas montane voles (*Microtus montanus*) are solitary and polygamous. Both species have similar levels of the circulating neurohypophyseal hormone vasopressin. However, montane voles lack vasopressin receptors in some critical areas of their brains in contrast to prairie voles. Artificial insertion of the gene for this receptor into the brains of montane voles causes them to change their phenotype from promiscuity to monogamy and behave like prairie voles.

In the same manner, corticotropin-releasing hormone (CRH) is usually described as the mediator of stress-induced arousal and anxiety. However, it also acts as a modulator of immunity with opposite effects depending on its site of production. In the brain, CRH downregulates inflammation through its effects on the pituitary–adrenal axis and the sympathetic nervous system. In the peripheral nervous system and accessory cells of the immune system, CRH promotes inflammation through direct effects on macrophages and lymphocytes.

Conclusion

Psychoneuroendocrinology of stress is a very active field of research that is covered by two specialized journals, *Psychoneuroendocrinology* and *Stress*, and can be found in more general scientific journals as well. This article has been restricted to the presentation of the way psychological factors influence neuroendocrine systems and, vice versa, the way neuroendocrine systems influence psychological functions. However, the field is much richer than what this presentation might indicate. At the anatomical level, the neuronal circuits that are involved in information processing of stressful stimuli can now be examined thanks to the

development of neuroimaging techniques. Using functional magnetic resonance imaging, for instance, it is possible to study the relationship between the salivary cortisol response to an emotional visual scene and specific brain structure activity based on signals dependent on blood-oxygen levels. The way this association between the hormonal response and specific brain region activity is modulated by various conditions, such as depression or posttraumatic stress, is important to understand how attentional resources to potentially threatening stimuli are affected by the condition under study. Another important development is the consideration of temporal variations in sensitivity to stressors. The study of possible alterations in the bidirectional relationships between hormones and behavior over the life span has become an important theme of research in psychoneuroendocrinology. In particular, currently, there is much interest in understanding how activation early in life of the stress hormonal systems can shape coping strategies and physiological response to adversity at adulthood.

See also: Brain Imaging; Emotion–Cognition Interactions; Emotions; Measuring Stress; Offensive and Defensive Aggression; Hormones and Memory; Regulation of the HPA Axis by Acute and Chronic Stress; Stress and Emotionality; Stress and Social Behavior.

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Psychosocial Influences on Immunity

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Glossary

- Dispositional optimism** – A trait that reflects generalized positive expectations about the future.
- Hostility** – A stable personality trait characterized by cynical mistrust, aggressive responding, and an overly antagonistic attitude.
- Negative affect** – The feelings associated with negative engagement with the environment, which include anger, distress, and fear; can be conceptualized as a state (reflecting short-term emotional responses) or as a trait.
- Positive affect** – The feelings associated with pleasurable engagement with the environment, which include happiness, excitement, and enthusiasm; can be conceptualized as a state (reflecting short-term emotional responses) or as a trait.
- Sociability** – A stable personality trait reflecting a tendency to affiliate that has been found in a variety of species; is similar to extraversion in the human.
- Type A behavioral profile** – A personality type comprising impatience, time-consciousness, competitiveness, hostility, and aggressiveness; was once thought to be a risk factor for coronary heart disease (CHD), but newer evidence implicates only the hostility component in CHD.

Introduction

Only within the past three decades has it been generally appreciated that the immune system is not an isolated system of the body, but rather is in intimate and continuous interaction with the central and peripheral nervous systems, and that this interaction is bidirectional. This realization has led to empirical support for the long-suspected idea that psychological and social factors are associated with disease processes, and has given rise to a new scientific discipline, psychoneuroimmunology (PNI). The focus of much of this research has been on establishing links between psychosocial factors (including personality, psychopathology, coping, and environmental events) and disease-relevant immune processes, such as inflammation and immunization; in understanding the physiological mechanisms associated with those links; and recently, on using this information therapeutically.

Brief Overview of the Immune System

There are two broad types of immune responses, typically referred to as innate (natural or nonspecific are synonyms) versus acquired (adaptive or specific). Innate immune responses are the first line of defense against pathogens, allowing the organism to combat the microbe while more targeted, specific, immune responses develop over days. For example, within hours of becoming infected with a virus, a cell will produce high levels of interferons (IFNs) – proteins that inhibit viral replication. IFNs also activate natural killer (NK) cells, which kill virally infected cells. Inflammation is another aspect of innate immunity. In acute situations, injured cells release substances that alter vascular permeability locally, and that recruit phagocytic cells. Innate immune responses such as NK-cell activity and inflammation develop automatically and with consistent strength. This is in contrast to acquired immunity, whose main cell type is the lymphocyte. When a pathogen is first encountered, the few lymphocytes that can detect that pathogen become activated and then differentiate and clonally expand. Over the course of the next several days to weeks, a highly specific response develops to a protein portion of that pathogen, referred to as an antigen. Once the pathogen has been eliminated, specific immune responses retain a memory of the encounter. Should that same pathogen be encountered a second time months or years later, memory lymphocytes will be able to rapidly upregulate their responses, and so this very intense and targeted immune response will occur much more rapidly with succeeding exposures. This provides the rationale for vaccination.

Specific immunity comes in two varieties – humoral and cell-mediated. Humoral immunity results from the production of antibodies by B lymphocytes (also referred to as immunoglobulins (Ig)). Antibodies are proteins that are secreted by B cells and are specific to a single antigen. Antibodies attach themselves to the pathogen for which they are specific, and neutralize the ability of that pathogen to infect or cause disease in the host. There are five broad types of antibodies, but the type that provides the bulk of humoral immunity in the blood compartment is the G subtype, referred to as IgG. Antibodies do not enter infected cells; consequently, they are most effective against extracellular microbes like bacteria. Cell-mediated immunity, on the other hand, involves development of a specific immune response designed to kill the infected cell. The main effector cell is the

CD8+ T-cell – often referred to as a cytotoxic T lymphocyte (CTL). (CD refers to a set of proteins on the surface of the cell that can be used to identify the type of immune cell.) The function of this cell is similar to that of the NK cell, but an NK cell will kill any virally infected cell, whereas a CTL will only kill a cell expressing its specific antigen.

It is important to note that the two distinctions made so far – namely, between innate and acquired and between humoral and cell-mediated immunities – are, in reality, much less distinct. Both the innate and acquired arms of the immune system typically work together – a specific immune response can amplify, focus, and direct the innate immune response, for example. Moreover, cells of the innate system, such as the phagocytic macrophages, serve as antigen-presenting cells – they kill foreign microbes and present peptide fragments of them (i.e., antigens) on their surface to cells of the specific immune system. A particular pathogen may also activate both cell-mediated and humoral responses. Viruses, for example, are obligatory intracellular parasites, requiring a cell's machinery to replicate. While they are inside a cell, they cannot be reached by the IgG; rather cell-mediated immune responses focus on killing the infected cell. Once the virus has replicated within a cell, however, it will spread itself by leaving the cell and circulating to target other, as-yet uninfected, cells. During this extracellular stage, it is susceptible to neutralization by antibody. In addition, it is also important to recognize that one very important T cell – the CD4+ T lymphocyte, referred to as a helper T-cell – helps facilitate a variety of immune responses, both innate and specific, as well as both cell-mediated and humoral responses (via different subtypes, referred to as T-helper 1 (Th1) and T-helper 2 (Th2) cells, respectively).

There are a number of ways that immune responses can be measured. One of the easiest is simply to draw a blood sample and determine the numbers of specific types of cells in the circulation. Unfortunately, enumerative measures such as these bear little relationship to immune function – the ability of the cells of the immune system to respond to an antigen. Only a small percentage of immune cells are present in the circulation; most of the action in an immune response takes place in lymphoid tissue such as lymph nodes or spleen, and there is generally little relationship between cell numbers in peripheral blood and the ability of the organism to mount an effective immune response to a pathogen.

Most contemporary studies focus on functional immune measures. One simple functional measure of humoral immunity is to immunize (vaccinate) an animal and measure the amount of IgG that is produced several weeks later. This *in vivo* measure reflects the integrated response of an individual's immune system, and can assess a primary response (if this is the first time the animal has

been immunized with that antigen) or a secondary response (how well the immune system responds to an antigen to which it had previously been exposed). A second, related measure is to assess the amount of antibody to a latent herpes virus. Normally, the immune system manages herpes virus infections, but under certain conditions – including stressful conditions – viral antigens reappear, stimulating B cells to produce more antibodies. Thus, unlike in the first example, where a high antibody titer is (presumably) beneficial, increased antibody to a herpes virus reflects the immune system's inability to successfully manage the latent infection. A third measure of immune function involves isolating the peripheral blood mononuclear cells (PBMCs; mostly lymphocytes) from a blood sample, and stimulating them with either mitogens or antigens (an antigen stimulates a small, specific set of cells, whereas a mitogen stimulates numerous sets of cells) in order to assess mitosis (proliferation). A variety of mitogens are used, including concanavalin A (ConA) and phytohemagglutinin (PHA) – both of which are T-cell mitogens – and pokeweed mitogen (PWM), which stimulates B and T cells. Once stimulated, a marker is incorporated into the culture that allows the amount of proliferation to be measured. A fourth functional measure assesses the ability of NK cells to lyse cells from defined human tumor cell lines. A fifth measure focuses on cytokines – proteins that are produced by a variety of cell types and have many functions, including promotion of inflammation (proinflammatory cytokines) and facilitating communication both within the immune system (e.g., between cells of the innate and adaptive immune systems) and between the immune and other physiological systems. Cytokines can be measured in plasma, or in the supernatant of mitogen-stimulated PBMCs. Finally, an *in vivo* measure of cellular immunity is the delayed-type hypersensitivity (DTH) response. This involves measuring the inflammatory response 48 or 72 h following exposure to a previously encountered antigen. DTH is typically performed using a skin-prick test, and the response that is measured is the size of the resulting wheal.

Mechanisms of Nervous–Immune System Interactions

It is now known that cells of the immune system such as lymphocytes contain a variety of receptors for non-immune-related ligands, including serotonin, opioids, and various neuropeptides (e.g., substance P, corticotropin-releasing factor (CRF), and somatostatin). The bulk of research in PNI that has focused on how psychosocial factors affect immunity, however, has focused on interactions between the two major stress-response systems and the immune system.

Sympathetic Nervous System (SNS) Influences on Immunity

Perhaps the most direct mechanism by which an organism's experience can get translated into an immune outcome involves innervation of primary (thymus, bone marrow) and secondary (lymph nodes, spleen, gut-associated lymphoid tissue, etc.) lymphoid organs by catecholaminergic fibers of the SNS. Of importance, there is no anatomical evidence of efferent parasympathetic innervation of any lymphoid organ, suggesting that there may have been survival value in having the immune system responsive to increased activity of the SNS. These sympathetic fibers enter in conjunction with the vasculature, but spread into parenchymal tissue and release norepinephrine (NE) non-synaptically in the vicinity of immune cells. NE binds to β_2 -adrenergic receptors (the predominant type of adrenergic receptor on immune cells), which are present on nearly all types of immune cells, the principal exception being the Th2 cell. Stimulation of the β -receptor elevates cyclic adenosine monophosphate (cAMP), initiating a cascade of intracellular signaling events. Epinephrine, produced and released by the adrenal medulla in response to SNS activation, serves as an endocrine mediator that stimulates the same receptors.

Functionally, activation of the SNS results in a variety of complex effects, depending on when cells of the immune system encounter NE relative to their being stimulated by an antigen. In terms of innate immunity, though, *in vitro* studies suggest that NE generally suppresses NK-cell and macrophage activity, and production of the proinflammatory cytokine tumor necrosis factor-alpha (TNF- α) by stimulated macrophages appears especially affected. NE also has effects on specific immunity, such as enhancing antibody production and suppressing CTL activity. Activation of the SNS can have disease-related consequences as well. For example, suppression of NK-cell activity by NE is associated with enhanced tumor metastasis – both of which effects can be prevented by administering a β_2 -adrenergic receptor antagonist. In addition, in lymph nodes, active replication of the simian immunodeficiency virus (the monkey form of human immunodeficiency virus (HIV)) occurs nearly fourfold higher in the vicinity of catecholaminergic nerves compared to areas devoid of SNS innervation. Finally, growing evidence indicates substantial plasticity in lymph node-innervation patterns. Stress can increase the density of innervation, which is associated with increased expression of neurotrophic factors such as nerve growth factor.

HPA Axis

Should a stressor persist for more than a few seconds, activity in the HPA-axis system is likely to be increased. Areas of the brain involved in processing emotion-related

information, such as the limbic system, influence release of CRF from neurons in the hypothalamus. CRF, acting as a hormone, mediates release into the circulation of adrenocorticotrophic hormone (ACTH) from the anterior pituitary, with consequent release of glucocorticoids (GCs; primarily corticosterone in rodents and cortisol in primates) from the adrenal cortex. Long before their mechanism of action was known, GCs were used clinically as anti-inflammatory agents. These and other effects of GCs result from the fact that they are steroid hormones, fat-soluble molecules that can traverse the cell membrane, attach to intracellular receptors, and act as transcription factors to activate or repress gene expression. The principal receptor type for GCs is the GC receptor (GR) – a protein that exists in virtually every nucleated cell. GRs can affect transcription directly, acting via GC response elements in promoter regions of genes, or indirectly, through interactions with other transcription factors, such as NF- κ B, a transcription factor that upregulates expression of proinflammatory genes.

Glucocorticoids exert numerous effects in the immune system. They decrease the numbers of leukocytes in peripheral blood, in contrast to catecholamines, which often lead to an immediate increase in numbers. GCs also downregulate genes that code for proinflammatory cytokines, such as interleukin (IL)-1 β , IL-6, and TNF- α , as well as the expression of cytokine genes associated with other aspects of the immune response such as specific immunity. Moreover, GCs can also affect expression of a variety of cytokine receptors. For example, during an immune response, local production of IL-12 by antigen-presenting cells can facilitate the differentiation of naive T cells into Th1 cells, which promote a cell-mediated immune response involving CTLs and macrophages. GCs can decrease the expression of the IL-12 receptor as well as the production of IL-12. Because Th1 and Th2 responses tend to be reciprocally regulated, the effect of GCs is to generally shift the balance toward a Th2 phenotype, facilitating humoral and allergic responses and inhibiting cellular responses. In addition, because mRNA expression of the GR is itself affected by GCs, typically in a negative fashion, the effects of chronic stress, in which GCs may be elevated for days, weeks, or longer, can alter regulatory processes that lead to adverse outcomes, particularly with respect to viral infections.

Examples of Topics under Active Study

Timing of Stressors: Prenatal Effects

Events occurring early in life typically have disproportionate influences on many different outcomes, largely because of the increased sensitivity to the environment during periods when biological systems are organizing. A recent emphasis on fetal programming in biology and

medicine has shown that the experience of the pregnant female can have consequences for offspring that last well into adulthood. While animal models for study of the effects of prenatal stress on immunity are invaluable, it is important to note that the major models – rodents and primates – differ substantially on when, relative to birth, the immune system develops. This fact – along with differences between laboratories in the nature and timing of the stressor, as well as the timing of the postnatal immune assessments – may explain the many conflicting results in this new field. Nevertheless, in general terms, the effects of prenatal stress on immunity are negative. In rodent models, prenatal stress has been associated with decreased cytotoxic function of NK cells, decreased phagocytosis by macrophages, and reduced IgG in serum. Effects on cellular immune function – as indexed by measurement of proliferative responses to mitogen stimulation – have been mixed, with increased proliferation reported in response to PHA and PWM, but decreased proliferation to ConA, relative to nonstressed controls. In nonhuman primate studies, prenatal stress during midgestation has been associated with a decreased proliferative response in a mixed lymphocyte culture compared to nonstressed controls, and this difference in immunity persisted through 2 years of age. Moreover, when PBMCs were stimulated *in vitro*, lower production of the inflammatory cytokines TNF- α and IL-6 was found in prenatally stressed animals.

The primate studies also included assessments of GC sensitivity, by incubating stimulated PBMCs with varying concentrations of the synthetic GC dexamethasone. As expected, dexamethasone inhibited TNF- α and IL-6 production in a dose-dependent fashion. However, addition of dexamethasone to the wells for the control animals resulted in cytokine concentrations that were similar to those found in the prenatally stressed animals' wells that did not contain dexamethasone. This suggests that the effects of prenatal stress on infant immunity may be mediated by the effects of the stressor on the mother's HPA-axis system. GCs are known to cross the placental barrier, and are considered a major candidate for mediating the effects of stress on fetal immune development. Demonstration of the importance of GCs as mediators has been shown in primate studies in which ACTH (the peptide that stimulates release of cortisol, but which itself does not cross the placenta) was administered to pregnant females. Offspring born to such females show reduced responses in mixed lymphocyte cultures and reduced proliferative responses to ConA. Other molecules have been proposed as possible mediators of the relationship between stress in the prenatal period and postnatal immune function. These include catecholamines, opioids, cytokines, and other steroids. Whether these potential mediators exert direct effects (e.g., by crossing the placenta and influencing development of

fetal immune organs) or indirect effects (e.g., by acting on nonimmune organs in the fetus, by altering placental function) is a source of continued interest.

Duration of Stressors: Acute versus Chronic Stress

Up to the past decade, stress was generally considered immunosuppressive. Recent evidence, however, suggests that acute stress (generally lasting minutes to hours) can be immunoenhancing, while immunosuppression is typically seen when stress is chronic (lasting for hours per day over a period of months to years). The immunoenhancing effect of acute stress has been conceptualized as adaptive – in the human's evolutionary history, many short-term stressors would have been encountered that could result in injury or infection. It would make sense that natural selection has shaped physiology to overcome such natural challenges (e.g., from an encounter with a predator); individuals capable of doing so would have greater reproductive success. Given this view, it is not surprising that the immunoenhancing effects of acute stress are more often seen in localized tissues that are likely to encounter challenges such as wounding or infection. For example, acute stress has been shown to enhance the cell-mediated skin DTH response to a previously encountered antigen.

In contrast to some of the data from studies of acute stress, chronic stress typically leads to immunosuppression. This is due, in large part, to chronic activation of the HPA axis and subsequent release of GCs which, as described above, suppress inflammation and promote a Th2 response. Why might GCs serve a suppressive function? It is important to recognize that immune responses can be destructive to normal tissue as well as to damaged/infected tissue. The HPA axis is an important regulator of immunity. This has been demonstrated in studies of adrenalectomized rodents who can die within a day or two following simple immunization – but the effects can be reversed if the animals are given corticosterone replacement treatment. These studies provided some of the strongest early evidence for the idea that there is a bidirectional relationship between the immune system and the HPA axis – infection can activate the HPA-axis system, which can then serve as a brake on the resulting immune response. Chronic stress taps into this system, not only by virtue of the elevated levels of GCs that can occur during chronic stress, but also by stress's ability to alter regulation of the HPA axis (and the HPA-immune axis) through its impact on GR number and binding in target cells.

Host Factors: Personality and Immunity

Links between personality and disease have been suspected for centuries, although mechanisms have been

unclear. Certainly, personality can affect health through non-immune-related mechanisms. Conscientious individuals, for example, may be less likely to smoke, more likely to take their medications regularly, and have more health check-ups that could result in earlier disease detection. The effects of personality on immunity can also be mediated via coping behavior. For example, in a study of the simian immunodeficiency virus infection in rhesus monkeys, some monkeys experienced socially stressful circumstances. Animals that were low in sociability – a stable personality trait reflecting a tendency to affiliate – showed more submissive behavior, and also greater expression of genes that are induced by IFNs. Animals high in sociability showed the opposite pattern. Mediational analyses, however, revealed that the effect of sociability on IFN-stimulated gene expression in PBMCs was mediated completely by display of submissive coping behavior.

Other research has focused on determining whether individuals with different personality characteristics are merely built differently. For example, hostility – a major component of the type A behavioral pattern – has long been associated with increased risk for heart disease, and there has been a growing recognition that heart disease reflects an inflammatory process. However, is hostility related to inflammation outside the context of heart disease? Evidence indicates that it is – healthy, middle-aged individuals scoring high on indicators of hostility had higher concentrations of inflammatory markers such as IL-6 and C-reactive protein in peripheral blood even after controlling for health practices, demographic factors, and a closely related psychological construct – negative affect. Animal studies have also provided evidence that personality is associated with constitutional differences. Monkeys that are low in sociability had a nearly threefold higher density of NE-secreting nerve fibers in their lymph nodes, compared to monkeys that were high in sociability. Low-sociable monkeys also showed greater expression of IFN- γ and of IL-4 mRNA in lymph nodes, and, functionally, low-sociable monkeys showed a lower IgG response 9 months after tetanus toxoid immunization. Of course, data like these are largely correlational and do not address the origins of the relationship between personality and immunity, or the nature of the mechanisms linking the two.

Positive Emotionality: Positive Affect and Optimism

While the bulk of research studying psychosocial influences on immunity have focused on stress, distress, and negative emotion, there is an emerging research area examining how positive emotional states impact immune function and health. Two constructs that have been examined include positive affect and optimism.

Positive affect refers to the feelings associated with pleasurable engagement with the environment, which include happiness, excitement, and enthusiasm. As with many emotion-related constructs, positive affect can be of brief duration (often referred to as state positive affect) or can reflect a stable disposition (trait positive affect). When considered as a trait, positive affect tends to be independent of negative affect, while state-like measures of the two constructs typically reveal strong negative correlations. In general, measures of state positive affect (e.g., induced by having subjects watch a humorous film) are associated most strongly with higher levels of secretory IgA measured in saliva. This antibody type is found principally on mucosal surfaces where it serves as a first line of defense against microbes. Interestingly, similar results have been found when negative affect has been induced, suggesting that it is activation of emotions – rather than the valence of the activated emotions – that affect immunity. The similarity in results may be related to activation of the SNS in both positive and negative emotional states. Longer-term assessments of trait positive affect have generally revealed positive effects. For example, the level of dispositional positive affect was found to be positively correlated with the *in vivo* antibody response to hepatitis B vaccination, even after controlling for dispositional negative affect. Other studies have suggested that trait positive affect is associated with greater NK-cell activity, better control of latent herpes viruses, and increased Th1 cytokine responses to antigenic stimulation *in vitro*.

Like positive affect, optimism can be considered as either short term (situational optimism) or more trait-like (dispositional optimism). It is the latter construct that has been examined in immune studies. Dispositional optimism is often defined as involving generalized positive expectations about the future, and several studies have shown that dispositional optimism is generally associated with positive health outcomes such as blood pressure and self-reports of health. Studies of immune function have been mixed, however. It has been suggested that the key to understanding the conflicting results relates to the qualities of the stressor – in general, when stressors are straightforward, brief, and controllable, dispositional optimism is associated with better immune function, and when stressors are difficult, prolonged, and uncontrollable, higher dispositional optimism is related to poorer immunity. Measures that have been examined include NK-cell activity and DTH responses. Psychological explanation of these results has suggested that during difficult tasks, those low in dispositional optimism typically disengage, while optimists – who typically show higher motivation compared to pessimists – remain engaged, which can result in greater effort, fatigue, and stress.

Treatments: Behavioral Interventions

Many PNI studies have demonstrated causal relationships between psychosocial factors and immune outcomes. A few studies are utilizing this information to try to identify behavioral interventions that can palliate existing disease conditions and promote better adjustment to diagnosis and treatment for major conditions. The principal pathways by which behavioral interventions such as exercise, relaxation training, biofeedback, massage, and cognitive behavioral stress management (CBSM) work is through modulation of the stress-response systems described above. In general, research has focused on medical conditions in which the immune system is implicated, such as acquired immune deficiency syndrome (AIDS), and conditions that are relatively long term, such as cancer and persisting inflammation. Application of basic PNI results to the clinical setting is in its infancy, but will be of increasing importance in the future.

One example of a successful application has been use of CBSM in HIV-infected people. The focus of this treatment is to provide the patient with techniques that affect appraisals of stressful events, facilitate more effective coping with stressors, and promote better interpersonal interaction (e.g., through anger management). Compared to controls, individuals assigned to CBSM treatments have been shown to have reduced emotional distress and increases in CD4+ and NK-cell numbers upon notification of their seropositive HIV status – a highly significant stressor. Other studies have also shown the CBSM can result in reduced depression and anxiety, as well as lower urinary cortisol and NE levels in HIV+ males. Six- to 12-month follow-ups of these males showed improvement in immune measures – including CD8+ numbers in peripheral blood – and these improvements were most evident for men who had shown the greatest reductions in hormone levels during the original CBSM intervention. Massage therapy has also shown significant effects in HIV+ males: increases in the number of CD8+ cells and NK cells in peripheral blood, increased NK-cell cytotoxicity *in vitro*, as well as reduced cortisol output and decreased anxiety. As with the CBSM study, follow-up again revealed that the men that had shown the greatest reduction in distress during the original intervention had a smaller reduction in T-cell numbers at the 24-month follow-up.

Acknowledgment

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See also: Animal Tests for Anxiety; Behavioral Development and Socialization; Cytokine Effects on Neuronal Processes and on Behavior; Depression; Evolution of Emotions; Fear, Anxiety, and Defensive Behaviors in Animals; Human Fear and Anxiety; Maternal Deprivation; Measuring Stress; Perinatal Influences on Behavior and Neuroendocrine Functions; Personality, Temperament, and Behavioral Syndromes; Regulation of the HPA Axis by Acute and Chronic Stress; Stress and Emotionality; Stress and Social Behavior; Psychoneuroendocrinology of Stress.

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Infant Bonding and Attachment

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Glossary

Altricial – This refers to animals that are relatively undeveloped at birth and require intensive parental care for survival. Both humans and rats are altricial.

Amygdala basolateral complex – The amygdala encompasses several nuclei with distinct functions. Among these nuclei are the basolateral complex, central, medial, and cortical nuclei. The basolateral complex can be further subdivided into the lateral and the basal nuclei, which are associated with plasticity and fear learning.

Attachment and developmental psychology – While no strict definition can be given for attachment, several research areas use this word differently. The animal literature, including neurobiology, views attachment as a bond that can occur rapidly between the infant and mother or any two individuals. Attachment with respect to the human literature and developmental psychology, which is generally synonymous with attachment theory, views attachment as a protracted process occurring over the first year of life and suggests a very complex cognitive representation of the bond between two individuals. To ensure no confusion, this article is from the viewpoint of the neurobiology of attachment. However, to ensure translation across disciplines and how attachment relates to humans, attachment and attachment theory will be briefly outlined.

Classical conditioning – Also called Pavlovian conditioning, this is a form of complex learning involving the association between two stimuli based on their contingency and predictive value. Procedurally, a neutral conditioned stimulus (CS, i.e., a tone or odor) is repeatedly paired with an unconditioned stimulus (US, i.e., a shock or food). During conditioning, the animal learns that the CS is associated with or predicts the occurrence of the US and the animals will behave toward the CS as though it is the US or prepare for the onset of the US. In fear conditioning, where a tone or odor predicts the occurrence of a shock, the animal will learn to avoid or freeze with fear when the learned CS is presented, suggesting the animal is preparing for the arrival of shock or danger.

Electroencephalography (EEG) – A technique that measures the brain's electrical activity produced by the electrical activity of neurons during their communication

with one another. This technique requires electrodes to be placed on the scalp and measures the neural activity of groups of neurons.

Freudian psychoanalysis – Psychoanalytic theory originated with Sigmund Freud and was based on his interactions with patients with mental illness. Freud believed that childhood experiences and the unconscious influenced the patient's mental health.

Functional magnetic resonance imaging (fMRI) – A brain scan used to measure the neural activity of the brain. It measures changes in brain hemodynamics that correspond to the increase in blood flow in the local vasculature that accompanies brain neural activity.

Imprinting – A phase of potentiated learning temporally limited to a particular age range or sensitive period. Here, we describe filial imprinting since the learning involves the social bond formed by the young to its mother, which results in the young following the mother.

Limbic system – A group of brain areas distributed in cortical and subcortical areas in both humans and rodents. The limbic system's roles include emotional and cognitive function. Brain areas typically included and their functions are: amygdala (emotional regulation, especially fear; learning, and stress regulation), hippocampus (learning and memory, as well as stress regulation), prefrontal cortex (decision making, attention, and autonomic function including stress regulation, learning and memory, and emotional regulation).

Magnetic resonance imaging (MRI) – A technique that can be used to image the brain and visualize the brain's structures. It uses a magnetic field to align the nuclear magnetization of hydrogen atoms in water/blood in the body.

Major histocompatibility complex (MHC) – A large gene family found in most animals and has an important role in the immune system. In mammals, including humans and rats, the MHC contributes to unique, genetically determined body odors. The volatile odors are transmitted through body fluids, such as sweat and urine.

Sensitive period – A temporally limited period or age range of accelerated learning, experience, or development.

Stress system – This system coordinates brain activity with emotional and physical stressors and controls the

release of the stress hormone corticosterone (in rats) or cortisol (in humans) from the adrenal glands. The stress hormone modulates brain activity to produce behavioral responses, adjust homeostasis, and produce an adaptive response for survival. The main components of the stress axis are the hypothalamus–pituitary–adrenal gland, and the amygdala–locus ceruleus.

Robert Hinde characterized imprinting, which suggested that attachment to the caregiver was innate or biologically determined. Specifically, imprinting was first characterized in chicks as a temporally limited sensitive period when the hatchlings learn to follow the real or foster mother. The mother then elicits a following response in the young. This early-life attachment lays the foundation for later-life choice/acceptance of a reproductive partner.

Second, research on maternal separation and social isolation in rhesus monkeys by Harry Harlow and his colleagues characterized the importance of affection and social interactions with the mother for social and cognitive development. Specifically, based on observations that human infants became distressed and ill during prolonged separation from the mother, Harry Harlow separated infant rhesus monkeys from their mothers. These infant monkeys appeared to mirror the strong emotional and physical stunting of orphaned and hospitalized infants separated from their mothers. Furthermore, both the orphaned monkeys and children showed enduring emotional and cognitive problems that continued into adulthood.

This early work was quickly followed by focused studies on rodents and nonhuman primates to better understand how and why the infant showed such dramatic effects when separated from the mother. Interestingly, attachment was first studied by the removal of the attachment figure and is referred to as the maternal deprivation or maternal separation paradigm. In general, within a few hours of separation, the stress axis is engaged and shows increases in the stress hormones, corticosterone and cortisol, and in adrenocorticotropic hormone (ACTH), which controls the stress response at the level of the pituitary, although recent research suggests there are ubiquitous effects throughout the brain.

In the 1981 book, *Roots of Human Behavior*, Myron Hofer described the unique role of sensory stimuli in controlling pup behavior, brain, and physiology and provided a clearer understanding of the infant's response to separation from the attachment figure. Hofer defined the mother as a 'hidden regulator' of pup behavior and physiology through her sensory stimulation of the pups. Indeed, it is the mother that maintains pups at homeostasis, with different stimuli and her temporal patterning controlling specific behavioral and physiological systems. Thus, maternal separation could be viewed as removal of sensory stimuli that were controlling homeostasis. For example, tactile stimulation increases growth hormone, warmth increases the neurotransmitter norepinephrine (NE), and maternal odor increases behavioral activity. Thus, removal of one or all of the regulating sensory stimuli produces deregulation in pups' behavior and brains. The long-term effects of maternal separation appear to produce an animal that is more behaviorally

Introduction

Humans are social animals, relying on emotional attachment to others for emotional well-being. Attachments are made throughout the life span and include the bond between any two organisms, although a particularly important attachment exists between an infant and the caregiver, as well as reproductive partners. Here, we focus on the attachment between the infant and the caregiver, and briefly review its psychology and neurobiology.

The Infant's Attachment to the Mother

At birth, the newborn infant transitions into a world filled with new sensory experiences, including new sights, sounds, textures, and temperatures. Beginning just moments after this dramatic transition, the infant must rapidly reorganize fetal behavior to postnatal behaviors that elicit nurturing from the caregiver. Indeed, the mother and infant rapidly engage in a finely tuned dance of social behaviors that enable both the infant and mother to learn about one another and support bonding and attachment. Two words have been used, almost interchangeably, to describe the infant's relationship with the parent or caregiver – bonding and attachment – which relate to a strong emotional bond between the infant and caregiver.

The importance of childhood for emotional well-being was first highlighted by Freud. However, it was not until the 1950s, with animal studies on maternal separation in primates and rats, as well as clinical observations of maternally separated hospitalized and orphaned children, that the study of early-life attachment and its importance for mental health came into experimental focus.

Animal Research and the Importance of Maternal Care of the Infant

The importance of the care and nurturing of infants by the mother was dramatically demonstrated around 1950 by a series of experiments in chicks, rodents, and nonhuman primates. First, Konrad Lorenz, Niko Tinbergen, and

responsive to stressful situations as suggested by increased fear, anxiety, cognitive impairment, anhedonia, and susceptibility to drug and alcohol abuse. The long-term effects of maternal deprivation also appear to alter maternal care, which is then transmitted nongenomically through the next generations.

John Bowlby and Attachment Theory

In the 1950s, the psychiatrist John Bowlby developed attachment theory and an expanded version of this theory is a critically important framework in developmental psychology. This theory was in response not only to Bowlby's training as a Freudian psychoanalyst, his personal clinical observations on disturbed children, but also to the emerging animal research of the 1950s described above, which suggested a biological system for an infant's attachment to the caregiver and its importance for mental health. Bowlby's patients and initial research project involved a group of maladjusted children and was entitled 'Forty-four juvenile thieves.' He identified a common factor in these children: all had been deprived of their mothers in childhood. A second major influence on Bowlby was Rene Spitz's and James Robertson's observations on the detrimental effects of separating the child from the mother during hospitalization and the resultant extreme emotional distress. Bowlby was also intrigued by the work of animal researchers who were characterizing the importance of early-life attachment, evolution of a biological attachment system in infancy, and the importance of maternal care on later mental well-being in other species.

Thus, through a synthesis of clinical and basic research, as well as the synthesis of research from biology

and psychology, Bowlby developed his theory of attachment. He focused on the importance of a biological system for infant's attachment to the caregiver and the importance of quality caregiving for normal development. Attachment theory's central core is that the child's relationship with the mother has profound enduring effects on cognitive and emotional development. The child seeks proximity to the mother or other caregiver, which provides protection and a sense of safety. The caregiver needs to be available to the child and provide a secure base for the child to explore the world and develop other relationships. The child builds a representational mental model of the self and others based on this early relationship, which then shapes the child's emotional and cognitive development. A caregiver that shows sensitivity to their needs, supports their behavioral expression, and is available when needed, will produce a healthy infant with a secure attachment. An unavailable, obtrusive, or psychologically or physically abusive caregiver produces an insecure attachment and disturbs emotional and cognitive development. Bowlby concluded that through evolution and due to the critical importance of attachment for survival, children are biologically preprogrammed to form attachments to their caregiver (Figure 1).

Developmental Psychology and Attachment: The Strange Situation Test

In the 1970s, Mary Ainsworth, a student and collaborator of John Bowlby, focused on the attachment quality issues of attachment theory and developed the 'strange situation' experimental paradigm. This test, which uses the infant's proximity seeking to the caregiver combined with the child's desire to explore a novel environment, was used

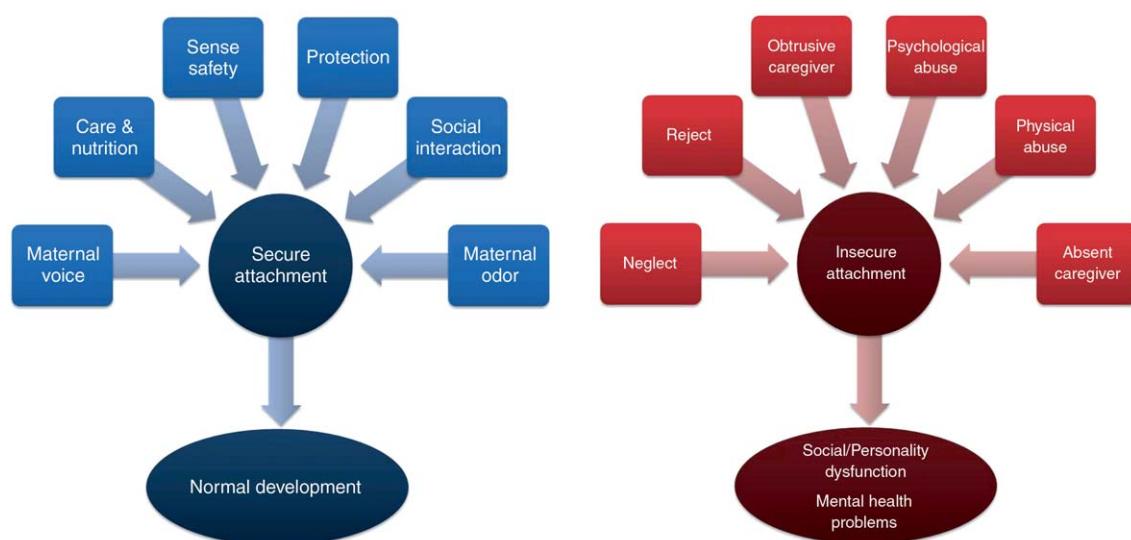


Figure 1 Environmental factors that contribute to secure (right) and insecure (left) attachment during infancy and its long-life effects.

to characterize different types/qualities of attachment. Based on this laboratory observation of mother-child separations and reunions, Ainsworth noted that most children have a secure attachment to their caregiver. This type of attachment is associated with a caregiver who is responsive and sensitive to the needs of the child, supports the child's ongoing behavior, and is available for the child. The child develops a sense of safety with this caregiver and uses the caregiver as a base for exploring the world and forming additional relationships. Ainsworth also identified characteristics of insecure attachments referred to as 'avoidant attachment,' which is associated with rejecting, interfering, or inconsistent caregiving and ambivalent attachment. An additional category of disordered attachment has been added more recently, which is most closely associated with child abuse. While attachment theory has not been without criticism, it has been a strong focus of developmental research within psychology and has greatly increased our understanding of the importance of early-life infant-caregiver attachments.

Clinical studies have found that early-life abuse is also associated with enduring effects within the brain. Although it is still unclear how attachment style in early life relates to the enduring effects of early-life abuse/trauma, caregiver abuse and neglect may be particularly damaging to the brain. These early-life events leave the child vulnerable to not only a wide range of social or personality problems such as hyperactivity, aggression, delinquency, or substance abuse, but also psychiatric problems such as borderline personality disorder, depression, posttraumatic stress disorder (PTSD), and schizophrenia. Child abuse is associated with abnormal brain responses associated with cognitive and emotional function and also in areas involved with regulation of the stress response, such as the amygdala, hippocampus, frontal cortex, and cerebellum. While a wide variety of techniques have been used to assess the relationship between abuse and human brain function, including functional magnetic resonance imaging (fMRI), MRI, and electroencephalography (EEG), due to ethical and practical restraints in human studies, these studies must rely on correlation. For a causal relationship, we must rely on animal studies that are reviewed below.

Early Translational Research: The First Postnatal Days and Attachment

In the 1980s, Marshall Klaus and John Kennell presented the concept of bonding in their classic book *Maternal Infant Bonding*, which suggested newborns had a sensitive period at birth that enabled rapid, robust learning about the mother. This idea was generated by animal research, which indicated that ewes require immediate contact with the lamb for acceptance and attachment. Klaus and Kennell asked whether mother-infant contact

immediately after birth was critical for attachment in humans and compared mother-infant dyads placed together or separated for the hours after birth. Their results suggested that mother and infant dyads that were together right after birth showed stronger attachment/bonding. This work was the catalyst for dramatic changes in hospital policies and mothers were permitted to keep their babies in their rooms. While this togetherness during the postpartum period is important, humans are resilient and infants separated from their mother at birth generally do form a strong attachment and quickly show bonding as strong as babies given immediate contact with their mother. More prolonged separations, as can occur with illness or prematurity, can require special attention for attachment. That is, unlike sheep bonding, human bonding is not a now-or-never occurrence, although more effort may be required when circumstances prevent early and consistent contact between the mother and infant. The success of adoptions, as well as the caregiver-infant relationships between the child, fathers, and other caregivers, illustrates human resilience for bonding beyond the early postpartum period and the importance of social interactions in human resiliency. It should be noted that while we now understand that human and sheep infant attachment differ, the animal work provided a new way of viewing human infant attachment and resulted in changing clinical practices in nurseries.

The Infant Shows Attachment Behavior Toward the Mother on the First Day of Life

More recent research efforts have complemented this early work on attachment and bonding by assessing the birth process and defining the events of early life. While the infant is a social being at birth, the process of building this social infant begins during the last trimester of pregnancy. The womb provides a rich auditory experience through the rhythm of the mother's heartbeat and blood flow. Due to the higher pitch of the female voice, the baby hears its mother's voice not only through the mother's abdomen but also transduced through her bones. The prenatal infant also swallows and inhales the amniotic fluid, which activates the taste receptors on the tongue and the olfactory receptors in the nose. Amniotic fluid is relatively distinctive for each mother because it has her unique olfactory signature through her diet and major histocompatibility complex (MHC). These prenatal odors appear to bridge pre- and postnatal life since the birthing room is typically filled with the odor of amniotic fluid.

At birth, the mother's voice and odors have powerful ability to guide the infant's motor patterns and emotional responses to facilitate interactions with the caregiver. These stimuli, which were learned and experienced *in utero*, lay the foundation for the infant's social behaviors

during the first few hours of birth. However, the postnatal infant quickly learns other characteristics of the mother as well as the bond and attachment between the mother and infant is strengthened. In the 1980s, DeCasper and Fifer showed that an infant just a few hours old will modify his or her sucking to hear a recording of the mother's voice. Her voice is also capable of soothing a fussy infant and bringing the infant to an optimal state for attending to the environment. The infant's visual system is also tuned to focus the infant's attention on the mother, with infants biologically predisposed to focus on the human face. Benoit Schaal and his colleagues, as well as work in our lab, have shown that maternal odors are also important to the newborn and are likely learned during late pregnancy, during birth as well as the early weeks of life. Importantly, the maternal odor is not a pheromone and can be learned by the infant. Indeed, a novel citral odor or perfume worn by the mother can become the maternal odor. The mother's odor has powerful effects on the infant's behavior: mother's odor can soothe a crying newborn and guide him or her toward the breast for feeding. Indeed, this work suggests that, when a mother holds her newborn infant, her odor can initiate a sequence of behaviors that ensure nipple attachment: cessation of the baby's crying, optimization of the infant's state, head turning toward the breast, and mouthing. Moreover, the maternal odor reduces the stress response by reducing the amount of cortisol released by the adrenal gland and helps infants develop a more organized sleep-wake cycle.

The Neurobiology of Attachment

As noted above, Bowlby's characterization of human attachment was strongly influenced by animal research, and for this reason, it is not surprising that Bowlby's attachment theory is readily applied to other species. Indeed, many altricial species must learn an attachment to their caregiver and must have a specialized biological system that supports infant attachment. Recent research on rat pups has been characterizing the attachment circuitry. While human attachment is certainly more complex than that seen in rat pups, animal models can provide insight into basic neural circuits for attachment. There are notable differences between rats and humans. First, humans rely on vision, audition, and olfaction for attachment, while pups rely only on olfaction. Second, and more importantly, the role of cognition in human attachment is more complex than that seen in rat pups. However, while rats and humans may not have identical neural circuits supporting attachment, some overlap may exist, such that general principles of the neurobiology of attachment will be useful.

Infant Rats Show Robust Rapid Learning of the Maternal Odor

Similarly to human infants, rat pups also face the daunting task of learning about their caregiver and attaching just minutes after birth. Since pups are born without vision and audition, they must rely on odors to approach the mother and exhibit appropriate behaviors, such as huddling and nipple attachment. While we initially thought the mother emitted a pheromone to control pups' behavior, we now understand that pups learn the maternal odor before and during the birth process and continue to learn the diet-dependent maternal odor throughout early development. Indeed, simply placing a novel odor (e.g., peppermint or orange) on the mother during interactions with pups is sufficient for pups to learn a new maternal odor. The maternal odor, whether natural or a peppermint odor paired with the mother, elicits approach from the pups and permits nipple attachment. Without the maternal odor, pups do not survive. This odor also retains value into adulthood, where it controls reproductive behaviors. To facilitate a neural analysis of attachment, pups are removed from the nest and maternal behavior is mimicked during pairings with an odor using a learning paradigm called 'classical conditioning.' This extra-nest attachment learning also enables the odor to take on characteristics of maternal odor, which produce approach responses and nipple attachment.

Attachment Learning Requires NE from the Locus Ceruleus

Attachment odor learning in rat pups is supported by both anatomical and physiological changes within the olfactory bulb, the brain area important in processing odor information. Importantly, the same neural response occurs to the natural maternal odor, or an artificial odor conditioned to become the maternal odor (e.g., placed on the mother or classically conditioned). This plasticity induced by olfactory-bulb learning is attributable to a large influx of NE released from the locus ceruleus (LC) into the olfactory bulb, which causes prolonged activation of the bulb's primary output neurons (mitral/tufted) and prevents habituation to repeated presentations of the odor. Notably, the abundant release of NE to the olfactory bulb is induced by the same stimuli that support attachment learning and preventing the LC from releasing NE also prevents attachment learning. Thus, enhanced NE during an odor presentation is sufficient to produce the plasticity within the olfactory bulb and pups' learning of a new maternal odor.

Sensitive Period for Attachment Learning

NE only supports attachment learning when pups are infants because the infant LC releases approximately 3 times more NE into the olfactory bulb than the adult LC. The abundant release of NE by the infant LC occurs for a number of reasons. Specifically, the LC responds to a very broad range of sensory stimuli, fails to habituate after repeated sensory stimulation, and does not have the functional LC's α_2 inhibitory autoreceptors that terminate the LC's excitatory response in adults. Indeed, a 1-s stimulus generally produces a 20–30-s response in the LC of the infant, but only a few milliseconds one in the adult. Thus, unique prolonged and robust functioning of the LC produces the abundant release of NE required to support the neural plasticity for the rapid and robust odor attachment learning exhibited by infant rats. The sensitive period ends as the LC matures and fails to release sufficient NE to support plasticity in the bulb.

Avoidance and Fear Learning Are Suppressed during the Sensitive Period by Attenuation of Amygdala Function

One characteristic of attachment listed by Bowlby was the child's persistence in learning and maintaining attachment to a caregiver, even when the caregiver inflicts the child with pain. This characteristic of attachment has also been demonstrated in rat pups. Indeed, attachment learning occurs equally well when a novel odor is paired with presumably pleasant stimuli such as milk, warmth, or tactile stimulation, or painful stimuli such as 0.5-mA shock or tail pinch. This pain-related attachment learning also exists within the natural environment of the nest.

Specifically, pups were placed with a mother rat that mistreated pups in the presence of a novel odor, yet the odor still supported a learned odor preference and nipple attachment at a level similar to natural maternal odor. That is, whether the mother nursed and groomed the pups or mistreated them by biting or trampling on them in the presence of a novel odor, all pups later showed robust approach responses to the newly learned artificial maternal odor.

Sensitive-period learning is usually characterized by enhanced learning. This paradoxical learning illustrates that limitations on learning also occur. For example, as noted above, shocking chicks during imprinting to the mother supports approach learning, while shock supports avoidance learning just hours after the imprinting-sensitive period closes. Similarly, shocking or mistreating an infant dog while interacting with a caregiver results in a strong attachment to the caregiver. This paradoxical attachment learning has also been demonstrated in non-human primates by Harlow and, more recently, when abused infant monkeys form strong attachments to an abusive caregiver. Furthermore, children tolerate considerable abuse while remaining strongly attached to an abusive caretaker. We have hypothesized that through selection pressure and evolution, the attachment system has evolved to ensure that the infant attaches to the caregiver regardless of the quality of caregiving received ([Figure 2](#)).

At least in rat pups, the neurobiology for this paradoxical attachment learning has been partially documented. Specifically, the amygdala, which is a brain area required for fear and avoidance learning in adult animals, does not participate in the infant odor learning. The assessment of

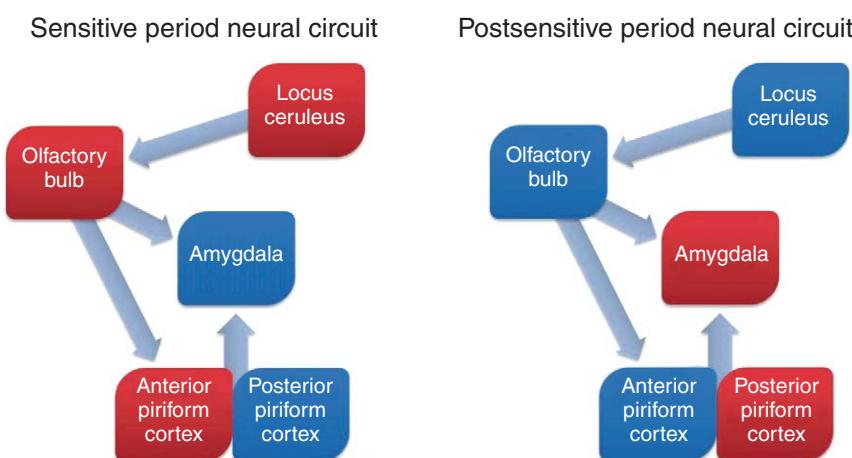


Figure 2 The unique neurobiology of infant learning and its transition to the more “adult-like” learning circuit in the brain of rat pups. Specifically, the neural basis of attachment learning with olfactory conditioning during the early life sensitive period for attachment uses the olfactory bulb, anterior piriform (olfactory) cortex and the locus coeruleus (red areas) to support approach learning regardless of the quality of care the infant receives. With maturation and the end of attachment learning, the neural circuit for learning changes so that the quality of care (pain or pleasant) becomes important in determining what is learned in older pups. The learning circuit for pain based learning then includes the posterior piriform cortex and the amygdala (red areas).

the neurobiology of infant attachment indicates infants possess a unique learning circuit that ensures rapid and robust learning of the maternal odor regardless of its association with a normally behaving mother, a mother mistreating her pups or painful stimuli presented in a classical conditioning paradigm outside the nest. Indeed, all these procedures appear to activate the same neural circuit: the olfactory bulb, anterior piriform of the olfactory cortex, and the LC. Briefly, during the presentation of a reward (maternal care, milk, stroking, etc.), the hyperfunctioning infant LC releases a huge amount of NE into the olfactory bulb. The combined stimulation of the olfactory bulb with an odor and NE is sufficient and necessary to support the neural plasticity within the bulb for learning of the maternal odor. This odor learning and the plasticity of the olfactory bulb is sufficient to produce a maternal odor that supports not only approach responses, but also the complex sequence of motor responses required for nipple attachment. However, pups' attachment learning with pain also requires suppression of the amygdala's plasticity, which is normally activated by pain and required for learning fear. It should be noted that electrophysiological studies indicate that the pain information reaches the amygdala, yet the amygdala fails to exhibit the plasticity required for fear learning.

Thus, attachment learning in rat pups not only appears to activate the attachment circuit but also inhibits amygdala plasticity if that attachment learning is associated with pain. The suppression of amygdala plasticity presumably prevents pups from learning to avoid the maternal odor and the care required for survival that is provided by the mother. This unique ability for pups to prevent avoidance/fear learning and suppression of amygdala function in early life is due to pups' naturally low levels of the stress hormone corticosterone, which is required by the infant for amygdala plasticity. Importantly, while the amygdala was suppressed during attachment learning, pups experiencing pain developed a smaller amygdala basolateral complex. Remarkably, simply experiencing the pain without attachment did not result in changes in amygdala size, indicating that pain within attachment is processed differently than pain without attachment.

Implications of Animal Studies for Understanding Human Attachment

Infant rat studies suggest that early attachment memories are formed within brain circuits that are different from those used later in life. They also suggest that the circuitry involved in attachment learned with painful stimulation is likely to be mediated by additional brain regions and hormonal facilitation. The comparison of normal attachment formation and pain-related attachment suggests similar behaviors, yet different neural

substrates that may lay the foundation for the enduring effects of early-life trauma.

The Mother's Attachment to the Infant

Birth is also a transition for the mother and requires considerable learning and adaptation that complements the infant's attachment. Unlike the infant, however, the human mother relies on years of experience of observing and experiencing infants suggesting a considerable importance of cognition and learning. Indeed, cognitive ideation of the mother's role in parenting and the attachment quality experienced in the mother's childhood have robust effects on the quality of mothering and attachment a mother provides her baby. Indeed, the social transmission of maternal behavior and its flexibility likely supports the diverse climates and cultures that support human populations. On the other hand, biology has a role in maternal behavior in humans. Remarkable consistency in emotional responses is associated with late pregnancy, and birth is associated with heightened sensory responsiveness, rapid learning about the baby, and a strong need to be with and hold the infant.

Summary and Conclusion

Attachment is a strong bond that can be formed at any stage of development in humans and other social animals. Robust attachments are formed at sensitive periods associated with reproduction, including the reciprocal mother–infant attachment associated with altricial animals such as humans and rats. While this article has primarily focused on the infant's attachment to the mother, attachment is a process of interactions between the mother and infant. While the infant's role in this relationship appears passive, minor changes in the infant's behavior toward the mother can produce confidence or weakening in the mother's nurturing skills and attachment. Indeed, the expression of appropriate responses by the infant to the mother, as well as the mother to the infant, is critical for the synchrony of attachment. Thus, both the infant and mother are active and critical participants in the mother–infant dance that requires subtle coordination between the mother and new baby. The vulnerability of bond formation in mothers of unresponsive infants, either because of illness or prematurity, illustrates the important role of the infant in this dyad.

Due to ethical and practical concerns, we must rely on animal studies to characterize a causal link between neurobiology and behavior in attachment. While humans and other animals may not use the same behaviors and neural structures for attachment, behavioral similarities suggest at least some overlapping neural circuits that may involve

amygdala suppression. More globally, cross-species comparisons may provide insight into unique ways of viewing attachment and potentials for intervening and preventing attachment difficulties.

See also: Animal Models of Learning and Memory; Behavioral Development and Socialization; Cognition: Learning and Memory: Pavlovian; Fear, Anxiety, and Defensive Behaviors in Animals; Maternal Deprivation; Neural Basis of Gender; Neural Substrates of Conditioned Fear and Anxiety; Stress and Emotionality.

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- <http://www.nichd.nih.gov> – National Institute of Health: National Institute of Child Health and Development
- <http://www.psychology.sunysb.edu> – Psychology Department – SUNY Stony Brook: Assessing attachment quality in adults.

Parental Behavior

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Glossary

Alloparental behavior – Parental behavior directed toward a conspecific infant by an individual who is not the infant's biological parent.

Amygdala – A neural region in the limbic system that is involved importantly in the regulation of fearfulness and defensive behaviors. Two important nuclei are the medial nucleus of the amygdala (MeA) and the central nucleus of the amygdala (CeA).

Corticotropin-releasing factor (CRF) – A neuropeptide produced in the brain which contributes to neural circuits regulating fearfulness, anxiety, and stress responsiveness.

Fos – A protein whose expression is increased in cells, including neurons, when the cell is affected by input signals, such as neurotransmitters or hormones. The detection of Fos expression within neurons is typically used as a marker of neural activity.

Lactogens – Peptide hormones – including pituitary prolactin and placental lactogens – which not only stimulate lactation, but also act on the brain in conjunction with the steroid hormone estradiol (produced by the ovary) to stimulate maternal behavior.

Medial preoptic area (MPOA) – A brain region located in the rostral hypothalamus that is essential for maternal behavior. It is the site where estradiol, prolactin, and oxytocin act to stimulate maternal responsiveness.

Mesolimbic dopamine (DA) system – A neural system originating from DA neurons in the ventral tegmental area (VTA) of the midbrain. These neurons have ascending projections to various parts of the subcortical telencephalon which include a projection to the nucleus accumbens (NACC). An important area to which the NACC projects is the ventral pallidum. Activation of the VTA–NACC DA system appears to regulate a variety of goal-directed or motivated behaviors, including maternal behavior.

Null mutation – A mutation produced in a transgenic mouse strain which eliminates the functional activity of a particular gene. It is also referred to as a knock-out mutation. For example, when a mouse line is produced with a null mutation of the prolactin receptor gene, then cells in the body can no longer produce the prolactin receptor protein.

Oxytocin – A neuropeptide that is produced in the brain and can function either as a hormone (when it is released into the general blood supply via the neural

lobe of the pituitary gland) or as a neurotransmitter (when it is released locally at synapses in the brain). Oxytocin (OT) acts on oxytocin receptors (OTRs) in the MPOA and VTA to stimulate the onset of maternal behavior. Estradiol can stimulate the synthesis of OTRs in the brain.

Introduction

Parental behavior in mammals includes maternal, paternal, and alloparental behavior. Most mammalian species show a polygynous or promiscuous mating system, paternal behavior does not occur, and uniparental maternal behavior is the dominant pattern. However, monogamous mating systems do occur in some mammals, such as prairie voles, California mice, and marmoset and tamarin monkeys. In such species, family groups can consist of mother, father, recently born young, and older subadult or adult siblings. In addition to the mother, the father and older siblings may care for the young for long periods of time.

Because maternal behavior is the dominant form of mammalian parenting, it will be our primary concern. The nature of maternal behavior is influenced by the development of the young at birth and the social group into which the young are born. Infants may be altricial (immature) and immobile at birth, precocial and mobile at birth, or intermediate between these two developmental extremes (semiprecocial and semimobile). Rodents, ungulates, and primates, respectively, will be used as representative examples of each case. Most rodent infants are helpless and incapable of temperature regulation at birth. They are born into a secluded nest site where the mother crouches over the young to nurse them and to keep them warm. She also licks them, which grooms them and facilitates urination and defecation. If the nest site is disturbed or if pups become displaced, the mother shows retrieval or transport behavior where she carries pups one at a time in her mouth to a new nest site or back to the original nest. Experimental studies have shown that most rodents do not form selective attachments to their own young: one can cross-foster young among litters and mothers will care for these young. The evolution of selective attachment mechanisms has not occurred because infants are not capable of wandering from one

nest to another and, therefore, confusion between own and alien young is not likely to occur. Ungulates, such as sheep and goats, give birth to precocial young which are relatively mature at birth, and these young are born into herds composed of unrelated individuals. Selective attachments between the mother and the calf occur rapidly after birth, and the mother will remain near her calf and nurse only her calf, while rejecting the advances of alien young. These attachment mechanisms prevent confusion between own and alien young under these conditions. Lastly, maternal responses in primates are adapted to young who are semiprecocial at birth. Initially, the mother is in constant contact with her offspring, nursing and grooming it, and the infant clings to its mother for transport. During movement, the mother carries the infant either ventrally or dorsally. As the infant develops it will begin to wander away from the mother, but the mother is usually aware of its location and, when necessary, will retrieve the infant. Selective maternal attachments develop in most primates, although this development is not as temporally rigid as that which occurs in sheep and goats, which is consistent with the semiprecocial nature of primate offspring.

Maternal behaviors are sometimes divided into appetitive and consummatory components. Appetitive components are proactive voluntary maternal behaviors such as approaching, guiding, grooming, and retrieving/transporting the young. The consummatory component is more reflexive and inactive in nature and primarily consists of nursing behavior. Appetitive maternal behaviors engage forebrain control mechanisms, while nursing responses may be controlled primarily by brainstem reflexive mechanisms.

There are two other important characteristics associated with the maternal condition: (1) maternal aggression, which is an increase in aggressiveness that presumably allows the mother to protect her young and (2) a postpartum decrease in fear- and anxiety-related behaviors, which may allow the mother to take reasonable risks in order to raise her young.

Hormonal Mechanisms Regulating Maternal Behavior

For most mammals, the hormonal events associated with pregnancy termination and the initiation of lactation are also involved in stimulating maternal responsiveness. Of the typical female mammal, nulliparous or virgin females do not show maternal behavior. Such females avoid infants and reject their advances. In contrast, newly parturient females will care for their own or alien young. In the case of those species which form selective attachments to specific young, learning mechanisms are involved: an ewe will act maternally to any lamb that is presented to

her at the time of birth, but once a bond is formed with that lamb, she will reject other lambs. The formation of this selective attachment is the result of olfactory learning mechanisms which allow the parturient mother to recognize the odor of the lamb to which she was exposed.

These findings from typical virgin and parturient mammals have led to the idea that hormones stimulate maternal responsiveness at parturition by acting on the brain to inhibit avoidance and rejection responses toward young infants while stimulating neural circuits which regulate approach and acceptance responses (**Figure 1**).

Most of the research on the endocrine basis of maternal behavior has been done on rats. The critical hormonal events include declining levels of progesterone and rising levels of estradiol and lactogens (pituitary prolactin and placental lactogens). Similar mechanisms are operative in sheep. In sheep, the vaginal and cervical stimulation which occurs at birth co-acts with these hormonal mechanisms.

Oxytocin is a hormone released by the posterior pituitary at parturition and it stimulates uterine contractions. Oxytocin – as a hormone – probably is not essential for maternal behavior since it has poor penetrance across the blood–brain barrier. However, oxytocin – serving as a neurotransmitter/neuromodulator – is also released into the brain of the parturient female where it acts to promote maternal behavior.

Although the endocrine events of pregnancy termination stimulate maternal behavior in the typical female mammal, other events must also be operative in those cases where paternal behavior or alloparental behavior occur, since these individuals are not exposed to those hormonal events. One proposal is that there is a basic and intact neural circuitry regulating parental behavior in all mammals of both sexes, but this circuitry is under varying intensities of inhibition, based on sex, genetic, developmental, hormonal, and experiential/environmental factors. When this parental circuitry is under low inhibition, infant stimuli may activate a certain degree of caregiving behavior in the absence of pregnancy-related hormones.

In some cases where long periods of infant dependency exist – which occur in primates, including the human – a monogamous mating system with high levels of maternal, paternal, and alloparental behavior may be important for infant survival. Evolutionary factors – including kin-selection mechanisms – may have influenced the formation of mechanisms which allow for high levels of parental behavior in the absence of the brain's exposure to the hormones of pregnancy and lactation. It should be noted, however, that in marmosets where alloparental behavior is shown by nulliparous females, when such females are treated with a hormone regimen that simulates the endocrine events associated with pregnancy termination their maternal responsiveness is further enhanced.

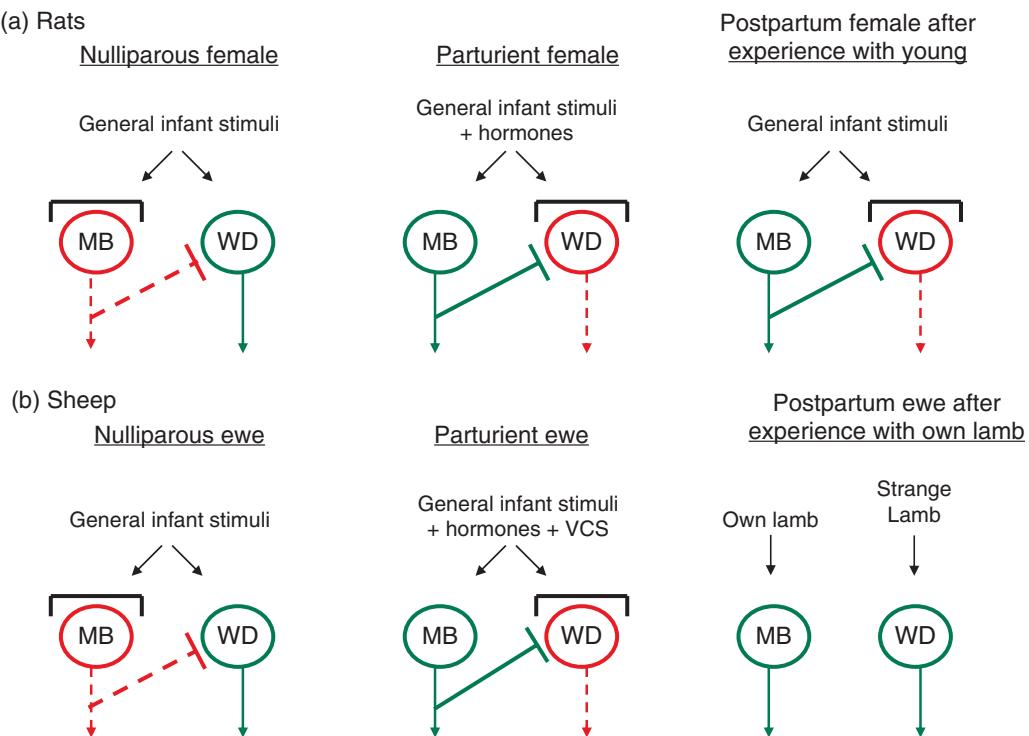


Figure 1 Neural models of the regulation of maternal behavior in rats (a) and sheep (b). Two neural systems are depicted: a maternal behavior (MB) system, which controls the occurrence of appropriate maternal responses, and a withdrawal (WD), rejection, or avoidance system, which prevents interaction with infants. A bracket over the neural system origination point indicates that the system cannot be stimulated by afferents carrying inputs from infant stimuli. When a system is shown in red dashed lines, it is not operative. When it is shown in green solid lines, it is operative. Lines ending in an arrow signify excitation and those ending in a bar represent inhibition. In rats, for nulliparous females, lamb stimuli activate the MB system, which is shown as inhibiting the WD system. In parturient and postpartum-experienced rats, general infant stimuli activate the MB system, which is shown as inhibiting the WD system. In nulliparous sheep, lamb stimuli activate the WD system. In parturient sheep that are exposed to young for the first time, a general infant stimulus activates the MB system and the WD system is not operative. After the ewe interacts with her lamb for several hours post partum, an olfactory learning process occurs so that the female's own (specific) lamb activates the MB system, while alien or strange (unfamiliar) lambs activate the WD system. VCS = vaginocervical stimulation.

Feral mice act like rats and sheep: nulliparous females avoid or attack, while parturient females care for the young. In contrast, as a result of selective breeding or inbreeding, most nulliparous females of laboratory strains of mice show maternal behavior toward young pups. These data show that experimental selection can modify the brain such that infant stimuli can activate maternal behavior in the absence of the hormones of pregnancy and lactation. Presumably, natural and kin-selection mechanisms can act similarly. Interestingly, in nulliparous female laboratory mice that would normally show maternal behavior toward the young, a null mutation of the prolactin receptor gene prevents this maternal behavior, while a null mutation of the prolactin gene does not. These results suggest a possible mechanism which might underlie alloparental behavior in nulliparous females of certain species: a brain factor other than prolactin may stimulate maternal responsiveness by activating the prolactin receptor in the brain.

In summary, although the hormonal events associated with pregnancy termination are important factors for stimulating the onset of maternal behavior in most

mammals, other factors can be involved which would allow for the occurrence of alloparental behavior in nulliparous females and also for the occurrence of paternal behavior. Some of these facts are summarized in **Figure 2**. Allomaternal behavior mechanisms in nulliparous females may allow for the occurrence of adoption in the human and other primates. In fact, even in the rat, something like adoption can occur. Although virgin nulliparous females are not initially maternally responsive, if such virgins are continuously housed with healthy foster pups, then after about 7 days of continuous exposure the virgins will show the full pattern of maternal behavior toward the foster pups even though they are not capable of lactating.

Neural Circuits Regulating Maternal Behavior

In the typical female mammal, hormones are proposed to act on the brain to depress avoidance/rejection circuits and to activate maternal behavior circuits regulating

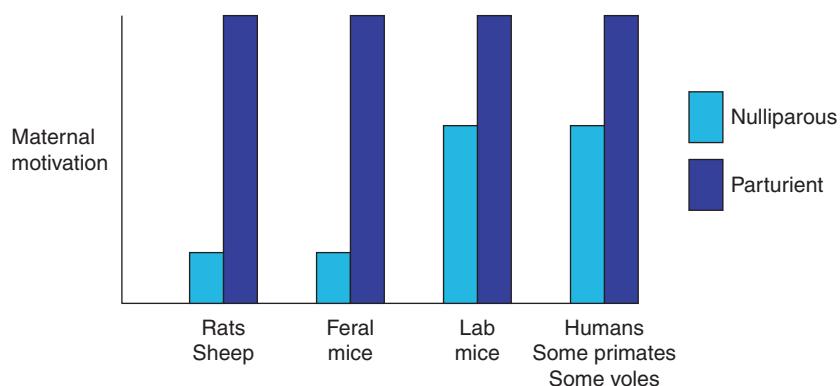


Figure 2 Comparative analysis of the level of maternal motivation or responsiveness in nulliparous and parturient females of a variety of species. For most female mammals, nulliparous females do not show maternal behavior upon an initial exposure to young, while parturient females – as a result of the neural effects of the hormones associated with pregnancy termination – do. In some species, however, nulliparous females show alloparental behavior and will care for young. However, even in these species the evidence indicates that pregnancy hormones boost maternal motivation to a level above that of the nulliparous female. For example, although nulliparous marmoset monkeys show alloparental behavior toward their young siblings, they will not perform an operant response at a high rate in order to gain access to infant stimuli. However, if such females are treated with a hormone regimen which simulates the endocrine pattern that occurs toward the end of pregnancy, they will perform the operant response at a very high rate. See Numan and Insel (2003) for the data that were used to develop this graph.

approach and acceptance behaviors (Figure 1). Research, primarily on rats, has uncovered the details of both the inhibitory and stimulatory circuits.

The Medial Preoptic Area (MPOA)

The MPOA plays a dominant role in the control of the appetitive aspects of maternal behavior in rats and other species. Electrical or excitotoxic amino acid lesions of the MPOA interfere with maternal behavior in rats, and retrieval behavior is disrupted to a much greater extent than is nursing. Such females, however, are capable of picking up and carrying other items, like candy, in their mouths. Postpartum females with MPOA lesions will not perform an operant response to gain access to pups, although they will do so to obtain food. Finally, Fos is expressed in MPOA during maternal behavior and also when postpartum females are searching in an environment previously associated with the presence of pups. All of these results suggest that the MPOA is necessary for proactive appetitive maternal responses. MPOA lesions also disrupt maternal behavior in mice, hamsters, and sheep.

The MPOA neurons contain receptors for estradiol, progesterone, and lactogens, and direct injection of either estradiol or lactogens into the MPOA stimulates maternal behavior in female rats who have been treated systemically with the other critical hormones. When maternal behavior is stimulated by hormone application to the MPOA, the full complex of retrieving, nest building, nursing behavior, and pup grooming is activated. One possibility is that hormone activation of the MPOA promotes output circuits which decrease avoidance behavior

and increase approach behavior and retrieval. Once the pups are in the nest with the female, proximal suckling and nuzzling stimuli from pups are likely to reflexively elicit nursing postures.

The Avoidance–Rejection Circuit

The typical response of the nulliparous female to young has given rise to the view that when a female is not primed with pregnancy hormones, infant stimuli gain access to a defensive circuit which promotes avoidance and rejection behaviors. Since the amygdala is involved in fear-related responsiveness, it is not surprising that it is involved in this defensive circuitry. Research has indicated that, in the absence of appropriate hormone priming, pup stimuli (including olfactory stimuli) activate a circuit from the medial amygdala (MeA) to the anterior hypothalamus (AHN) to the periaqueductal gray (PAG) in the midbrain which promotes defensive responses. The best evidence for this proposal is that lesions to points along this circuit stimulate maternal behavior in females who have been suboptimally primed with hormones (in terms of dosage and the actual hormones administered).

MPOA Output Circuits: Inhibition of Avoidance and Stimulation of Approach

The MPOA neurons which express Fos during maternal behavior project to the AHN and PAG. Since a proportion of Fos-expressing MPOA neurons also contain the inhibitory neurotransmitter γ -aminobutyric acid (GABA), the hormonally primed MPOA may inhibit the

avoidance circuit, which fosters the onset of maternal behavior at parturition.

For maternal behavior to occur, not only must avoidance be depressed, but proactive appetitive maternal responses must be stimulated. The MPOA neurons which express Fos during maternal behavior also project to the ventral tegmental area (VTA) in the midbrain. The VTA contains the dopamine (DA) neurons which give rise to the ascending mesolimbic DAergic system, and one site of termination of these DA neurons is the nucleus accumbens (NACC) in the telencephalon. Research has indicated that VTA-DA projections to the NACC are involved in the regulation of a variety of appetitive proactive voluntary responses. The proposed link between the MPOA and the VTA may form a connection between a specific appetitive system (MPOA: maternal motivation) and a general appetitive motivational system (NACC), which when exposed to DA promotes appropriate responsiveness to biologically significant stimuli.

Dopamine is released into the NACC during maternal behavior in rats and disruption of DA action in the NACC interferes with retrieval behavior without depressing nursing behavior. There are two types of DA receptors, D₁

and D₂, and research has emphasized the importance of D₁ receptors: microinjection of D₁ antagonists, but not D₂ antagonists, into the NACC disrupts appetitive maternal responses in postpartum rats. Significantly, microinjection of D₁, but not D₂, agonists into the NACC activates the full onset of maternal behavior in female rats that have been suboptimally primed with hormones. Finally, neural disconnections between the MPOA and the mesolimbic DAergic system disrupt maternal behavior.

Additional research has led to the following neural model for the regulation of proactive voluntary maternal responses: when the MPOA is hormonally primed, it can be activated by infant-related stimuli and the MPOA efferents in turn activate VTA-DA neurons. DA release into the NACC acts on D₁ receptors to depress the responsiveness of the NACC and this acts to release the ventral pallidum (VP) from inhibition. The disinhibited VP is then capable of being stimulated by afferent pup stimuli derived from limbic sources, leading to appetitive maternal behavior.

Figure 3 shows a diagram of the brain circuits which may represent the core neural circuitry for parental behavior in mammals.

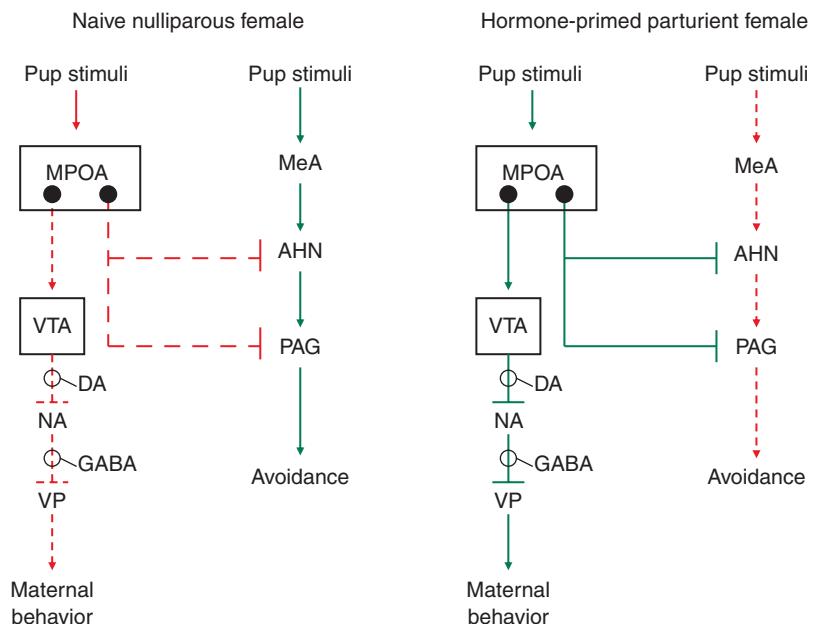


Figure 3 Neural model of a dual systems-control mechanism regulating maternal behavior in the rat. Systems represented in dashed red lines are not operative, while those shown in solid green lines are functional. Axons ending in an arrow indicate excitation and those ending in a bar represent inhibition. This figure is an elaboration of **Figure 1**, based on the findings from research on rats (see Numan, 2006). In the nulliparous female, pup stimuli activate avoidance, withdrawal, or defensive responses by activating a neural pathway from the medial amygdala (MeA) to the anterior hypothalamic nucleus (AHN) to the periaqueductal gray (PAG). As a result of exposure of the brain to the hormones of pregnancy and pregnancy termination, the maternal behavior system becomes operative. Hormones act on the medial preoptic area (MPOA), which becomes responsive to pup stimuli. The outputs of the MPOA inhibit the avoidance system and also activate proactive maternal responses via stimulation of the ventral tegmental area (VTA), which gives rise to the mesolimbic dopamine (DA) system. Subsequent DA release into the nucleus accumbens (NA) suppresses inhibitory GABAergic projections to the ventral pallidum (VP). An active VP is necessary for appropriate maternal responsiveness.

Oxytocin (OT) and Maternal Behavior

Other than DA, most of the research on the neurochemical control of maternal behavior has focused on OT, which exerts powerful modulatory influences at critical nodes within some of the neural circuits we have described. The parvocellular cells of the paraventricular hypothalamic nucleus (PVN) are one site of OT production, and some of these neurons terminate within the brain which allows OT to act locally as a neurotransmitter or neuromodulator.

The hormonal events associated with late pregnancy and the vaginal and cervical stimulation at birth influence both the synthesis and release of OT into the brain. The current view is that OT release into diverse neural sites is important for the initiation of maternal responsiveness at birth, but once maternal behavior has become established, OT no longer plays an essential role in the maintenance of the behavior. For example, PVN lesions performed during pregnancy disrupt the onset of maternal behavior at parturition in rats, but such lesions are not disruptive if produced after maternal behavior has become established. Similar results are obtained with more selective methods: intracerebral injections of OT receptor (OTR) antagonists during parturition disrupt the onset of maternal behavior, but similar injections postpartum are ineffective. Although OT is not essential for the continuance of maternal behavior, it may exert subtle modulatory influences on the degree of maternal grooming of infants and on the particular nursing postures the mother shows.

The hormonal and other events of late pregnancy, particularly rising estradiol levels, also increase the synthesis and expression of OTRs – which would allow critical brain nuclei to respond to OT. In rats, OTR expression increases in the MPOA and VTA at parturition and evidence shows that microinjection of an OTR antagonist directly into either the MPOA or VTA disrupts the onset

of maternal behavior at parturition. There is also some work on prairie voles which suggests a role for OT action on the NA in maternal behavior control. Therefore, at the time of parturition, OT may act at critical nodes along the circuitry regulating proactive maternal responses and such action may allow these circuits to work at optimal efficiency (**Figure 4**).

In addition to OT's influence on appetitive maternal responding, OT – in conjunction with MPOA effects – may downregulate activity in fear and anxiety-related neural circuitry, and this influence may continue into the postpartum period. We return to this issue in our discussion on postpartum maternal aggression and anxiety reduction.

Paternal Behavior

Although paternal behavior is rare in mammals, it does occur. Its neural mechanisms may be similar to maternal behavior, since it is unlikely that distinct paternal circuits evolved in each mammalian species that naturally displays the behavior. It is more likely that dual parental-control mechanisms exist in all mammals of both sexes: circuitry through which infant stimuli can elicit (1) defensive/withdrawal responses and (2) appetitive parental responses. The developmental effects of perinatal testosterone secretion may act to enhance the defensive circuitry in the typical male mammal when compared to his female counterpart.

Since male mammals are not exposed to the endocrine events of pregnancy and pregnancy termination, when paternal behavior naturally occurs, some other factors must be involved in downregulating the putative avoidance circuits and upregulating the appetitive parental circuits. Evolutionary forces may have operated on

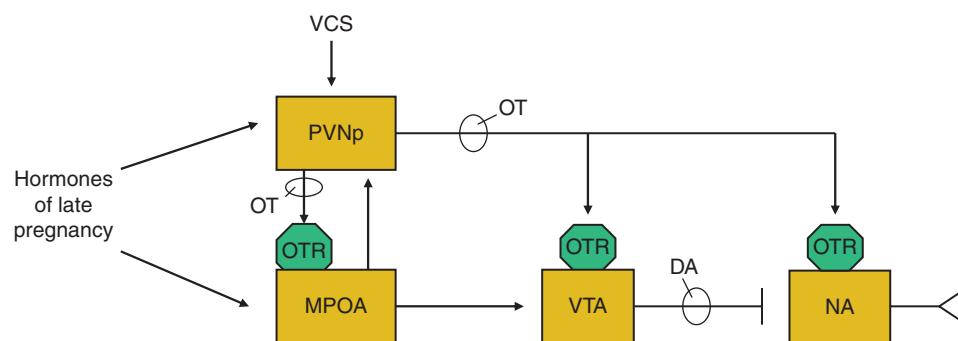


Figure 4 The coordinating role of oxytocinergic neural pathways in the regulation of the onset of maternal behavior. The parvocellular neurons of the paraventricular hypothalamic nucleus (PVNp) release oxytocin (OT) into various neural sites. The hormones of late pregnancy and the vaginocervical stimulation (VCS) which occurs during parturition promote the synthesis and release of OT. Pregnancy hormones also stimulate the synthesis of OT receptors (OTR) in the medial preoptic area (MPOA) and ventral tegmental area (VTA). At parturition, OT action on the MPOA, VTA, and, possibly, nucleus accumbens (NA) promotes the efficient operation of the MPOA–VTA–NA circuit which is essential for proactive maternal responses. DA = dopamine.

males of naturally paternal species to allow specialized stimuli and experiences to influence the critical circuitry.

Some evidence to support these views comes from rats, which are promiscuous breeders and not naturally paternal. Laboratory male rats initially avoid or attack pups, but if they are experimentally housed with healthy foster pups on a daily basis they will eventually show parental behavior after about 7–10 days as would virgin females. Furthermore, if one treats male rats with hormones which simulate the pattern that occurs in females at the end of pregnancy, their parental behavior is facilitated. Finally, MPOA lesions disrupt, and estradiol application to MPOA facilitates, parental responsiveness in male rats. Therefore, the neural mechanism underlying parental behavior exists in male rats, but under natural conditions the male is not exposed to factors which decrease avoidance of infants and stimulate parental behavior.

With respect to cases of naturally occurring parental behavior, it is known that Fos is expressed in the MPOA of paternal prairie voles, suggesting that this critical region is active. More importantly, in the naturally paternal California mouse, MPOA lesions disrupt paternal behavior.

Very little experimental research has been done on the factors which might stimulate paternal behavior in biparental mammals: hormonal factors, the experience of being biparentally reared, previous experience with young, and copulation and continued cohabitation with a mate may all play a role, and the specific factors involved may differ for different species. A popular idea with respect to endocrine influences, derived from research on avian species where paternal behavior is common, is that declining levels of testosterone and rising levels of prolactin stimulate paternal behavior. For male mammals, however, the supportive evidence is only correlational and not entirely convincing. For example, the increase in prolactin secretion which occurs in paternal marmosets and tamarins may be the result of infant contact rather than the cause of paternal behavior. In fact, in marmosets, experimental analysis has failed to support a causal link between prolactin and paternal behavior: drug treatments which depress prolactin release do not disrupt paternal behavior.

Some of the most promising ideas with respect to the mechanisms which may activate paternal circuitry in some species come from research on the domestic house mouse and on the biparental California mouse. Laboratory male mice (derived from the domestic house mouse) are not naturally paternal. However, either a null mutation of the progesterone receptor gene or treatment with a progesterone receptor antagonist depresses infanticide or avoidance and stimulates parental behavior. These results suggest that progesterone depresses paternal responding in these males, a mechanism which may also be involved in the California mouse. Unmated males of this species are typically infanticidal. However, after a

male mates with a female he becomes paternal once the female gives birth, and both copulatory stimuli and pheromones from the parturient female may be involved in this effect. There is also some evidence for the involvement of vasopressin (a neuropeptide with a chemical structure similar to oxytocin) in some of these effects. Furthermore, plasma progesterone levels are higher in nonpaternal males than in paternal males and the likely source of secretion is the adrenal cortex, since progesterone levels are not affected by castration. In addition, testosterone plays a positive role in the paternal behavior of this species and there is also an increase in aromatase activity (the enzyme which converts testosterone to estradiol) in the MPOA of paternal males.

For the California mouse, a preliminary and partial hypothetical model for paternal behavior can be offered: In unmated males, the aversive circuitry is dominant, the male attacks or avoids pups and this may be associated with adrenal release of progesterone. As a result of mating and subsequent cohabitation with a female – which includes pheromonal stimulation from the postpartum female – aromatase increases in the MPOA where testosterone is converted to estradiol, and estradiol stimulation of MPOA may then allow infant stimuli to activate appetitive paternal responses. In addition, the aversion circuitry is downregulated and progesterone levels fall.

Maternal Aggression and Fear Reduction in Postpartum Females

Postpartum females show a general decrease in fearfulness to a wide variety of novel or threatening stimuli and an increase in aggressive behavior aimed at conspecifics or potential predators that approach the infant (maternal aggression). The evidence which is currently available suggests that the hormonal events associated with late pregnancy and parturition modify the brain so that these two motivational changes occur and then subsequent proximal contact with infants maintains a high level of aggression and fear reduction.

With respect to postpartum fear reduction: it is general in nature, which distinguishes it from the specific reduction in aversion to novel infant stimuli that was proposed as necessary for the onset of infant-directed behaviors in primiparous females. Although MPOA projections to the aversion circuitry may be involved in the depression of avoidance responses to infant-related stimuli, other neural mechanisms may co-act with this MPOA effect to reduce fear-related behaviors to other stimuli. Fear reduction may be necessary for the mother to take reasonable risks in caring for her offspring, which would include displays of maternal aggression. Many studies have found an inverse relationship between levels of fearfulness and levels of maternal aggression in postpartum mothers.

Maternal aggression obviously has a neural circuitry which is independent of those circuits which regulate fearfulness and anxiety, but a reduction in fearfulness may be necessary for the maternal aggression circuitry to operate properly.

Neural projections from the central nucleus of the amygdala (CeA) to the PAG are involved in potentiating fear-related responses to a wide variety of stimuli. Corticotropin-releasing factor (CRF) action at the level of the CeA has anxiogenic effects, and intracerebroventricular injection of CRF depresses maternal aggression in postpartum mice. OT neural pathways – presumably derived from the PVN – not only facilitate the onset of infant-directed maternal behaviors, but also have general anxiolytic effects. OT is released into the CeA during maternal aggression and direct action of OT in the CeA increases maternal aggression and decreases fearfulness. Other studies have shown that OT also acts on PAG to decrease fearfulness in postpartum females. Furthermore, PAG lesions decrease fear-related behaviors and increase maternal aggression in postpartum rats. One can propose that in the postpartum female there is a downregulation of CRF neural systems and an upregulation of OT neural systems, which results in a level of fear-reduction which has a permissive effect on the occurrence of appropriate levels of maternal aggression.

Some studies have found that very low levels of fearfulness in postpartum rats are actually associated with low levels of maternal aggression. Such females may simply be passive and not concerned about potential threats to their offspring. Therefore, the relationship between fearfulness and maternal aggression is probably best described by an inverted-U-shaped curve: an optimal level of fearfulness or protectiveness may be necessary for the female to be vigilant and to display appropriate maternal aggression.

Although postpartum human females also show decreased anxiety, the occurrence of postpartum depression and anxiety is a significant problem. It can be suggested that a dysregulation of some of the systems we have just described – which may involve gene–environment interactions – can lead to high postpartum anxiety, depression and its associated anhedonia, and faulty maternal responses.

Experiential Influences on Maternal Behavior: A Window into the Mechanisms Which May Underlie Dysfunctions in Human Parental Behavior

How does animal research help us understand the neurobiological underpinnings of child abuse and neglect that are displayed by some human parents? Human children who have been abused or neglected by their parents tend to become abusive/neglectful parents themselves. Although

this could derive from genetic inheritance, it is possible that the early adverse effects of being abused and/or neglected affected the child's brain development so that, as adults, they show faulty parental behavior. Research on rhesus monkeys supports this view: motherless mother monkeys (monkeys raised without their mothers) grow up to show aberrant or absent maternal behavior to their own offspring. Such females also show high levels of anxiety. Furthermore, some rhesus monkey mothers are naturally abusive. Research has shown that infants born to nonabusive mothers who are cross-fostered to abusive mothers at birth tend to become abusive mothers when they have their own offspring in adulthood. Complete maternal deprivation in rats also results in the development of poor maternal behavior in the affected offspring.

Although gene–environment interactions are likely to be involved, these results show that early, adverse rearing conditions provide an experiential input that can lead to the development of poor maternal behavior. Possible mechanisms might include: (1) disrupted functioning of the MPOA output circuits which regulate maternal behavior; (2) increased activity in neural circuits regulating fear, anxiety, and stress responsivity; and (3) decreased activity within the mesolimbic DA system. We explore the possibility that mechanism (1) may play a contributory role in this article.

Rat mothers show variations in their maternal behavior: Some mothers are highly attentive to their pups and show high levels of licking/grooming behavior (HLG mothers), while others are less attentive, showing low levels of licking/grooming (LLG mothers). These are natural variations and we are not describing dysfunctional maternal behavior; all of these mothers rear their pups to weaning. Interestingly, when tested in a strange environment, HLG mothers are less fearful (they show more exploratory behavior) than LLG moms. HLG mothers have more estrogen receptors (ERs) and OTRs in the MPOA than do LLG moms, and during the expression of maternal behavior, more DA is released into their NA. What these behavioral and neural data suggest is that differences in the attentiveness of mothers may be due to differences in the organization of the MPOA output circuits: perhaps, because of greater numbers of ERs and OTRs in the MPOA, MPOA outputs to both the mesolimbic DA system and to the avoidance/rejection circuit are potentiated, and this leads to a more attentive and less fearful maternal style.

Cross-fostering studies show that infants develop the maternal style of the parent by which they were raised by: an infant raised by a HLG biological or foster mother grows up to become a HLG mother, expresses low fearfulness, and has high numbers of ERs in the MPOA. Just the opposite occurs in females raised by a LLG mother: as adults, such female offspring show higher fearfulness, low licking and grooming, and low numbers of ERs in the MPOA ([Figure 5](#)).

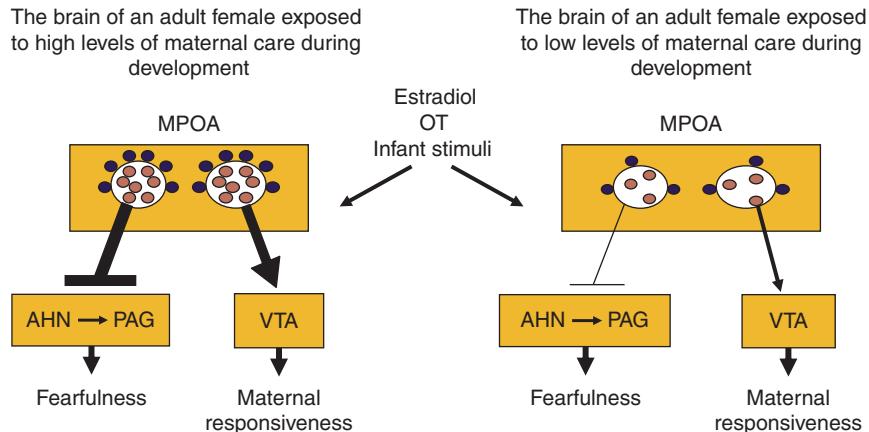


Figure 5 Experiential influences on the medial preoptic area (MPOA) development and maternal responsiveness. This figure is partly based on research reviewed by Champagne (2008) and by Kaffman and Meaney (2007). Female rat offspring that are exposed to high levels of maternal care – when compared to young that receive lower levels of maternal care – grow up to show higher levels of maternal care to their own offspring and such females are also less fearful or anxious than their low-maternal-care counterparts. Importantly, female young that receive high levels of care from their mothers develop an MPOA which contains more estrogen receptors (ERs: intracellular small circles within the MPOA cell bodies) and oxytocin receptors (OTRs: small circles on the membrane of the MPOA neurons). One can speculate that the enhanced expression of ERs and OTRs in the MPOA allows the MPOA neural circuits outlined in **Figure 3** to work more effectively. Therefore, females that have been well cared for by their mothers should grow up to show higher levels of maternal behavior to their own offspring while also being less fearful. Other abbreviations: AHN = anterior hypothalamic nucleus; OT = oxytocin; PAG = periaqueductal gray; VTA = ventral tegmental area. Axons ending in an arrow indicate excitation and those ending in a bar represent inhibition. The thickness of axons signifies the strength of the synaptic connection.

The implication of these data is that by understanding the underlying processes which influence natural variations in maternal responsiveness, one can gain insight into some of the mechanisms through which extreme adverse early-life events – such as parental abuse, neglect, or deprivation – might affect the brain of the developing child so that s/he develops truly dysfunctional parental behavior in adulthood. One possibility is that the MPOA output circuits are so disorganized by environmental insults interacting with certain genotypes that parental responsiveness to infants becomes abnormal or pathological.

See also: Animal Tests for Anxiety; Control of Food Intake; Hormonal Contributions to Arousal and Motivation; Incentive Motivation and Incentive Salience; Mammalian Parental Behavior and Neurohormonal Determinants; Maternal Deprivation; Motivation; Neural and Pharmacological Substrates of Aggression; Neural Bases of Defensive Aggression; Neural Substrates of Conditioned Fear and Anxiety; Neural Substrates of Unconditioned Fear, Defense, and Anxiety; Neurobiology of Offensive Aggression; Offensive and Defensive Aggression; Play Behavior; Stress and Reward; Thermoregulation.

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Play Behavior

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Glossary

Antimuscarinics – Drugs that act as antagonists at the muscarinic class of synapses using the neurotransmitter acetylcholine. High doses eliminate playfulness without producing abnormal locomotion.

Attention-deficit hyperactivity disorder (ADHD) – A childhood disorder that has been hypothesized to result from insufficient play.

Brain-derived neurotrophic factor (BDNF) – The protein product of a gene that is upregulated during play.

cFos – The protein product of an immediate early gene that is rapidly upregulated when a neuron fires, making it a marker for neuronal activity.

Insulin-like growth factor-1 (IGF-1) – The protein product of a gene that is upregulated during play.

Mesolimbic dopaminergic pathway – One of the brain pathways in which neuronal cell bodies in the midbrain that produce and release the neuromodulator dopamine project to the forebrain. Lesions and electrical stimulation of this pathway affect play along with many other behaviors.

MK-801 – A drug that acts as an antagonist at *N*-methyl-D-aspartate (NMDA) glutamate receptors. It eliminates playfulness without producing abnormal locomotion.

Opioids – Endogenously produced neuromodulators with opiate-like effects that are involved in play through their role in social dominance.

Parafascicular complex – A region in the thalamus where nonspecific somatosensory inputs converge. Lesions of this complex reduce play by rats, as do lesions of the dorsomedial thalamus.

comparison to mammals, there is little well-established scientific information about the play of other classes of animals, aside from modest systematic behavioral work on birds. Whether other chordates and invertebrates show any playful behaviors remains unresolved. Accordingly, neuroscientific work has been conducted almost exclusively on laboratory rats. The most common variant of play in rats, distinct rough-and-tumble social engagement, is clearly an evolved function of the brain since the executive structures for such activities are situated subneocortically. There are currently sufficient high-quality data to be confident that there are, in fact, evolutionarily dedicated subcortical brain networks for the generation of social playfulness.

Historically, the scientific analysis of play has been hampered by biases ranging from the mistaken views that play simply reflects a form of exploratory activity, that it is a superfluous (excess-energy) behavior of youth, and that it may serve no adaptive psycho-behavioral functions, except perhaps for increasing physical fitness. In fact, we now know that mammalian play is functional – a basic brain–mind tool for living and learning about the social and physical environments in which young mammals find themselves. However, the neuroscientific analysis of play remains in its infancy, partly because it is not widely recognized as an easily studied fundamental emotional function of the mammalian brain that has profound societal as well as psychiatric implications.

The complexities and idiosyncrasies of play, not to mention definitional problems, discouraged investigators from initiating sustained research programs on the neural basis of play until recently. Because of conceptual and methodological advances, the experimental analysis of rough-and-tumble social play blossomed in the early 1980s. Several investigators found that it was remarkably easy, with excellent interobserver reliabilities, to obtain and quantify social play in laboratory rats, permitting rigorous neuroscientific research. This could be emulated in some other species (e.g., degus and gerbils), but has not been so far. Perhaps, the most attractive alternative laboratory species, mice, may not work well. Most strains do not exhibit clear and distinct play behaviors comparable to rats, perhaps because play has been bred out of them. (That is, it is possible that the ‘popcorn’ phase of early development reflects a play urge that was inadvertently minimized through selection practices since it was disruptive to

Play as a Basic Process of the Mammalian Brain

Playfulness is one of the most prominent behaviors of young animals across most mammalian species. However, play has remained enigmatic at a scientific level, partly because it has been one of the last primary-process emotional processes of the brain to receive experimental attention. Despite this, social play is a prominent, easily recognized, and operationalized behavior sequence in the young of most mammalian species. In

animal husbandry issues.) Several wild strains of mice, such as grasshopper mice, have been found to exhibit distinct rat-like play sequences.

We are still left with the dilemma of how to understand and discuss brain processes for complex affective behavior sequences systematically. Play is surely constituted of many highly interactive brain processes. This article focuses on the possibility that there are distinct executive, command networks for the most common social variant of play in rats – rough-and-tumble social engagement. However, there are other types of play.

Types of Play and Operational Definitions

There appear to exist several distinct kinds of play. They include most prominently, in order of ascending attention in behavioral neuroscience: object-play, locomotor-play, and, most importantly, social-play, also known as ‘rough-and-tumble play’ or ‘play-fighting.’ It is not known to what extent these forms of play are controlled by shared brain processes and whether they have distinctive functions in promoting developmental adaptations for future life challenges. To delimit the topic to the form that has the most abundant neuroscience evidence (modest though it is), the focus here is on social play, mainly as studied in laboratory rat models. However, even with this form of play, a variety of behaviors are exhibited across species – with herbivores commonly exhibiting more running, prancing, and chasing, while carnivores and omnivores exhibit play that has a much clearer pouncing attack,

predatory and wrestling-fighting character. They appear to be sham battles – tests of strength just for the fun of it.

Although the above complexities may preclude any simple universal definition of social play, rough-and-tumble play is still so distinctive that few people would mistake it for serious aggression, even though there are typically clear winners (so-called top-dogs) and losers (bottom-dogs) in play encounters. Despite this, all members of play pairs find this activity highly rewarding. Both winners and losers run down mazes to obtain play equally rapidly. Thus, a critical affective feature of play is that the social activity is highly rewarding.

Presented here is a behavioral description of play in rats that may serve as a provisional descriptive definition: when two rat pups are placed together in a nonthreatening environment, they rapidly begin to exhibit vigorous fighting. Animals chase and pounce on each other, sometimes unilaterally, sometimes mutually with rapid role reversals. They repeatedly poke and nip each other, often at the nape of the neck but also on the ventral surface when one animal is pinned.

Operational Definitions and Measurement of Play

From the above observations one can derive three main measures of playfulness that are easily quantified: chasing, dorsal contacts that seem designed to solicit play from another animal, and pinning, where one animal is on top and the other, on its back, below in a classic wrestling pose (see Figure 1). Across days, one animal of a stable play

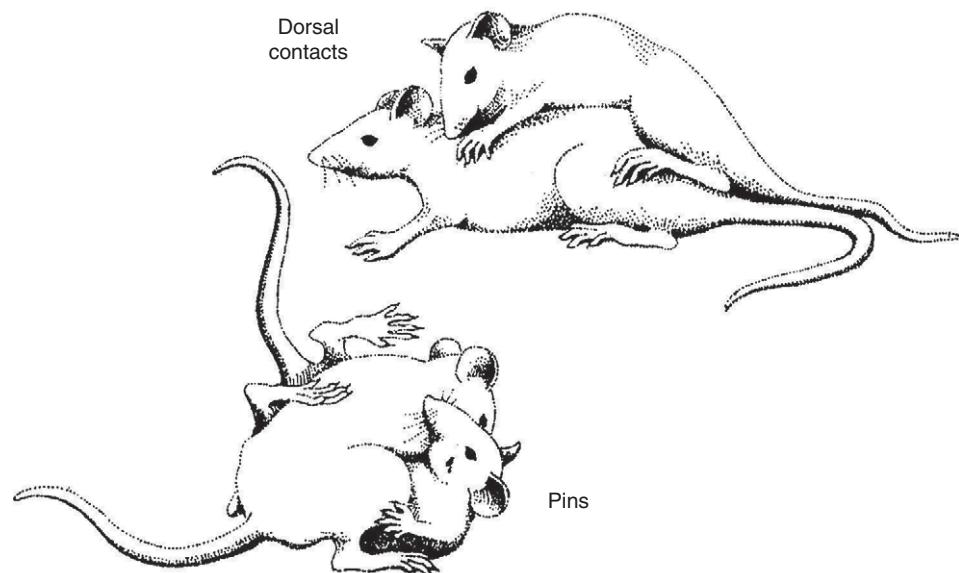


Figure 1 Two major postures that can be used as proxies to index overall amounts of rough-and-tumble play, with dorsal contacts being more related to the appetitive components of play and pins (especially the longer-duration pins) the consummatory components. Reprinted from Panksepp J (1998) *Affective Neuroscience: The Foundations of Human and Animal Emotions*. New York: Oxford University Press.

pair tends to become dominant, being the ‘top-dog’ about 70% of the time, while the other animal gets to be on top in about 30% of the pins. The durations of these indices of playfulness are quite variable, so the harvesting of behavior-duration measures is useful. Of course, as with all complex sequences of behaviors, with frame-by-frame video analysis, one can increase precision and the number of submeasures, such as quantifications of body rotations and the timing of subtle interactions, but the decision to use simple versus complex measures (e.g., pins and dorsal contacts along with overall platform activity measures vs. the many that can be obtained from frame-by-frame analysis) depends on the question one is asking. If one simply needs indices of overall amounts of play, the simple indices that can be obtained as play occurs suffice, and if the play is conducted on a sensitive activity platform one can get a measure of overall playful activity that typically correlates highly with the distinct bodily measures.

The use of such proxy measures for a complex holistic process is typical in most scientific endeavors. For instance, a biochemist typically develops a detection assay for a specific molecule, without attempting a full description of the molecule of interest. If, however, one needs ethologically complete descriptions of playfulness, then a fine-grained, slowed-down, video-analysis becomes essential.

Major Model Systems and Behavioral Descriptions

When play is studied in semi-natural, group-housing conditions, one must use a focal observation procedure where one periodically samples the behavior of designated animals. Using such procedures, it is hard to control many variables, including influences of unobserved social learning. In contrast, the paired-encounter procedure, where play-deprived animals are given short daily play periods, yields more consistent, sustained, and vigorous play activities. Play increases systematically with hours of social deprivation, closely resembling other regulated processes such as feeding and drinking.

Developmentally, play exhibits an inverted-U type of trajectory, with play indices increasing into the middle juvenile ages (days 30–40 in rats), then diminishing gradually as animals approach puberty. Playfulness continues at low levels well into adulthood, with females exhibiting generally higher levels of adult play than males. It is generally believed that juvenile males play more than juvenile females, and that this reflects a differential genetic disposition. There are reasons to doubt that, since gender differences are observed mostly in focal observation studies, where most of the social histories of animals have not been observed. Gender differences are rarely observed using the paired-encounter procedure,

where every play episode has been documented. If one allows mixed-sex play, however, males tend to prevail and remain more playful. However, this reflects the fact that the smaller and lighter females cannot compete as effectively with the bigger males. It would appear that the gender differences in play are largely socially learned. Likewise, in one of the few formal scientific studies of social physical play in like-sexed pairs of human boys and girls, no robust gender effects in the ethologically measured incidence of play behaviors were observed.

The sensory system that is most important for sustaining play in laboratory rats is the somatosensory system. Anesthetization of the anterior dorsal region of the back is the most sensitive region for obtaining play reductions as monitored by pins but not dorsal contact, suggesting that the motivation for play remains intact. Similar effects can be obtained by lesioning the spinothalamic tract carrying somatosensory information, and, at the thalamic level, the clearest and most specific lesion effects are obtained where somatosensory information diverges into nonspecific, presumably affective, projection areas (e.g., parafascicular nucleus, see below). Other equally complex behaviors such as food foraging are not disrupted by such damage.

Anything that provokes anxiety (e.g., cat smell) or induces other negative affective states (e.g., hunger) dramatically reduces playfulness. The ability of a single meal to return hunger-reduced play to normal could be used as a sensitive measure of normal satiety. Many drugs reduce feeding only because they make animals feel sick or bad in other ways. Play is one of the few behaviors that could be used as a measure of normal satiety through behavioral activation as opposed to sedation.

Play Vocalizations

During short play periods, rats exhibit abundant short (~50 ms) 50-kHz ultrasonic vocalizations (USVs), which come in two forms, the flat calls and the more complex frequency-modulated (FM) chirps which start at around 50 kHz but end with a sinusoidal FM tail. The latter are most abundant in socially rewarding situations, while the flat variants often prevail during serious fighting. Animals like to listen to the FM chirps, but they seem to be neutral about the flat calls. Rats avoid the anxiety-indicative long 22-kHz USVs.

During a half-hour play session, play diminishes systematically partly because of play satiety and partly because of an increasing number of affectively negative interactions. The changing affective balance during prolonged play sessions (15–30 min) is well indexed by gradually diminishing 50-kHz USVs, which index positive affect, and gradually increasing rates of 22-kHz USVs, which index negative affect, especially mild

anxiety. It is noteworthy that outright fear, as provoked by imminent footshock, shuts down both types of USVs.

The 50-kHz FM chirps indicative of positive affect can be most powerfully evoked by tickling (human hand-play), and the rewarding aspects of play are directly related to the amounts of this laughter-type of response. Animals become readily bonded to the human who has tickled them. This vocal marker of playfulness – 50-kHz USVs – has been genetically amplified through selective breeding, yielding lines with phenotypic differences in playfulness and a variety of related social activities.

Functions of Play

The abundance of functional theories, all with a dearth of evidence, generally agree that social play serves to practice social, emotional, and cognitive skills needed in the future. Play seems to be an experience-expectant process. Some theories specifically emphasize that play prepares animals for dealing with a variety of unpredictable events, but the evidence base is slim.

The empirical results tend to show that animals that have been play deprived are generally more aggressive and less capable of navigating complex social situations and complex learning tasks. Play-deprived animals are not deficient in simple classical and operant-conditioning tasks, nor do they exhibit deficiencies in straightforward instinctual behaviors such as copulation. For instance, adult males deprived of juvenile play approach, mount, and copulate with hormone-primed females just as rapidly as play-experienced animals. However, play-inexperienced rats are not as effective in competing with others for sexual resources as play-experienced animals.

The likelihood that play is a regulated process of the brain is indicated by highly systematic elevations of play with increasing hours of play deprivation and systematic diminishing of play with satiation. Likewise, play seems to be regulated across the life span. If animals are not allowed adequate play early in development, they play more at subsequent ages if given the opportunity.

The Neuroscience of Play

Neuroanatomy of Play

Only recently has it been recognized that there exist primary-process, genetically determined, subcortically situated play circuitries in the brain (i.e., probably genetically determined dispositions for social physical play). Playfulness seems to be an intrinsic aspect of mammalian brain organization since rats that have never been allowed to play when young (housed in isolation from 15 days of age when their eyes open until the beginning of their peak play period about 2 weeks later) play essentially normally when given their very first opportunity to play. Further

evidence for the intrinsic nature of social play is the fact that play fighting in laboratory rats survives radical neocortication of 3-day-old animals, long before play circuitry has matured in the brain. These animals exhibit as much playfulness as neurologically intact animals, even though certain measures are diminished (e.g., pins) perhaps because of modest motor abnormalities. The decorticates compete effectively with the normals, as measured by play dominance. In addition, animals that have had complete cerebellar lesions at 3 days of age still exhibit robust play urges and behaviors during juvenile development, even though their motor coordination is so poor that their social engagements are far from normal.

Using more restricted lesions, critical circuitry for play has so far only been identified in the medial thalamus, with the parafascicular complex, where nonspecific somatosensory inputs converge, having so far yielded the most specific reductions of play. Damage to many other brain areas can disrupt play (e.g., especially along the lateral hypothalamic, mesolimbic dopamine-rich medial forebrain bundle), but such damage reduces many other motivated behaviors equally. Certain forms of brain damage, such as frontal cortical and septal area lesions, can actually increase playfulness. Neuronal marker studies indicate that abundant cells in thalamic midline and dorsal midbrain regions are aroused in play, and play can promote expression of genes for neuronal growth factors such as brain-derived neurotrophic factor (BDNF) and others (see section titled ‘Pharmacological and neurochemical analysis of play’).

Electrical Stimulation of the Brain and Play

Unlike other primary-process emotional systems, play circuitry has not yet been effectively mapped with localized brain-stimulation procedures. A clear vocal marker of playfulness has been so mapped, however. Using localized brain stimulation, the simple marker for positive playful affect, namely the 50-kHz FM-USVs, has been mapped and optimal sites are situated along the trajectory of the mesolimbic dopamine pathways. Through the use of a variety of converging lesion and pharmacological manipulations, the response has been shown to be modulated positively by dopamine in the brain.

Pharmacological and Neurochemical Analysis of Play

Only a few pharmacological agents have been found to increase play. Most prominent among them are low doses of opioids such as morphine, α_2 -adrenoreceptor antagonists such as idazoxan and, perhaps, endocannabinoid facilitators. Opioids can also regulate the social

dominance that emerges in playful encounters, with opiate antagonists reducing and low doses of opiate agonists facilitating social dominance. In this context, it is interesting that opioid antagonists such as naloxone mildly reduce play, even though the animal's social motivation is increased. This may be because, without opioids, the animal feels socially insecure and is not getting as much reward from the social interaction. Indeed, it has been shown that there is abundant endogenous opioid release in the brain during social play.

A great number of pharmacological manipulations can reduce play, including dopamine- and muscarinic acetylcholine-blockade. If one wishes to make one animal of a pair unplayful so as to monitor the play-motivation (i.e., play solicitations such as dorsal contacts) of the other animal, one can use high doses of antimuscarinics or N-methyl-D-aspartate (NMDA) glutamatergic receptor antagonists such as MK-801 to generate very nonplayful animals that still locomote relatively normally. High doses of opioids and dopamine-blocking agents can produce cataleptic target animals that essentially remain immobile during the monitoring of play solicitations. These immobile targets are less socially attractive than animals that move around.

In general, every drug that makes animals feel bad – as monitored by place aversions – reduces play. Only a few agents can elevate play, and usually only at low doses, especially with opioids and alcohol. All psychostimulants that facilitate dopaminergic activity reduce play, even though dopamine release is amplified in the middle of play. This paradox may reflect the fact that dynamically modulated dopamine release is needed to sustain play; drugs that ‘freeze’ dopamine release at high, sustained levels are apparently not compatible with the dynamic, rapidly fluctuating behavior patterns that characterize play.

Gene Expression and Regulation and Play

Using the immediate-early-gene product cFos as an indication of neural activity, it is clear that only a limited number of subcortical brain areas participate in play: regions such as the medial thalamus, along with the polysensory regions of the dorsal tectum, as well as the affectively more positive regions of the medial midbrain. Although the neocortex is not needed for play, it is dramatically aroused during play. Why might that be? Perhaps because play helps program various complex social skills into the neocortex, which resembles a *tabula rasa* at birth. Preliminary genetic evidence suggests play may be important in the epigenetic programming of the higher regions of the brain; gene microarrays indicate that a great number of genes in the cortex are up- or down-regulated during play. Thus, in simple terms, play may

facilitate the developmental construction of the ‘higher social brain.’ A growth factor gene expression that is upregulated as a result of play is BDNF – which has been implicated in depressive disorders. From this, one might speculate that the positive affect from abundant play may tend to reverse depressive symptoms. At the present time, the only gene that has been studied at a functional level is that which manufactures insulin-like growth factor-1 (IGF-1). The protein products of this gene are released vigorously during play, but the amplified gene expression leads to significantly higher protein levels a few hours later. The protein, administered into the ventricular system, promotes positive affective responses, and recent functional studies have demonstrated that IGF-1 administered into the ventricular system can modify a variety of social and related emotional behaviors.

Play and Psychiatric Considerations

The genetic analysis of social behaviors, including play, remains in its infancy. What genes apparently provide are a few rough-and-ready emotional brain tools for animals to behave prosocially – for instance, to be motivated to play, have sex, and show maternal behavior – while the psychobehavioral refinements of these potentials, as in programming the neocortex, must be largely learned with the assistance of various epigenetic/developmental processes.

Although there are bound to be many higher psychological functions that can be refined through play, one possibility is that play is especially important in facilitating the maturation and refinement of frontal lobe executive functions. Attention-deficit hyperactivity disorder (ADHD) is a childhood disorder where such maturation is deficient; it is possible that this type of tendency is elevated when children do not get enough social physical play. This idea has been modeled in rats. Indeed, young animals that have been deprived of play tend to be more impulsive and playful when they are older than young rats that had abundant play. Children diagnosed with ADHD do have more slow-wave activity in their frontal lobes, which are typically slightly smaller (~5%) especially on the right side. Following imposed frontal lobe damage, rats are very much more impulsive and they play more than normal. Such symptoms are diminished if animals are allowed abundant daily access to rough-and-tumble play. In this context, it is important to recall that the psychostimulants used to treat ADHD uniformly reduce play in animal models. Likewise, sick and hungry animals and children also do not play much.

It is worth noting that animals deprived of social play throughout adolescence grow up to be more aggressive animals that are not as responsive to social cues. This has

also been evident in preliminary human studies, where violent, young, adult criminals in the Texas Prison system were found to have childhood upbringings that were very deficient in play opportunities. It is to be anticipated that play can facilitate the maturation of the fully social brain and the various sensitivities and empathetic attitudes that may go along with abundant playfulness in childhood.

Toward a Functional Understanding of Play and its Role in Society

Although the study of the functions and brain substrates of social play remains in its infancy, there are many reasons to believe that physical play is involved in the epigenetic programming of higher brain social functions. If we are to understand how the prosocial functions of the neocortex emerge in our children, we may need to devote more effort to the scientific study of play-induced epigenetic progressions in the brains of animals.

When it comes to developing new social policies related to insuring adequate physical play for our children, perhaps we should follow Plato's advice and encourage more free play – "those natural modes of amusement which children find out for themselves when they meet." In *The Republic* [section IV] he insisted that "our children from their earliest years must take part in all the more lawful forms of play, for if they are not surrounded with such an atmosphere they can never grow up to be well conducted and virtuous citizens." Pursuant to such ideas, there is a greater need to understand the functions and neuroscientific foundations of social play in animal models.

See also: Behavioral Development and Socialization; Control of Food Intake; Feeding; Pleasure; Regulation of the HPA Axis by Acute and Chronic Stress; Stress and Brain Morphology; Stress and Emotionality; Stress and Reward; Stress and Social Behavior.

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Relevant Websites

<http://www.nifplay.org> – The National Institute for Play.

Social Bonding and Attachment

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Glossary

Arginine vasopressin (AVP) – A nine-amino-acid neuropeptide synthesized in the hypothalamus, including the supraoptic and paraventricular nucleus, as well as the suprachiasmatic nucleus. AVP is also synthesized in the medial amygdala (AMY) and bed nucleus of the stria terminalis (BNST) and released in the lateral septum (LS). AVP in the AMY–BNST–LS axis is androgen dependent.

Corticotropin-releasing factor (CRF) – A secretogog for adrenocorticotropic hormone (ACTH) which has been implicated in various forms of behavior and anxiety.

Dopamine – A catecholamine neurotransmitter associated with movement as well as behavioral activation and incentive seeking.

Oxytocin (OT) – A nine-amino-acid neuropeptide synthesized primarily in the hypothalamus, including the supraoptic nucleus and paraventricular nucleus, and historically associated with birth and lactation.

Social monogamy – A social system characterized by pair bonding and biparental behavior.

Vagus – The tenth cranial nerve supplies innervation to visceral organs. The source nuclei of the motor/efferent components of the vagus are in the dorsal motor nucleus of the vagus (DMX) and the nucleus ambiguus. The nucleus ambiguus is located ventral to the DMX in an area known as the ventral vagal complex (VVC), which also contains the source nuclei of the special visceral efferent pathways regulating the striated muscles of the face and head.

Vertebrates, and especially mammals, are dependent on other members of their species for reproduction and, in some cases, for basic survival. Social relationships can be of varying intensities and durations, ranging from casual, nonselective interactions to selective comparatively long-lasting interactions or mental representations that may last for the lifetime of the individual. The purpose of this article is to describe central neurobiological processes that have been associated with the causes and consequences of mammalian sociality and selective social bonds.

Mammalian infants are nutritionally dependent for part of their lives on the mother. In addition, most

mammals produce their young through live birth. In mammals, the neuroendocrine systems necessary for birth, lactation, and maternal behavior are also at the core of the neurobiology of adult social bonds. In mammals, the most consistent social bonds are also between the mother and infant. In fact, the biology of the mother–infant interaction may be the physiological prototype for mammalian sociality. However, relationships structured around social bonds can form throughout the lifespan, and are integral to the formation of families or other social groups. The biology of social bonds probably originates from endocrine and autonomic processes necessary for very basic physiological processes such as water balance, the management of homeostasis, and reproduction.

Several neurotransmitters and neuromodulators, including compounds that are also implicated in reproduction and homeostasis, such as oxytocin (OT), arginine vasopressin (AVP), and corticotropin-releasing factor (CRF), have been implicated in social bond formation. The autonomic nervous system, comprised of both sympathetic and parasympathetic components, mediates emotional states and regulates various bodily organs. The same neurotransmitters that are involved in sociality play a role in the regulation of the autonomic nervous system, allowing integration of social behaviors with physiological and autonomic states.

Species differences in the capacity to form social bonds are common, and this variation has provided clues to the specific neurobiology of social bonding. For example, sheep, which exhibit selective maternal behaviors, and socially monogamous rodents, such as prairie voles, have proven particularly useful models for the analysis of the physiology of mammalian social bonds.

Attachment and Bonding

In the field of psychology, the term ‘attachment’ has been typically used in reference to the responses of a young child to the presence or absence of its mother or another caretaker. This somewhat narrow use of the term may also be used to describe ‘attachment theory’ which is usually restricted to human behavior. The more general term ‘bonding’ is used here to refer to selective social relationships that may occur in humans or other animals. In the context of neurobiology, social bonds may be measured by behavioral, autonomic, or endocrine responses that are differentially expressed toward one

individual versus another. The behaviors used to index social bonds may include positive behaviors, such as the preference of a mother for her own young or selective partner preferences between adults. Proximity and social contact are commonly used to index pair bonding in animals. In addition, defensive aggression, usually toward a stranger or intruder, may also be used as a measure of social bonding. In this context maternal defense or mate guarding may also be expressions of social bonds.

The first steps in the formation of social bonds, whether between a mother and infant or two adults, require social proximity, social engagement, and individual recognition. However, social cues can be perceived or interpreted as either positive or negative. Typically, positive signals elicit approach, while negative signals are followed by avoidance or withdrawal. The most basic elements of sociality are influenced by the physiological condition of the social partners, including sensory and autonomic processes which can in turn influence reactivity to various sensory cues. Sensory cues from a potential partner – such as odors, physical traits, and auditory signals – are received and filtered through neural networks that are influenced by endocrine processes. In addition, autonomic processes, necessary for social engagement, social learning, and social bonding, are dynamically affected by the same hormonal factors that regulate reproduction. Studies of maternal behavior have provided specific clues to the neuroendocrine mechanisms necessary for the formation or expression of social bonds between adults. There is strong evidence in mammals that both maternal–infant bonding and adult pair bond formation rely on neuropeptide hormones, including OT and AVP.

OT and Vasopressin

The major sources of OT and AVP are in the brainstem, including the paraventricular and supraoptic nuclei. OT and AVP are released into the blood supply at the posterior pituitary (also known as the ‘neurohypophysis’). These hypophyseal hormones act on various peripheral tissues. They are also secreted into the brain where they have their major behavioral effects. Of particular importance to sex differences in social behavior are an androgen-dependent set of AVP cell bodies in the medial amygdala and bed nucleus of the stria terminalis. Processes from these AVP secreting cells extend into the lateral septum. In this article this system is termed the medial amygdala–bed nucleus of the stria terminalis–lateral septum (AMY–BNST–LS) axis.

In general, OT and AVP are synthesized in separate cells, and tend to have dynamic, often opposing effects on both behavior and physiology. OT has only one known receptor subtype (OT receptor, OTR). The same OTR is

found in brain, uterus, and mammary tissue. AVP has three receptor subtypes; of these the AVP V1a receptor subtype is particularly abundant in the nervous system. OT and AVP are selective for their own receptors, and may also bind to and affect each other’s receptors, allowing peptidergic interactions and complexity in this system.

Social Engagement, Social Bonding, and the Autonomic Nervous System

Fundamental to the formation of pair bonds are social engagement behaviors, most simply measured by the reduction of physical distance between prospective partners. Engagement is followed by recognition of another individual as either familiar or unfamiliar. In time, especially in socially monogamous species, a social preference is expressed, usually for the familiar partner. Autonomic function is necessary for all forms of affective and emotional experience, including the interactive and contingent behaviors leading to the formation of selective social bonds.

The origins of social engagement are more fully understood in the context of the evolution of the mammalian autonomic nervous system. The mammalian nervous system evolved from phylogenetically older vertebrates. During this evolution, new autonomic mechanisms emerged that supported the metabolic needs of the complex, oxygen-dependent mammalian nervous system. Associated with the phylogenetic transition to mammals was the evolution of unique mechanisms for social communication and associated selective sociality, regulated in part by the autonomic nervous system.

It is common to divide the autonomic nervous system into sympathetic and parasympathetic branches. The sympathetic nervous system is, in general, linked to mobilization behaviors and an increase in metabolic output, while the parasympathetic nervous system is involved in calming and immobilizing behaviors. Anatomically, sympathetic pathways are integrated with nerves exiting the spinal cord, while the primary nerve of the parasympathetic nervous system exits the brainstem as the vagus or the tenth cranial nerve. Social engagement and bonding require a turning off of the primary fight and flight defense systems. The phylogenetically more recently evolved myelinated vagus dampens sympathetic activation and reduces the sense of danger associated with engaging prospective mates. Thus, social bonding and attachment are related to the effectiveness of the ventral vagus in dampening sympathetic activation, reducing defensive behaviors, and facilitating social engagement through the special visceral efferent regulation of the muscles of the face and head.

The vagus nerve is actually a bundle of fibers, including both motor/efferent and sensory/afferent components. Approximately 80% of the vagal fibers are afferent and carry information from the viscera to a brain-stem region known as ‘nucleus tractus solitarius (NTS).’ Output from the NTS contributes to the regulation of higher brain functions, including modulation of arousal and bodily state.

The two source nuclei of the efferent vagus include the phylogenetically older unmyelinated, dorsal motor nucleus of the vagus (DMX) and the more recently evolved myelinated portion of the vagus, which originates in the nucleus ambiguus. The nucleus ambiguus is located ventral to the DMX in an area known as the ventral vagal complex (VVC), which also contains the source nuclei for the special visceral efferent pathways that regulate the striated muscles of the face and head, through cranial nerves V, VII, IX, X, and XI. Actions of the ventral vagal complex allow the reduction in fear and the concurrent emergence of calm states necessary for social engagement and bonding. The special visceral efferent pathways, in coordination with the ventral vagus, form a social engagement system that integrates calming with the movements of the striated muscles of the face and head. In a context of relative safety, facial expressions and vocal intonations necessary for social engagement and social communication can emerge.

Neuropeptide receptors are found in autonomic source nuclei, where they serve to regulate sympathetic and parasympathetic functions, and in this capacity can influence social bond formation. For example, OTRs are abundant in the DMX. The DMX and the unmyelinated vagus have been implicated in various types of conditioning, such as taste aversion (e.g., nausea) and passive avoidance. The OT pathways may thus dampen both sympathoadrenal and DMX (unmyelinated) vagal reactivity, allowing reductions in anxiety and fear, and concurrently permitting proximity and social bonds. Within the brainstem, OT may act directly on the source nuclei of the DMX to protect the autonomic nervous system from surging, especially under conditions such as parturition or consensual sexual behavior, which require immobility without fear. A sense of safety may be essential to the formation of a social bond. In this context, OT may change the detection of immobilization from that of life threat to that of safety.

In contrast, both OT and AVP V1a receptors are found in the VVC and may functionally modulate the inhibitory action of the vagus on the heart. This enables the vagus to function as a bidirectional system characterized by dynamically increasing and decreasing the influence of the vagal brake on heart rate and supporting the functions of the SVE, without requiring increases in sympathetic activation. Treatment with OT and AVP has the capacity to increase sociality in general. However, both OT and

AVP (in conjunction with dopamine) may be essential to allow the expression of selective social behaviors, including social preferences, and the more mobilized states required for defense of a mate.

OT may also regulate the most primitive defensive response system, characterized by immobilization. Under stressful social conditions, in which avoidance or escape are not an option, a more primitive strategy, immobilization, based upon phylogenetically older neural systems, may emerge. For example, during subordination, the capacity to immobilize and not struggle, or even form social bonds toward a dominant partner, becomes part of an adaptive system. This primitive system is associated with an unmyelinated vagal pathway that triggers bradycardia, apnea, vasovagal syncope, and defecation as adaptive survival strategies to reduce metabolic demands and feign death. Unfortunately, this system, which functions adaptively for reptiles, amphibia, and fish, can be lethal for mammals. The neurophysiological properties of OT may also permit physical immobility without fear in prosocial settings, permitting sleep, digestion, growth, restoration, and reproduction.

Sex Differences in Sociality

Sex differences in steroid and peptide hormones, either during development or in adulthood, may be important in explaining sex differences in the factors that regulate social bonding. In general, males seem to be more capable than females of engaging in active social behaviors and may be more vigilant in response to threats, sometimes termed ‘active coping.’ Females, in contrast, are more likely to show reproductive behavioral patterns characterized by immobility, including those associated with birth, lactation, and female sexual postures such as lordosis. In females, stressors or severe threats may elicit passive coping. Based on animal models, it seems likely that an essential aspect of both parental behavior and pair bonding is the capacity to overcome neophobia and social anxiety. At optimal levels, both OT and AVP can reduce anxiety, which in turn can permit or facilitate social engagement with a novel partner. This in turn may lead to social bond formation, although the mechanisms for pair bonding may involve a more active pattern of behavior in males than females.

OT synthesis and, in some cases, the OTR are enhanced in the presence of estrogen. In addition, in females the relative absence of AVP, especially in areas such as the AMY–BNST–LS axis, may help to explain sex differences in the reaction of females versus males to stressful experiences. This axis may play a fundamental role in the sexually dimorphic expression of social behaviors.

Centrally administered AVP, acting on the V1a receptor subtype, has been implicated in social bonding in both sexes and higher levels of or increased sensitivity to AVP in the AMY–BNST–LS axis could help to explain sex differences in social behaviors. For example, following mating males of socially monogamous species become selectively aggressive toward strangers. V1a receptor stimulation may be of particular importance to active defensive behaviors associated with guarding a mate as well as an offspring. AVP also has a role in the regulation of the autonomic nervous system, including both sympathetic and parasympathetic activity. AVP helps to provide another level of integration between social physiology, emotional responses, and social behavior. The observed effect of AVP on social bonding can be mediated through shifts in autonomic regulation, providing a substrate for mobilization. Alternatively, autonomic effects of OT are associated with immobility or passive coping.

Male parental or alloparental behavior shares some features with maternal behavior. However, male parental behavior does not involve lactation and may also have a somewhat different neurochemistry, possibly requiring for expression both OT and AVP. Spontaneous alloparental behavior in reproductively naive male prairie voles is more reliably exhibited than in females. It is likely that female parental behavior reflects the dependence of females on OT which would normally be released by birth and lactation, but which may not be fully activated in nonreproductive females. This is one of the many examples of evidence for sex differences in the regulation of social behaviors, possibly because of sex differences in the roles of AVP and OT.

In humans, intranasal OT increased the willingness of human males to ‘trust’ others or to detect subtle emotional cues in pictures of human eyes. In contrast, men given AVP showed increases in frowning, and also rated neutral facial expressions as ‘unfriendly.’ Under the same conditions, women given AVP smiled more and reported more positive, affiliative responses to unfamiliar neutral faces. Reasons for this sex difference are not well understood, but may be because in females, the actions of exogenous AVP are primarily through effects on the OT (rather than AVP) receptor.

The Effect of Social Isolation

Experiments in animals provide an opportunity to examine in more depth, the physiological consequences of the absence of a social partner. Prairie voles have a human-like parasympathetic control of the heart, with high levels of vagal activity, unique in rodents. In this species, separation from a partner, followed by prolonged isolation, was associated with increases in heart rate, decreases in parasympathetic function, increased reactivity to stressors,

and increases in measures used to index anhedonia and depression. Following the disruption of a social bond increases in both central and peripheral measures of OT have been found, especially in females. Elevations in OT may provide physiological compensation and coping against negative consequences of isolation. In prairie voles, chronic OT injections were capable of preventing or reversing the cardiac and behavioral effects following prolonged separation (i.e., isolation) from a partner.

Possible Neural Substrates for Behavioral Effects of OT and Vasopressin

The full circuitry for selective sociality has not been described. However, V1a receptors in the lateral septum have been specifically implicated in social recognition in male mice. The lateral septum projects to the dopaminergic nucleus accumbens (NAcc), and through this connection may reinforce selective social behaviors.

Research in mice has also implicated OT and AVP in social recognition. For example, mice in which the gene for OT, or the OTR, or the gene for the AVP V1a receptor were ‘knocked out’ failed to show social recognition as measured by reactions to a familiar animal. In each case, these knock-out mice are not asocial, but do fail to show the selective sociality used to index social recognition. These studies support the more general hypothesis that both OT and AVP may be required for the expression of selective sociality.

The amygdala and its connections play a role in the integration of reactions to various kinds of sensory stimuli, including approach and avoidance. In human males, intranasal administration of OT inhibited the activity of the amygdala, especially after exposure to fear-associated stimuli; intranasal OT also altered downstream connections to brainstem structures involved in the regulation of the autonomic nervous system. AVP, acting centrally (especially in the AMY–BNST–LS axis), as well as other peptides, such as CRF, may elevate vigilance and defensiveness. Behaviors mediated by the central amygdala may also mediate stimulus-specific behavioral responses to fear, while the BNST has been implicated in experiences related to anxiety. At least some of the defensive actions of CRF or AVP may be downregulated by OT. Among the neural targets for OT are receptors in the paraventricular nucleus (PVN) and the posterior pituitary (neurohypophysis), where they may dampen both pain responses and defensive behavioral and autonomic reactions.

Opioids and Pair Bonding

Several other neurochemical systems, including those which rely on endogenous opioids, have been implicated in both maternal care and later sociality. In sheep and

primates, blocking opioid systems with naloxone in a mother is followed by reductions in these behaviors, although these mothers are still capable of suckling their young. Social interactions in infants are also regulated by opioids, although these effects may be species specific. In guinea pigs, which are precocial at birth, opiate injection diminished distress vocalizations. As the endogenous opioids also play a role in the regulation of the release of OT and AVP, as well as the hypothalamic–pituitary–adrenal (HPA) axis, they may also influence social behaviors indirectly.

Dopamine and Pair Bonding

Dopamine plays a role in behavioral activation and incentive seeking. Brain regions with high levels of dopaminergic input, including the medial prefrontal cortex (mPFC) and (NAcc), have also been implicated in suckling and the emergence of positive responses to an infant. Interactions between OT and dopamine in the NAcc have also been implicated in both maternal behavior and pair bonding in prairie voles. OTRs are especially abundant in the shell of the NAcc in socially monogamous species, including prairie voles, but not in nonmonogamous voles. Administration of a general dopamine receptor antagonist in the NAcc blocked mating-induced partner preferences. In contrast, treatment with a dopamine agonist facilitated pair bonding, suggesting an essential role for dopamine in pair bond formation. The action of dopamine on social bond formation and maintenance of these bonds is mediated through different subtypes of dopamine receptors. D2 receptors are necessary for pair bond formation. In contrast, D1 receptors play a role in the maintenance of social bonds, including an increase in selective and defensive aggression, which can help to protect the pair bond.

The NAcc is also a major input to the ventral pallidum and there is evidence that AVP and dopamine may interact in the ventral pallidum to facilitate pair bonding, possibly by overcoming fear or anxiety and reinforcing the bond. Prairie voles, in comparison to nonmonogamous vole species, have high levels of V1a receptors in the ventral pallidum. Blocking the V1a receptor in this region inhibited partner preference formation in male prairie voles. In addition, upregulation of V1a receptors in the ventral pallidum (using a viral vector) facilitated pair bonding in male prairie voles. Overexpression of the prairie vole, V1a receptor in the ventral pallidum of montane voles, a nonmonogamous species, also facilitated partner preferences. Species-specific microsatellites in the promoter region of the gene for the V1a receptor may alter the expression of this receptor, especially in brain regions necessary for pair bonding. Differences in the co-localization of dopamine, OT and AVP may help

to explain species or individual differences in the capacity to form pair bonds.

'Stress' Hormones and Pair Bonding

CRF is another neuropeptide that has been implicated in birth, maternal behavior, anxiety, and adult social bonding. The effects of CRF on pair bonding in prairie voles have thus far been studied only in males. The effects of CRF were dose dependent; moderate doses facilitated pair bonding, but higher doses did not, possibly because high levels of CRF can induce anxiety. Moreover, CRF receptors are sexually dimorphic, at least in prairie voles, which might also contribute to sex differences in pair bond formation. CRF and AVP are both associated with increased release of the adrenal steroid, corticosterone. It is possible that a cocktail of 'stress' hormones may facilitate pair bonding in males. CRF administered directly into the NAcc also facilitated pair bonding in male prairie voles, but was not effective in nonmonogamous vole species. Species differences exist in CRF receptors, with higher levels of the CRF type 2 receptor and lower levels of CRF type 1 receptor in the NAcc in socially monogamous species of voles. CRF may also modulate the release of dopamine, possibly contributing to the effects of CRF on pair bonding.

Developmental Factors Can Alter Social Bonding

Neuroendocrine systems involved in social bonding undergo long-lasting modifications as a function of early experience. This epigenetic model may help to explain the origins of traits, sometimes called 'personality' or 'temperament,' as well as individual differences in behavior. Understanding of these systems may also offer insights into the development of pathological or maladaptive behaviors.

Genetic differences are one source of variance in social behavior, including the tendency to form social bonds. However, genetic differences are not sufficient to explain individual variations in social behaviors. Among the behaviors and neural systems that are changed by early experience are those necessary for pair bond formation. For example, female prairie voles that were deliberately maintained with minimal disturbance during the pre-weaning period did not, in later life, form pair bonds.

Manipulations of OT and AVP in the postnatal period are also capable of programming individual differences in sociality. In female prairie voles, exposure to exogenous OT during neonatal life has a dose-dependent capacity to facilitate a later pair bond and enhanced subsequent hypothalamic synthesis of OT. In contrast, even brief

neonatal exposure to an OT receptor antagonist (OTA) disrupted subsequent social behaviors, including the tendency to form social bonds, to exhibit parental behaviors, and to manage anxiety or stress. OTA treatment also disrupted the expression of the AVP, V1a receptor, with consequences that were particularly dramatic in males. Many of the consequences of early peptide manipulations on the nervous system are sexually dimorphic and correspond to sex differences in behavior. The androgen dependence of AVP in the AMY–BNST–LS axis and the sexually dimorphic capacity of an OTA to downregulate both AVP receptors and AVP may help explain the fact that exposure to OTA was especially disruptive to male behavior. Conversely, in males (but not females), early OT exposure upregulated V1a receptors in the ventral pallidum, consistent with the finding that increases in AVP V1a receptors in the ventral pallidum can facilitate pair bonding in male prairie voles.

Early Experiences and Social Bonding

The biological mechanisms underlying traits, including the capacity to form affiliative bonds, are dynamic and capable of being influenced by early behavioral experiences. These effects are often on the same systems that regulate sociality in adulthood. For example, physiological and behavioral changes associated with pregnancy, birth, lactation, and the management of infants during the postpartum period can produce long-lasting changes in behavior. The widespread use of exogenous OT ('Pitocin') to induce or augment labor and more recently the use of OT antagonists also hold the potential to influence the parent and offspring. Even apparently simple decisions, such that the amount of time an infant is touched or receives other forms of social stimulation, can retune the developing mammalian nervous system. Human breast milk contains OT and related hormones, which may be eliminated in infant formulas. Moreover, sucking behavior engages the same neural circuits involved in social engagement. Thus, the decision to breast-feed, with both nutritional and behavioral consequences, could also be a neural exercise in the social context of nursing. Breast-feeding might strengthen the bond with the mother through the joint mechanisms of increasing OT and possibly dopamine as well. Physiological and behavioral experiences, including those associated with birth and parenting, can influence subsequent sociality and attachment. A deeper understanding of the causes and consequences of these

processes remains a major challenge for the neurosciences of the twenty-first century.

See also: Infant Bonding and Attachment; Maternal Deprivation; Perinatal Influences on Behavior and Neuroendocrine Functions; Stress and Social Behavior.

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Attention and Speed of Information Processing

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Glossary

Bottom-up – A strategy of information processing that arises from sensory stimuli present in a scene. In a bottom-up approach, physically salient stimuli, such as a bright light, bias the information to be processed.

Diffusion tensor imaging (DTI) – The structural noninvasive neuroimaging technique that enables the measurement of the integrity of cerebral white matter.

Electroencephalogram (EEG) – The electrophysiological noninvasive functional neuroimaging technique that enables the measurement of electrical brain activity by electrodes attached to the scalp.

Event-related potentials (ERPs) – The voltage fluctuations evident in the EEG recording that are induced by changes in brain activity temporally associated to the occurrence of sensory, motor, or cognitive events.

Functional magnetic resonance imaging (fMRI) – The metabolic noninvasive functional neuroimaging technique that enables the measurement of changes in cerebral blood oxygenation levels in the brain that are associated to sensory, motor, or cognitive events.

Speed of information processing (SIP) – The amount of information that can be processed per unit of time, or velocity at which a variety of cognitive processes can be carried out.

Top-down – A strategy of information processing important for guiding a goal-directed behavior. Information processing is guided in a top-down manner when expectations and goals determine what is relevant and what will be processed.

Working memory – The mental ability to maintain and/or manipulate information during brief periods of time in order to make use of this information to perform a particular task or to achieve a goal.

Definition

Attention could be defined as the mental ability to generate and maintain an activation state that allows information processing. It also allows the selection of specific

information from among multiple sources. This includes internal and external stimulation, memories, thoughts, or even actions. Attention may be considered a complex system of specialized subprocesses through which it addresses precision, speed, and continuity to behavior.

Functional dissociation between different attentional components is difficult to establish when considering everyday activities. However, researchers have traditionally described the independence of at least three functional systems that cooperate and work closely together. Current cognitive neuroscience has named these attentional networks as: alerting, orienting, and executive attention.

Alerting

Alerting relates to an increase and maintenance of the readiness response in preparation for an impending stimulus. This capacity is considered the basis of attention onto which other attentional mechanisms rest. The terms arousal, vigilance, and sustained attention have been used interchangeably to define ‘long-term attention.’ However, findings from functional neuroimaging, clinical, and animal studies have suggested the presence of neuroanatomical dissociations between the neuronal circuits underlying these attentional abilities.

The term ‘arousal’ has been defined as a continuum of physiological reactivity, sleep and excitement being the two extremes of the continuum. Arousal represents a basic attentional function that determines the efficacy of higher-level cortical attentional systems and general cognitive capacity.

The psychological construct ‘sustained attention’ is the ability to maintain attention to a particular stimulus, location, or task, for prolonged periods of time. Sustained attention has been frequently used synonymously with the term ‘vigilance.’ However, vigilance is thought of as a long-term process for periods of minutes and hours, whereas sustained attention, a shorter-term process for seconds and minutes.

Experimental Tasks

Arousal can be operationalized in terms of the degree of synchronization/desynchronization of the brain electrical

fields as measured in the electroencephalogram (EEG). In general, when the arousal state increases, a desynchronization in the recorded EEG activity is observed (i.e., small amplitudes and fast frequency rhythms). In neuropsychological and clinical practice, alerting has been also measured by different behavioral scales and tasks. Among the commonly used procedures to address alertness after brain damage, we can find the Glasgow Coma Scale (GCS), the Galveston Orientation and Amnesia Test (GOAT), the Westmead posttraumatic amnesia scale, or the Mental Control subscale from the Wechsler Memory Scale.

Vigilance tasks involve detecting stimuli with a low rate of appearance in long-lasting monotonous tasks. Prototypically, vigilance or warning tasks assess how quickly a subject can respond to certain auditory or visual warning signals.

Sustained attention tasks involve responding stimuli with a high rate of appearance such that subjects must keep monotonously responding to target stimuli. Thus, task length is not a critical variable. In a clinical context, it is common to use different versions of the Continuous Performance Test and the Symbol Digit Modality Test (SDMT).

Related Anatomy

The ability of arousal-inducing stimuli to trigger attentional processing is mediated bottom-up through noradrenergic projections from the locus ceruleus to the thalamus and the basal forebrain. This arousal system would be a lower-order system triggering, but not directly mediating, higher-order attentional mechanisms.

Visual and auditory vigilance tasks have been associated by functional neuroimaging with a decrease on right frontal activation as a function of time on task. This decrement correlates with behavioral measures of vigilance decrement, such as the slowing down in reaction times (RTs) to target stimulus.

Human neuropsychological and neuroimaging studies have also pointed to an amodal right frontoparietal-thalamic system involved in sustaining attention even for short periods of time. In addition, animal experimental research supports the basal forebrain corticopetal cholinergic projection as a major component of the circuit. Therefore, while frontoparietal regions would be related to the attentional requirements of sustained attention, thalamic signals would be related to the influence of arousal levels on sustained attention (**Figure 1**).

Clinical Impairment

Deficit in the alerting system vary from coma states to normal vigil response, and could include poor response to stimulation, lack of orienting reflexes, sleepiness, etc. Patients with severe central nervous system disease often show marked alerting difficulties.

The distinction between vigilance and sustained attention became especially relevant in the clinical context. Most patients with mild/moderate brain injury show no significant difficulties in carrying out vigilance tasks. By contrast, many patients show sustained attention difficulties when processing information presented at a high rate. These sustained attention difficulties may have a strong impact on daily activities.

Orienting

Orienting is the most studied attentional network. It is defined as the ability to select specific information from among multiple sensory stimuli or features. Selective attention is closely related to orientation of attention. While orienting of attention refers to directing attention to one particular location or stimulus, selective attention implies attending to one location or stimulus in favor of another.

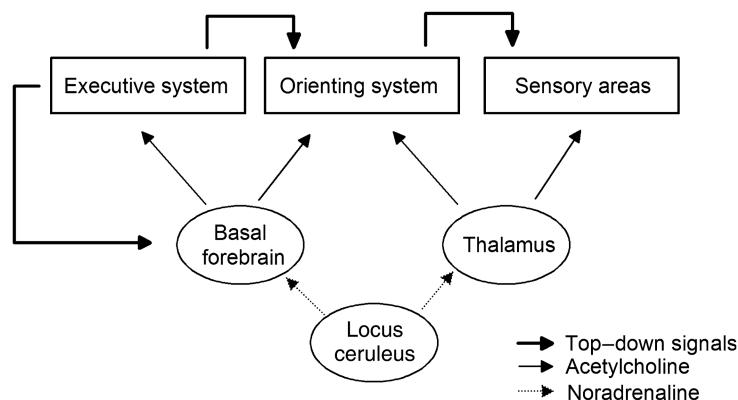


Figure 1 Schematic illustration of the major components of the neuronal network mediating sustained attention and its relation with executive and orienting networks.

Orienting attention to a stimulus enhances behavioral responses reducing RTs and error rates, as compared to nonattended features. This improved performance might be achieved by a relative increase in neural activity in a given sensory system. Accordingly, ‘bias competition’ models of attention suggest that the neural systems involved in cognitive control provide top-down signals to the different brain systems responsible for object’s representation. These signals will enhance attended perceptual objects or features and will increase the contrast with nonattended ones. Orienting and selective attention studies focus on the neural consequences of these top-down signals over the neural representations of the target attended features. These consequences may involve enhancement or reduction of the brain activation elicited by attended events. For example, orienting to a particular type of visual stimulus or to a particular aspect of the stimulus (e.g., its color) increases activity in the region where the specific task-relevant information is represented (e.g., specific color areas of the visual cortex). In this way, attention can modulate sensory processing in early stages of the visual processing stream.

Most research in orienting has been done in the context of visuospatial tasks. However, neural activity increases in response to attentional mechanisms have also been shown in the motor domain (i.e., preparatory orienting of attention toward a particular motor intention) and in the domain of working memory or long-term memory (i.e., the orienting of attention to a given number of a sequence that had to be maintained in memory).

Experimental Tasks

The oddball paradigm constitutes a classical example of selective attention tasks. Subjects are instructed to respond or to ignore stimulus presented sequentially in the center of a screen according to predetermined perceptual features (e.g., the color or shape of a stimulus). The main variables manipulated in this type of paradigm are: stimulus type and frequency. Target events are infrequent and must be covert or overtly responded by the subject. Standard events are more frequent and should be ignored. It is also possible to include other low-frequency distracting stimuli, such as deviants (similar to targets) and novelties (not previously presented or informed), allowing a variety of experimental manipulations. For instance, it is well established that RTs to targets usually increase if they occur after infrequent deviants and novel stimuli.

In a typical spatial orienting task, the experimenter cues the participant’s attention to a given location of the space before a target stimulus appears. This can be achieved by means of either visual or auditory cues. In valid trials, the cue and the target are placed in the same location; in invalid trials, cue and target positions do not

match. RTs in the valid condition are subtracted from those in the invalid condition to yield an efficiency score. Comparison between these two conditions and a no-cued condition usually shows a small benefit of orienting to a validly cued location, and a larger cost for invalidly cued locations.

Visual search tasks involve an active scan of a visual display for a particular object or feature (the target) among other objects or features (the distractors). In the visual search task, targets defined by a single feature (e.g., red dot among a number of blue dots) are easily detected in parallel, and detection time does not increase with the number of distractors in the display. In contrast, targets defined by the conjunction of two or more features (e.g., color and orientation of lines) are more difficult to detect and require serial search. For these targets, detection time increases as a function of the number of distractors in the display, suggesting that the integration of features into objects is an attention-dependent process.

Related Anatomy

Intracranial research, studies with patients with focal brain lesions, functional neuroimaging (fMRI) studies, and scalp-recorded event-related potentials (ERPs) converge suggesting that a widespread cortical network gives rise to the controlled detection of target events during the detection of targets from an oddball-like task. The temporoparietal junction, the medial temporal cortex, and the lateral prefrontal cortex (PFC) are the brain regions that have been consistently associated to target detection mechanisms.

The anatomical brain networks involved in orienting are similar for spatial orienting and visual search tasks. Posterior parietal and frontal areas are reliably involved in visual spatial orienting. This brain network is more pronounced in the right hemisphere, and its activation is independent of the direction of the attentional shifts, except at primary sensory areas. Activation in the parietal cortex is centered along the intraparietal sulcus and often extends into the superior parietal lobule. Frontal activations occur in the anterior cingulate, supplementary motor areas, and in the frontal eye fields. Other regions that frequently appear in studies of the spatial orienting network are the pulvinar, superior colliculus, temporo-parietal junction, and superior temporal lobe. These areas seem to be involved in specific subroutines in the orienting process. Further activations in the superior parietal lobule have been related to shifting attention between spatial locations as compared to maintaining attention on fixed positions. Lesions of the temporoparietal junction and superior temporal lobe disrupt the ability to disengage attention from a particular location. Finally, the frontal eye fields and superior colliculus might be

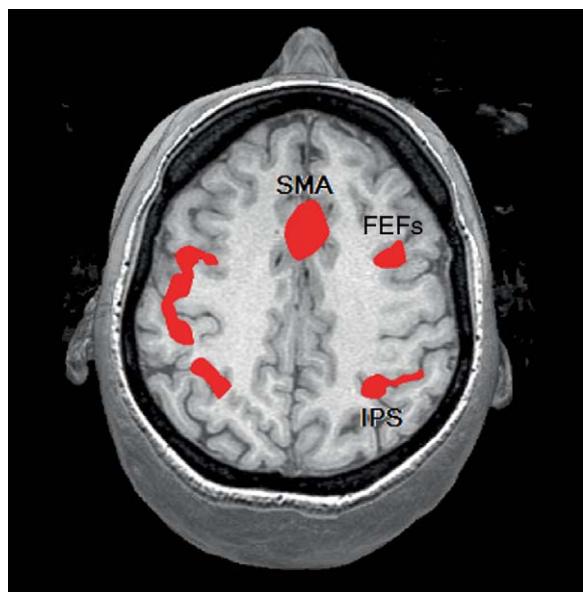


Figure 2 Brain areas involved in visual spatial orienting of attention: intraparietal sulcus (IPS), supplementary motor area (SMA), and frontal eye fields (FEFs).

primarily involved in the participation of overt eye movements in spatial attentional shifts (**Figure 2**).

Describing the brain network involved in nonspatial orienting of attention is currently an active research topic. Preliminary data on spatial and nonspatial orienting of attention seems to suggest both a partial overlap and a partial specialization of their neural systems. For instance, occipital, parietal, and frontal regions participate on spatial and temporal attention (i.e., attending to a given moment in time). However, while the right inferior parietal lobule seems specific for spatial orienting, the left inferior parietal lobule and the left inferior lateral premotor cortex seem specific for temporal orienting.

Clinical Impairment

Hemispatial neglect (or neglect) is one of the most prevalent and impairing deficits of the spatial orienting

component after brain damage. It is defined as a lack of attention for stimuli toward the side of space opposite to their unilateral lesion. Patients fail to report, respond, or orient to meaningful stimuli, suggesting that this failure cannot be attributed to primary motor (e.g., hemiplegia) or sensory dysfunction (e.g., hemianopia). Double dissociations can be found pointing to different subtypes of neglect. These are distinguished by input (attentional) or output (intentional) demands, the distribution (personal, spatial, and representational), the system affected (visual, tactile, or motor neglect), and the method used to elicit the signs (unilateral or bilateral stimuli). Patients may also exhibit viewer-centered or stimulus-centered neglect. Sensory extinctions are often considered mild forms of neglect, but are sometimes considered separate entities. According to the severity of symptoms, one cannot copy simple figures, does not eat the left side of the plate, dresses only the right half of one's body, reads only the right half of the pages or the words involving difficulties in texts comprehension, and, in most severe cases, may even deny that the left side of one's own body belongs to them (**Figure 3**). Neglect courses with lack of awareness of the deficit (i.e., anosognosia). This syndrome is observed primarily after unilateral lesions in the right parietal cortex, the supramarginal gyrus, and extends into subcortical areas. Lesions in the lateral and ventral portions of the frontal lobe and of the subcortical nuclei have also been implicated. The use of dopaminergic and noradrenergic drugs has been proposed to ameliorate neglect symptoms.

Executive Attention

Executive functions are required in situations that involve planning or decision making, error detection, novel or not well-learned responses, conditions judged to be difficult or dangerous, regulation of thoughts and feelings, and the overcoming of habitual actions. Executive attention involves effortful and controlled performance in tasks in which the response is not fully determined by the

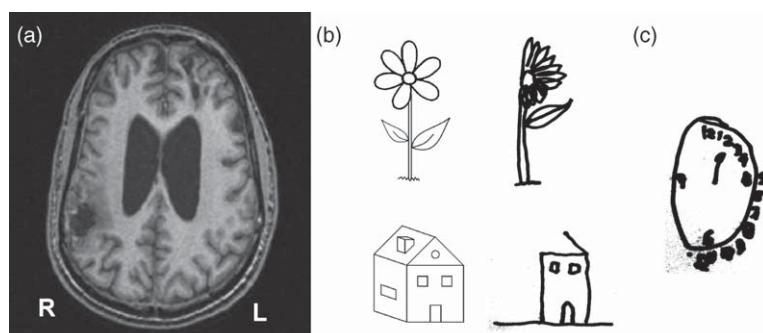


Figure 3 (a) Magnetic resonance image showing a right parietal lesion. (b) Examples of copied drawings by a patient suffering neglect. (c) Spontaneous clock drawing by the same patient.

stimulus. According to this broad definition, executive attention is not a single cognitive construct or neural mechanism but different subcomponent operations interacting within different brain regions.

A top-down mechanism for executive control must fulfill some requirements. First, it must provide a source of activity that can exert biasing signals to other structures. Second, the system must have access to a wide range of representations in other brain regions to be able to influence them. Third, it must be able to maintain information active in working memory against distractions until a goal is achieved, but should also be flexible enough to update its representations when needed. Finally, the allocation of control depends on a system able to detect conflict in processing. Conflict happens in response to the coactivation of competing representations or processing pathways.

Experimental Tasks

Several executive tasks have been used to isolate different components of executive attention and their neural basis.

Executive attention is commonly measured using tasks in which there is incompatibility between stimulus or response dimensions, as in the classic Stroop and flankers tasks. For instance, the purpose of the Stroop task is to assess one of the most fundamental aspects of cognitive control and goal-directed behavior: the ability to select a weaker task-relevant response, in the face of a competing and stronger but task-irrelevant one that the subject has to inhibit. This is generally achieved asking the subject to name the ink color of a word whose meaning is incongruent with it (e.g., to name the red ink color of the word blue).

Similarly, in the Eriksen flanker task, participants have to respond as fast and accurately as possible to a target arrow briefly presented in the center of the screen. When the target points to the right, the right button has to be pressed, and when the target points to the left, a response with the left button is required. The target arrow is accompanied by irrelevant flanker arrows displayed in the left and right sides of the target one (i.e., '> > > >' or '< < > < <' for congruent and incongruent trials, respectively). The most general finding using this task is that responses become slower under high conflict demands as compared to low- or nonconflict ones. This extra time is thought to reflect the time consumed by the operation of an executive attentional mechanism.

Research using task-switching experiments focuses on how the system is flexibly reconfigured moment by moment. A common denominator across different task-switching paradigms is that participants are asked to switch repeatedly among two or more simple tasks (e.g., classifying target stimulus according to their color or to their shape). The most general finding has been that

responses become slower and less accurate when switching than when repeating a task, a phenomenon referred to as 'behavioral switch cost.' This cost may reflect the time taken by control operations for task-set reconfiguration – a sort of mental gear changing – that must happen before the appropriate task-specific processes can proceed. Task-set reconfiguration can include shifting attention between stimulus attributes, abstract categories, goals (what to do), and condition–action rules (how to do it) into procedural working memory.

The Go/No-go task provides a simple paradigm to investigate response inhibition and response competition. In a typical Go/No-go experiment, subjects are instructed to respond by key press to the go stimulus (e.g., the letter A) and to withhold responses to No-go stimulus (e.g. the letter X). The number of correctly withheld responses to the No-go events, as well as the number of false-alarm responses and RTs, are frequently used as measures of inhibitory ability.

The novelty oddball task has been employed to study behavioral and neural responses to novel events. In this paradigm, an improbable series of unique and unexpected novel events are presented, in addition to targets and standards. As novel events are unexpected, this experimental procedure closely mimics the real-world situations of attentional shifting. Motor responses are slower and more error-prone after novels, a phenomenon that has been related to the dynamic shifting of attention from the processing of the ongoing task to the processing of the unexpected novel event.

Many of these tasks are used in the clinical context, with necessary adaptations to fit the needs of patients. Some of the most commonly used are the Stroop test, Paced Auditory Serial addition Test (PASAT), the Trail Making Test (TMT), or the Wisconsin Card Sorting Test (WCST).

Related Anatomy

Lesion and brain imaging studies using executive tasks usually describe a network that includes the anterior cingulate and supplementary motor area, the orbitofrontal cortex, the dorsolateral PFC, and portions of the basal ganglia and the thalamus (**Figure 4**). While there are evidences about functional specialization within these brain regions, several models of executive attention and prefrontal function suggest an interaction between them to achieve complex attentional functions.

The anterior cingulate cortex (ACC) is known to be an important node in the executive attention network. Different portions of this brain structure have consistently shown activation during error detection, divided attention, conflict monitoring, word generation, and task-switching performance. A fundamental executive ability related to the function of the ACC is dealing with conflict.

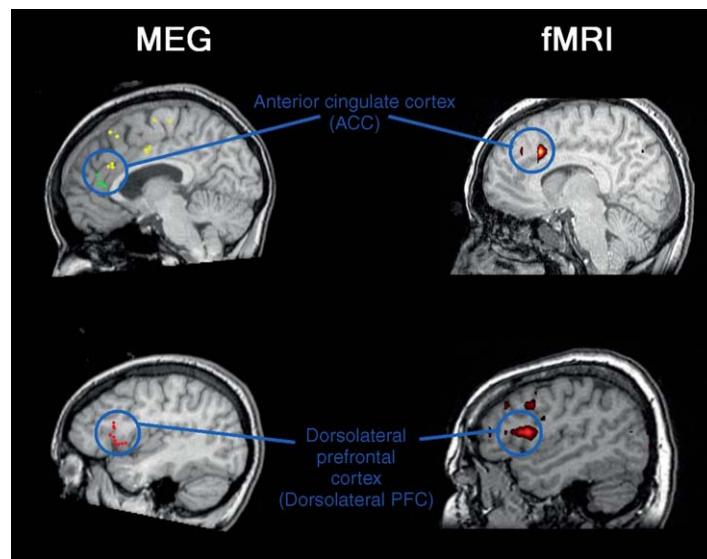


Figure 4 Brain areas activated by No-go stimulus in a Go/No-go task during magnetoencephalographic (MEG; left) and functional magnetic resonance imaging recordings (fMRI; right).

For some authors the ACC would be critical for monitoring and detecting conflict between processing pathways. According to this extended view, conflict signals would engage other prefrontal regions for conflict resolution. By contrast, other authors suggest that the ACC can subserve both conflict detection and conflict resolution directly. Recent findings have started to support the idea that different cognitive functions are mediated by different portions of the ACC.

The ACC seems insufficient by itself to achieve attentional control. Other prefrontal areas in the lateral PFC are central in creating and maintaining attentional sets. The proposal of dorsolateral PFC playing a determinant role in maintaining attentional sets is largely coherent with the well established assumption that this brain region plays a key role in the manipulation and maintaining of the contents of working memory. However, brain areas involved in working memory seem to depend on the type of information being processed. For example, functional imaging studies on humans show that verbal working memory tasks activate the ventrolateral PFC and language areas in the left temporal and inferior parietal cortex. In contrast, visuospatial working memory tasks activate the dorsolateral PFC, inferior parietal cortex on the right side, and high-order visual areas in the occipital cortex.

The inferior frontal gyrus (IFG) has been also related to the executive network by virtue of its connections with temporoparietal brain regions. This ventral PFC area seems to be active whenever low-frequency, unexpected, or previously learned relevant cues break down current processing. This is consistent with the proposed role of IFG in breaking down an outdated attended set in response to new or behaviorally relevant sensory events.

Clinical Impairment

Disturbance of different components of the executive system could lead to different impairments.

One main deficit of executive control of attention is characterized by the inability to accurately select appropriate stimuli or goals in each situation. The patient is unable to overcome salient stimuli or irrelevant responses for the present task. The most severe manifestation of this deficit is the so-called ‘utilization behavior’ where behavior is extremely guided by external stimulation.

Another deficit is the presence of perseverative responses or the difficulty to abandon a task or a *modus operandi* that is no longer adequate and does not lead to achieve goals. These patients show difficulties in adjusting their behavior to the changing requirements of the environment. At the same time, this can lead to troubleshooting difficulties, due to a rigid approach, stereotypical, and recurrent behavior. This is a complex deficit that has been related to not only cortical (e.g., prefrontal lobes and hippocampus) but also subcortical dysfunctions (e.g., caudate nucleus).

Dopamine depletions in frontostriatal circuits are thought to generate many executive deficits. For instance, it has been demonstrated that Parkinson’s disease patients usually exhibit deficits in executive mechanisms such as novelty detection, task switching, inhibiting No-go responses, and working memory. In addition, it has been shown that treatment with dopaminergic agonists improves attentional performance in Parkinson’s disease and attention-deficit hyperactivity disorder (ADHD). This constitutes evidence about the relevance of dopaminergic modulation in attentional performance.

Speed of Information Processing

Speed of information processing or SIP is not an attentional function *per se*, but a result of how information is processed, based on the properties of the system where cognition is implemented. However, an accurate attentional functioning frequently depends on how fast these mechanisms are implemented. For instance, when a potentially dangerous stimulus is to be detected, an efficient attentional system must be not only accurate, but also fast, in order to produce an adaptive response.

In addition, the relevance of studying the relationship between attentional processes and SIP became highlighted by the use of time measures in several attentional tasks. Both in clinical and experimental contexts it is often difficult to establish the extent to which a given time measure of attentional performance is mediated by proper attentional or SIP factors. However, this dissociation is very relevant to theoretical models and clinical practice.

The term SIP has been commonly used in cognitive neuroscience and while there is little debate about its meaning, it has not been clearly defined. SIP reflects the amount of information that can be processed per unit of time, or even the speed at which a variety of processes can be carried out. It can also be defined as the result of the time taken to perceive and process information, as well as the time to prepare and execute a response.

Experimental Tasks

The assessment of SIP can be achieved by different methods according to the selected level of analysis. These levels could involve measuring physiological signals of peripheral nerve conduction, the study of event-related brain potentials, timed neuropsychological tests to assess performance at a behavioral level, and specific neuroimaging techniques to study the underlying neuroanatomy.

The study of nerve conduction velocity was initiated early in neuroscience. More recently, electromyography and single-cell recordings from peripheral nerves have established a speed conduction that varies in a range between 0.5 and 90 m s^{-1} , as a function of certain physical and chemical conditions of the axon. In addition, ERPs have been used to measure the time course of sensory, motor, and cognitive brain responses, similar to the latency or the amplitude of particular components of the ERPs.

At the behavioral level, the introduction of mental chronometry allowed to measure cognitive speed using the subtraction method. This method implies the subtraction of RTs obtained in a simple task from those obtained in a similar task with an added cognitive element. Thus,

time differences between conditions reflect the additional resources consumed by the added cognitive element.

Standardized neuropsychological tests provide measures of mental slowness at a behavioral level, of which SDMT and symbol search (both configuring the speed of processing index in Wechsler Adult Intelligence Scale-III) are good examples. Whereas almost any test would serve as long as the execution time is monitored, the use of computerized methods would enhance the accuracy of time measures.

Lastly, recent advances in neuroimaging have provided new techniques to examine the neural correlates of SIP. High-spatial-resolution techniques, such as diffusion tensor imaging (DTI), are allowing for the first time to study *in vivo* the integrity of axons and validate some of the scenarios under discussion.

Related Anatomy

SIP has been related to white matter more than gray matter structures. Neuroimaging and behavioral studies have suggested a relationship between SIP and structural aspects of neural wiring, such as nerve diameter, integrity of myelin sheathing, the number of ion channels, and the efficiency of synaptic neurotransmission (**Figure 5**). Differences in speed of processing are thought to depend critically on nerve conduction velocity determined by axonal diameter and degree of myelination. As volume of brain white matter increases during development, so does the SIP.

Although white matter seems to be a key structural factor determining SIP, different cortical regions may be related to variations in speed of response. For instance, RTs usually increase after lesions in supplementary motor areas necessary to organize complex responses and activate unpracticed motor patterns. Longer RTs are also found after right frontal lobe lesions related to deficits in monitoring abilities necessary to prepare the processing of forthcoming events.

Clinical Impairment

The term ‘bradyphrenia’ defines a deficit in SIP. Particularly, patients cannot process information as quickly as healthy individuals. It is thought to be one of the most pronounced, striking, pervasive, and reliable phenomena of brain damage. Contrary to the view that suggests that bradyphrenia is a deficit of a specific information processing stage, it seems to be a general and global phenomenon.

Mental slowness seems to be a primary cognitive deficit in aging, multiple sclerosis, Parkinson’s disease, and traumatic brain-injury patients, but it is also evident in other central nervous system diseases, such as stroke.

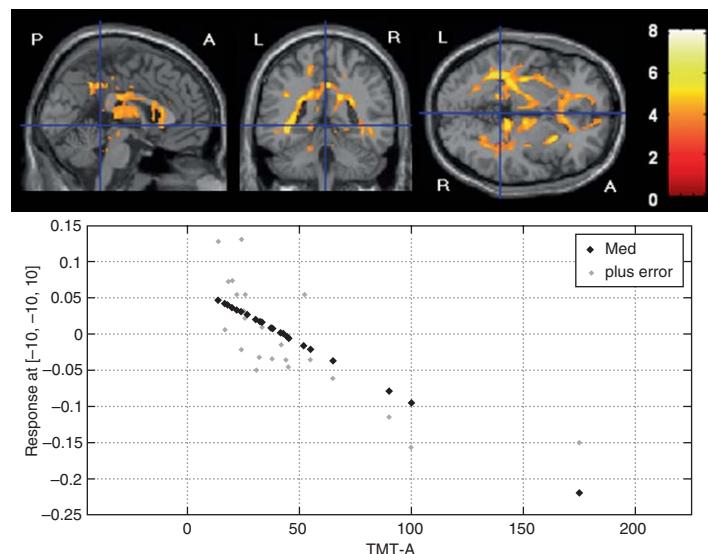


Figure 5 Correlation between trail making test part A completion time in seconds (TMT-A) and mean fractional anisotropy (FA) for main white matter structures. Line of best fit is presented for significant correlations.

Deficits in SIP impact various other cognitive abilities. As stated before, cognitive slowness may cause other attention deficits in a variety of tasks. Thus, the dissociation between attention and SIP is critical in order to detect the underlying deficit and to establish an adequate rehabilitation program for each patient.

Conclusion

Attention has been considered a complex and nonunitary process. Behavioral, functional neuroimaging, electrical scalp recording, as well as developmental and genetic studies in animals and humans provide converging evidences for the existence of at least three independent attentional brain networks. As depicted in Figure 1, alerting would relate to the activity of noradrenergic connections from the locus ceruleus to different subcortical areas, constituting one of the more basic attentional mechanisms. Through these projections, the alerting system might potentiate (generate and maintain an activation state) the efficiency of the other attentional networks. On the other hand, the orienting network relates to the ability to select (amplifying and inhibiting) specific information from both external (e.g., the environment) and internal sources (e.g., memories or thoughts). The efficiency of this network seems to rely on the integrity of a dorsal parietal–frontal system responsible of the neural representation of the attended features (e.g., spatial locations, perceptual attributes). Executive attention relates to a variety of subcomponent operations responsible for the top-down (intentional) guidance of behavior. Neural structures such as ACC, dorsolateral

PFC, or the inferior frontal cortex would interact to each other to modulate the neural activation of subordinate brain systems. SIP is not an attentional mechanism *per se*. It is a property of the system where attention is implemented and could modulate the efficiency of the attentional networks. White matter integrity seems to be a key factor for an adequate SIP. In spite of the relative independence among these networks, their interaction is continuously required for an adaptive behavior. This complex interaction can be hindered by different pathological states such as acquired brain injury, degenerative diseases, and developmental changes (in the child and in the aging process). As a result of alterations in these components, dissociations and different profiles of impairment can be observed in the clinical context.

See also: Aging and Cognition; Animal Models of Learning and Memory; Brain Imaging; Cognition: Attention and Impulsivity; Cognitive Control in the Service of Self-Regulation; From Sensation to Perception; Neural Basis of Attention-Deficit/Hyperactivity Disorder; Neural Basis of Working Memory; Short-Term Memory: Psychological and Neural Aspects; Voluntary Movement: Control, Learning and Memory.

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From Sensation to Perception

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Glossary

Apparent motion – The perceptual experience of continuous movement generated by the sequential presentation of two still images flashed in positions which are spatially offset in the visual field. This phenomenon was first studied by Max Wertheimer, the founder of the Gestalt School of Psychology, in his 1912 work: *Experimental Studies on the Seeing of Motion*.

Hallucinations and entoptic vision – Hallucination refers to a false sensory perception in the absence of an actual external stimulus. Various mental episodes can induce the vivid report by the conscious subject of activity patterns witnessing the inner architecture of specific regions of the brain, such as visual cortex. For instance, inner visions can be experienced following retrobulbar blockade of the optic nerves, during acute episodes of aura migraines (fortifications) or after taking hallucinogens such as cannabis or mescaline. Clinical analysis of these visual disturbances describes only four constant geometrical patterns, consistently reported by human subjects: (1) tunnels and funnels, (2) spirals, (3) lattices (honeycombs and triangles), and (4) cobwebs. As proposed by Christopher W. Tyler, these entoptic (vision-from-inside) geometric patterns must be regarded as indicators of some further selective process in the thalamo-cortical pathway, “a kind of functional Golgi stain by which certain neural activities are elevated into consciousness while the majority of possible discharges remain ignored.”

Mach bands – Mach bands are an optical illusion, named after the physicist and philosopher Ernst Mach. The stimulus is composed of two sectors of uniform luminance, one high (light) and one low (dark), separated by an intermediate zone with a monotonic gradient of luminance which decreases linearly when progressing from the light to the dark sector borders. Humans perceive two narrow bands of different brightness that are not present in the physical stimulus. They are situated on both sides of the gradient zone, and are perceived as respectively brighter than the light sector and darker than the lower luminance region.

Minimal discharge field – The minimal discharge field is the part of the receptive field within which the presence of an impulse-like stimulus increases the probability of firing or the number of spikes emitted by a given neuron. This definition excludes the possible recruitment of summation and interaction processes occurring across

stimuli in space and time. The surrounding zone, from which only subthreshold activity is generated (i.e., modulation of the membrane potential or changes in membrane conductance without spike activity), defines the ‘silent periphery’ or ‘surround’ of the receptive field.

Receptive field – The receptive field of a neuron is defined by the region of the sensory space within which the presence of a stimulus significantly modifies its activity. In the visual system, the receptive field is the region of the retina where a local change of luminance (relative to the background) modulates (enhances or suppresses) the firing rate or the probability of discharge of the recorded neuron. In the auditory system, receptive fields can be locations or volumes in space, and are also defined as compact regions in the temporal frequency domain. In the somatosensory system, receptive fields are regions of the body surface or internal organs. A special case of tactile receptive fields are those corresponding to the whiskers, specialized hairs which form a discretized mosaic of haptic sensors located on each side of the snout in mammals.

Sensory coding – Information in the brain, either evoked by sensory input or autonomously recalled in the form of memories, is represented by changes in neural activity, both excitatory and suppressive (although this latter case is less studied). These changes are expressed in the modulation of the firing rate of single neurons (the neuronal doctrine), which, in certain cases, becomes tuned to specific features of the sensory environment (e.g., feature detectors) or in the spatiotemporal pattern of spiking of activity distributed across neuronal populations (assembly coding). Sparse coding refers to the special case where stimulus information is encoded by the selective recruitment of a few cells active at irregular points in time. Information theory shows that sparse coding results in an independent component analysis of the sensory scene: it reduces redundancy and maximizes mutual information between the few active units.

Introduction

When facing a natural visual scene, human subjects have an immediate conscious perception of the elementary features that compose it (segmentation), as well as of the

higher-order global objects that emerge from their associations (binding), although not necessarily in this order. Contours, colors, textured surfaces, shapes, and three-dimensional objects pop out unambiguously, in a fraction of a second to seconds, according to the background context. For centuries, multiple theories have been proposed to explain the remarkable perceptual capacities of animals and humans in figure-ground segregation and identification of their immediate environment. The structuralist theory of Edward Titchener, dating back to the very beginning of the twentieth century, asserted that sensations were the basic elements of perception. In this context, sensations were considered as the simplest elementary building blocks open to introspection, on the basis of which complex perceptions could be synthesized. In this article, the concept of sensation has a different meaning than that used by the structuralists. Here we take into account the fact that the central nervous system is immersed in its environment with which it communicates through sensory channels and modalities and on which it exerts actions through specialized effectors. Closer to the enaction theory and to the spike-based computational approaches, we consider sensations as the result of the initial processing step corresponding to the transduction of the sensory input detected by the peripheral sensory organs (eye, ear, skin, etc.) into a spatiotemporally formatted spike-based input stream.

Through the example of a few sensory illusions, this article deals with the long-standing question of the link between the stimulus quantified in physical terms and the integrative process realized by the brain before any action in return takes place. Two sequential steps are classically

distinguished during low-level perception, the sequence of physiological events in central neural structures, which gives rise to the emergence of a conscious account of the percept at the psychological/behavioral level. However, animals and humans cannot be reduced to the status of passive receivers facing an external physical reality; thus, other components have to be included, such as motivation, attention, expectation, action, decision, and memory. These internally generated modulations activate top-down processes which construct unconscious hypotheses about the outer world.

We have restrained the scope of this article to neuronal correlates of perception that have been localized in the primary sensory cortices of the mammalian brain: these central networks appear as a major crossroads where feed-forward, and recurrent and feedback processing, merge to form a contextualized perception of peripersonal space. The case studies that we have chosen to illustrate offer examples of situated perception that can be related in neural terms to the Gestalt theory. They support two views which are not mutually exclusive: when driven by external sensations and bottom-up activation, the cortical neuronal machinery generates an automatic interpretation of our environment through built-in compositionality mechanisms; when driven by top-down feedback, the same network computes unconscious inferences from sensory events based on predictive knowledge derived from the past. Consequently, the primal sketch of our sensory periphery is continuously updated and modulated by the feedback or proactive context of our thoughts, intentions, and actions (**Figure 1c**).

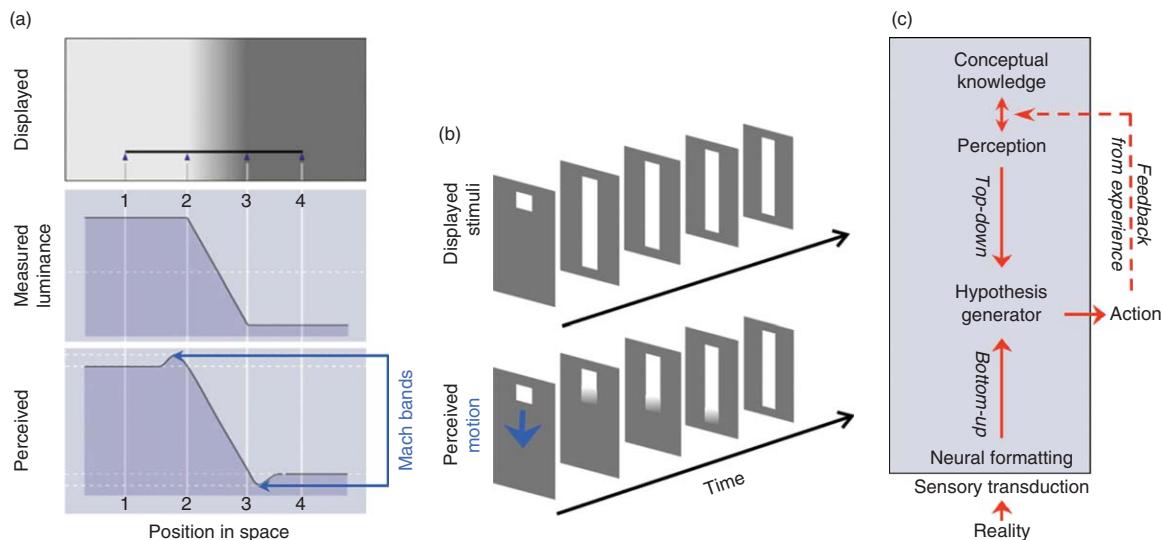


Figure 1 Sensation, perception and illusion. (a) Mach bands: from top to bottom, displayed luminance profile, measured luminance gradient in positions 1–4, perceived luminance profile. The Mach illusion (blue arrows) corresponds to the perceived lighter (position 2) and darker (position 3) bands. (b) Line-motion illusion: top, spatio-temporal sequence of displayed stimuli (square followed by a bar); bottom, perceived downwards motion (blue arrow). (c) A conceptual framework (inspired from Richard Gregory).

For more than half a century, beginning with the pioneering research on the visual system led by David Hubel and Torsten Wiesel, hundreds of laboratories throughout the world have tried to identify the neuronal mechanisms that would explain the remarkable sensory recognition performances of the mammalian brain, never equalled by any artificial device. This research initially targeted cortical primary visual areas, since they form the earliest stage in the functional hierarchy of the visual system where the emergence of high-order feature encoding (orientation, curvature, etc.) and elementary mechanisms for computing geometrical invariants were demonstrated. However, what applies to vision generally holds for the other senses too. All sensory cortical areas are considered as the seat of memory of our early sensory experience and their functional specialization has been shown to mirror the multimodal perceptual development of the organism, at least during critical periods of postnatal life.

In the case of the visual system, the bi-dimensional distribution of luminance and contrast in the retina is captured by specialized transducer cells, the photoreceptors, where light energy is translated into neural electrical activity. This electrical message is then transferred from retina to subcortical centers, including the thalamus. The ascending pathway associated with conscious perception is the retino-thalamo-cortical pathway and most of the processing in terms of segmentation and binding is thought to occur at the cortical stage. Three remarkable features of the early-perceptual pathway are: (1) the preservation of a topographic representation of space at all intermediate stages of the hierarchy, which provides some form of isomorphism between the sensory image and the feed-forward imprint formed at the next step of integration; (2) the existence of multiple loops (feedback from cortex to thalamus, feedback from higher cortical areas to primary sensory cortices) that coexist with the powerful feed-forward transmission; and (3) an overwhelming dominance (in terms of relative number of synapses per neurons) of connections (recurrent and lateral) intrinsic to each of the cortical areas, relative to the feed-forward contingent. Thus, as soon as a few tens of milliseconds after a stimulus impinges on the retina (bottom-up processing), the change in neuronal activity, departing from the level of irregular and sparse ongoing activity level, propagates through the nested thalamo-cortical architecture.

These complex activation dynamics are observed not only for exteroceptive sensations arising from the periphery, but also during interoceptive reactivation: for example, the recall of a visual memory (top-down processing) or an abnormal imbalance between cortical excitation and inhibition. In this latter case, remarkable illusions, which do not involve attention-related processes, are revealed during migraines and can be

amplified by hallucinogenic drugs. It is thought that the paroxysmal activity state of the cortex shuts off the processing of retinal activity and is interpreted by the human observer as an illusory constellation of geometrical patterns. The topological structure of these highly specific planforms is closely related to the functional architecture of the cortical analyser. Thus, central neural structures, such as the visual cortex, do more than mirroring visual experience. Entoptic hallucinations provide a vivid proof to the observer/patient that his own cortical activity generates a geometrical construct in the absence of sensory input. This construct is interpreted by the mind as a projection of an inner state expressed in retinal-encoded coordinates.

Feed-Forward Imprint of the Sensory Periphery

The predominant view of sensory processing posits that the filtering properties of the neurons that operate at the different successive steps of the functional integration ladder, from periphery to central cortical areas, become progressively more and more elaborate. This dogma is best illustrated by the hierarchical simple-complex-hypercomplex model of David Hubel and Torsten Wiesel and the grandmother concept of Horace Barlow. The critical attributes of a visual scene that trigger electrical activity of neurons in retina, thalamus, primary cortex, and higher-order cortices indeed show increasing complexity.

While retinal ganglion cells are specialized contrast detectors with a spatially isotropic, opponent (ON vs. OFF) concentric organization, simple neurons in the primary cortex show elongated receptive fields with spatially segregated ON and OFF subzones, indicative of the breakdown of circular symmetry. Consequently, their firing frequency is tuned for specific features, such as orientation and movement direction. Higher-order visual cortical areas of the dorsal pathway, such as the medio-temporal area, extract information about the motion flow by integrating information over larger areas of the visual space. Even more illustrative of this complexification is the finding of neurons in the inferior-temporal lobe of the ventral pathway that fire action potentials selectively to images of faces, animals, or other complex objects. The role of sensory experience in shaping perception is such that there exist neurons in the human medial temporal lobe that are activated selectively when reacting to the picture of famous personalities or even to the sight of their written names. This recognition performance is learned from the social environment of the human observer through different communication channels (radio, TV, movies, speech, etc.) and is dependent on various reward processes. It suggests an explicit

code, at least in superior areas of the ventral visual pathway, which might be linked to an abstract representation of iconic memories.

The ultrafast dynamics observed during pop-out recognition raises a paradoxical challenge in terms of correlation between neural structure and perceptual function. At the behavioral level in humans and monkeys, during a recognition task requiring the discrimination of animal versus nonanimals, the response onset delay measured by a forced choice saccadic paradigm can be as much as 100 ms (although it is not proven that the motor decision is based on the conscious identification and naming of the animal targeted by the saccade). Taking into account the number of integrative steps through the retino-thalamo-cortical pathway needed to reach prefrontal areas, it has been argued that most of the information processing relies on the first few evoked spikes propagating through a specialized fast pathway. Nonetheless, as we shall see in the next section, it is hard to reconcile an exclusively feed-forward processing by the thalamo-cortical pathway with our knowledge of the anatomical connectivity of sensory cortex and, in particular, the very great number of feedback synaptic contacts.

Beyond the Receptive Field of Sensory Neurons: Context Matters

In the primary visual cortex, the integrative properties of visual neurons result from the relative contributions of three sources of afferent connectivity: (1) feed-forward excitation from the lateral geniculate nucleus, the principal thalamic relay between the retina and the visual cortex; (2) feed-forward and local feedback inhibition from intracortical interneurons; and (3) recurrent excitation from other cortical neurons, both within (lateral or horizontal) and outside primary visual cortex, which provides the largest number of synaptic contacts. Geniculate feed-forward connections provide visuotopic information to the primary visual cortex that is spatially coextensive with the cortical neuron's minimal discharge field. On average, the extent of this receptive area is relatively small for neurons in the primary visual cortex, spanning only 1–4° of visual angle in the cat and 10 times less in the monkey for central and parafoveal vision. One might then suppose that primary visual cortex neurons only integrate luminance information locally. However, it has been well documented that visual cortical neurons exhibit modulation of responses from far beyond the spatial boundaries of the minimal discharge field. Quantitative analyses of anatomical data in the primary visual cortex of the cat show that axons from the principal neurons of the lateral geniculate nucleus account for no more than 5% of the total

excitatory synapses onto pyramidal neurons of layer IV, the thalamo-recipient layer of the cortex. Consequently, the large majority of excitatory synapses originate from other cortical neurons within the visual cortex and from other cortical areas that project back to the primary visual cortex. It seems unlikely then that the geniculate nucleus alone could fully control the activity of a given cortical neuron, and this seems particularly true for cortical neurons in supra- and infra-granular layers (above and below layer IV). Thus, visual cortex neurons are connected to a widespread network of horizontal and feedback connections that give access to spatially and temporally distributed information over large parts of the visual field.

There is accumulating evidence concerning the functional consequences of this distributed and recurrent connectivity scheme. For example, stimuli outside the minimal discharge field can have potent and complex modulatory influences when presented simultaneously with stimuli inside the receptive field. Neuronal responses in the primary visual areas of the alert macaque monkey to a single oriented line presented in the center of the neuron's classical receptive field are strongly modulated by the presence of textured patterns of oriented lines presented in the surround of the receptive field. This has been reported most often when the orientation of the surround elements matches that of the center element but has also been observed, although more rarely, when it does not. The first mechanism is a form of response suppression resulting from the collinearity of the stimuli, whereas the latter one appears to be triggered by the orientation contrast between center and surround.

These observations, documented at the cellular level, have their counterparts in psychophysical studies where the perception of an object's attributes depends on the spatial context in which a stimulus is presented. For instance, the finding that primary cortical neurons respond to orientation contrast supports a possible role of primary visual cortex in the mediation of perceptual segregation of texture borders and in perceptual pop-out. Low-level mechanisms can also be implicated in the perception of the apparent lightness of a surface, which is known to be strongly modulated by the spatial context in which it is embedded.

Sensory Illusions as the Perceptual Expression of Stimulus Inference by the Brain

Tout ce que j'ai reçu jusqu'à présent pour le plus vrai et assuré, je l'ai appris des sens, ou par les sens : or j'ai quelques fois éprouvé que ces sens étaient trompeurs, et il est de la prudence de ne se fier jamais entièrement à ceux qui nous ont une fois trompés.

Everything that I have learned so far, to be the most true and reliable, I have learned through my senses: and yet, I have sometimes felt that these senses were misleading, and that it is prudent to never rely entirely on that which has once mislead us. (*Méditations Métaphysiques*, Première méditation, R. Descartes, 1647.)

Hermann von Helmholtz described visual perceptions as unconscious inferences from sensory data and knowledge derived from the past. According to this view, perceptions are regarded as predictive hypotheses made by our brain, which are projected by the mind into the external physical space and accepted as our most immediate reality. The reflexive neural processes to which they correspond find remarkable counterparts in the low-level center-surround interactions that have been described by electrophysiologists in the primary visual cortex, as well as in the contextual perceptual effects described by experimental psychologists. Some of the best-known illusions, such as Mach bands (Figure 1a) and apparent motion, open explanatory windows on the inner functioning of the brain, since they result in an apparent contradiction between the physical nature of the stimulus present at the periphery and that reported consciously by the subject (here, luminance bands and continuous motion). The Mach band was a key to the elucidation of center-surround antagonism in retinal and higher-order sensory receptive fields. Its signature, in terms of neural correlates, is that of a Mexican hat where focal excitation is surrounded (or opposed) by widespread inhibition. Another example is apparent motion, where the percept of continuous motion emerges from sequential static activations. Max Wertheimer, who was one of the founders of the Gestalt school of psychology, proposed a seminal theory of perceptual grouping in his *Experimental Studies on the Seeing of Motion* (1912). This theory predicts the emergence of coherent percepts of global shape and motion from the temporal staggering of static presentations of elementary spatial features. It assumes the existence of mental processes which favor associations of visual elements in space (according to spatial proximity and similarity in contrast polarity) as well as in time (continuity and common fate). Originally called the beta phenomenon by Wertheimer, the apparent-motion illusion is a powerful dynamic effect induced when a visual target is flashed successively, immobile, in different positions in the visual field ordered along a virtual trajectory. Although, at each moment in time, the image that is presented to the observer is stationary, the subject reports the perception of the continuous motion of the object along the trajectory defined by the association path, which links the various positions explored in succession. The strength of the percept depends on the complexity of the test stimulus, including its shape and texture, on the duration of the static presentations, on the inter-stimulus

interval, and on the spatial offset between the explored positions.

A series of experimental observations makes it likely that these psychophysical effects are the result of activity waves propagating laterally across the layer plane (which is the projection plane of the periphery) within the primary visual cortex. At the single-unit level, the principal orientation axes of the receptive field of neurons that communicate through long-range horizontal connections are often co-aligned. Horizontal cortical connections are thought to facilitate the response of cells with collinear orientation preference and to reduce their response otherwise. At a more integrated level, recent local field potential studies show the spontaneous propagation of horizontal travelling waves that most often link cortical loci sharing the same orientation preference. Similarly, at the psychophysical level, numerous studies have described facilitatory or suppressive changes in the ability to detect a central target when adding a contextual periphery. They showed, in particular, that low-contrast visual contour elements are easier to detect when presented in the context of collinear flankers, confirming that lateral connectivity in visual cortex may participate in establishing such facilitation. Interestingly, the subthreshold synaptic integration field, recorded intracellularly at the cortical level, is within the same range as the perceptual association field reported by psychophysicists, and thus it is most likely that it provides a cellular substrate for these perceptual effects.

Case Studies for Visual and Haptic Perception: Apparent Motion and Funnelling

While the spatial contextual effect described above can be easily interpreted in the framework of the association field, the temporal determinants of this collinear facilitation have been less well explored. The perception of speed can be differentially modulated during apparent motion sequences of oriented stimuli by adjusting the collinearity and alignment of the local orientation with respect to the motion axis. When observers are asked to discriminate during a forced choice task between the relative speeds of two apparent-motion sequences composed of three identical Gabor patches (an oriented sinusoidal luminance grating whose modulation is weighted by a bi-dimensional Gaussian function) with positional offset, whose orientation is either collinear to the apparent motion axis or cross-oriented to it, a speed-up illusion is observed. For the same physical speed of the two apparent-motion sequences, a Gabor patch moving along its orientation axis appears much faster than a Gabor patch oriented at an angle away from the motion axis.

This psychophysical effect may be quantified by the ratio between the speed of the test sequence and that of the reference sequence for which the subject reports equality in perceived speed. The perceptual bias is as strong as threefold in humans, and its strength explains why observers find in the great majority of the cases that collinear sequences are faster than noncollinear ones even if the two composite stimuli have the same physical speed. The hypothesis that the speeding up is induced when the horizontal wave travels ahead or in phase with the feed-forward inputs from the thalamic stage is supported both by computational models and by intracellular observations made in cat primary visual cortex for the same stimulus configurations. These different data strongly support the hypothesis of a dynamic neural association field: oriented contours should propagate facilitation across space (collinearity) as well as across time (common fate). In this latter case, synergy should be observed when the feed-forward flow travels in phase with the lateral intracortical activity wave evoked by the apparent motion sequence.

The line-motion effect is another illusion from the same family, induced by asynchronous static presentations (**Figure 1b**). In this latter case, the cue feature is a uniform luminance square, followed by a bar of the same luminance, one end of which encroaches on the previously flashed square. For adequate inter-stimulus intervals and presentation durations, the human subject reports a continuous movement of one border, perceived as the smooth morphing of the square into the elongated bar. The line-motion illusion has been studied using voltage-sensitive-dye imaging of activity propagation, in a secondary visual cortical area of the anesthetized cat. The aim was to obtain a direct visualization of the spatial spread of the facilitation induced by the cue stimulus. For this purpose, the responses to a flashed small square and a long bar alone were compared with a stimulus configuration producing the line-motion effect, that is, a square briefly preceding the bar. In the associative condition, a spreading, low-amplitude activation wave was induced, extending far beyond the retinotopic representation of the initial cue. The observed propagation speed was consistent with the conduction velocity of horizontal axons reported in electrophysiological experiments or inferred with voltage-sensitive-dye techniques. Furthermore, this work demonstrated that the cue square, even though physically immobile, induced a propagating wave of cortical depolarization, indistinguishable from the spatiotemporal pattern produced by continuous motion of the same square. Although it is likely that similar low-level mechanisms may underlie both the speeding-up and line motion effects, it should be noted that the temporal parameters that maximize the line-motion effect (a delay of 100–200 ms between the presentation of the inducing spot and the flashed bar) are longer than the

fast and brief (<100 ms) motion sequences used for the speeding-up effect.

Context-dependent modulation of cortical activity has been observed in other sensory modalities as well. These modulations and the presence of neuronal signals correlated with the direction of apparent tactile motion have been explored in the whisker-to-barrel cortex system of the rat. Rodents localize objects and discriminate textures by scanning their surface with their facial vibrissae. The exploratory movements of the vibrissae generate spatio-temporally complex sequences of tactile contacts. The sensory whiskers in the mystacial pad of the rat are mapped onto the thalamo-recipient layer of primary somatosensory cortex as distinct units, named barrels. The detailed description of the organization of the cortical barrel field into discrete architectonic modules has triggered a large number of functional studies in recent decades. In particular, numerous anatomical and extracellular electrophysiological studies have demonstrated a one-to-one correspondence between a mystacial vibrissa and its matching cortical barrel, leading to the concept of a structural as well as functional imprint of the sensory periphery in the cortical representation. Recent observations, using whole cell and intracellular recordings of synaptic responses evoked by individual whisker deflections, have challenged the original notion of a segregated mosaic cortex in the primary somatosensory cortex of adult rats. The cortical spread function, which measures the pattern of divergence of information, is in fact extensive, encompassing several barrels. The spatial extent of subthreshold receptive fields in the vibrissal cortical representation is similar to that we have previously described in the visual cortex: it suggests that the rat barrel cortex has a wide array of cortico-cortical horizontal connections that, together with the multi-whisker thalamic input, provide a potential substrate for complex nonlinear temporal and spatial interactions. Consequently, context-dependent modulations of responses through the cortico-cortical network might have profound effects on neuronal receptive fields.

Recently, the dynamics of spatiotemporal integration in the somatosensory cortex (S1) were reevaluated by using experimental stimuli similar to those encountered naturally during tactile (haptic) exploration. This study took advantage of a newly developed stimulation device that, in the rat, allows the controlled application of large-scale spatiotemporal patterns of stimulation, by the parallel and independent activation of the almost-complete mystacial pad (stimulation matrix of 25 macrovibrissae). It compared two modes of stimulation: in the first, the vibrissal system was probed with random sequences of independent single-whisker deflections in one direction in the second, the stimulation of several whiskers was coordinated so as to generate an apparent global motion in a given direction. The electrophysiological recordings in S1 cortical neurons showed that the

second stimulus (global motion) drove the neurons with a direction preference that could not be predicted from the responses generated from single whiskers when moved independently. These results suggest that individual neurons have the capacity to integrate and extract collective information from the entire whisker pad. As in the visual cortex, we conclude that tactile perception emerges from collective invariants or global properties of the full field input as well as from local independent features.

Another famous illusion, still related to lateral intracortical interaction, but expressed with a sensory modality other than vision, is the tactile funnelling effect. This is characterized by the percept of spatial mislocalization and increased tactile intensity at a central skin location that is not directly stimulated. When stimulating the skin at three co-aligned points, inputs at lateral sites are funnelled centrally so that the perceived intensity at the central site is greater than that perceived when stimulated alone. With two-point stimulation, a funnelled sensation is produced and extends to an unstimulated part of the skin. This illusion has been reported on the forearm, palm, and fingers. A recent functional correlate has been reported in area 3b of the primary somatosensory cortex (S1) of monkeys, where optical imaging showed that simultaneous stimulation of two fingertips produces a single focal cortical activation between the single fingertip activation regions. Thus, in contrast to the traditional view of an isomorphism between the body and its cortical imprint in S1, the topography of the functionally evoked map reflects the perceived rather than the physical location of the peripheral stimulation. Such contextual influences from beyond the classical receptive field are likely to be determined by mechanisms dependent on intracortical distance, center and surround interactions, and cortical feedback as described earlier in the case of visual cortex.

Adaptation of Cortical Sensory Processing to the Statistical Structure of Our Natural Environment

As beautifully expressed by William James, the founder of the American School of Experimental Psychology, one hallmark feature of perception is the “constant fit between the mind and the world” in which we dwell. Continuous immersion in a natural environment irregularly updated by motor exploration causes cortical neurons of different modalities to adapt their function to process specific spatiotemporal power spectrum statistics ($1/f^\alpha$). Neural representations of our peri-personal sensory environment in the cortex are the result of an ever-active optimization of the fit between brain representations and the physical features of the environment that are experienced during

epigenetic development. For instance, during natural vision, informative sensory input is generally gained from the whole visual field and there is now ample evidence that the activity patterns of neurons in the primary visual cortex evoked by full-field exposure to natural scenes differ radically from the activity elicited by simpler and local stimuli. In particular, the most evident qualitative change in response to natural stimuli is the induction of a sparser regime of activity spatially distributed across the cortical network.

Intracellular recordings and voltage-sensitive-dye imaging in the mammalian primary visual cortex show that stimulus-locked variability and network correlations (a measure of information redundancy) both decrease with stimulus complexity, whereas the temporal precision and sparseness of the neural code increase. While drifting gratings evoke highly variable and dense visual responses, stimuli with richer spatiotemporal structures force the cortical network dynamics to become more reproducible at the subthreshold-membrane-potential level and sparser and more reliable at the spiking level. Thus, the precision of the neural code changes with the complexity of the visual input statistics and their closeness to those of natural scenes. These findings contradict the frequently asserted view that the neural code used to represent sensory information in mammalian visual cortex is noisy and redundant. This apparent low efficiency could be due partly to the choice of visual stimuli used in most previous studies: luminance spots, bars, and gratings are typically of low dimension when compared with the high dimensionality of the natural sensory environment and of limited neuroethological value.

The sparsening of activity produced by natural scenes results from the recruitment of dynamic nonlinearities linked mostly to center-surround interactions. A dramatic contextual reformatting of sensory cortical representations has been reported in awake behaving macaques, when extending the presentation of natural-like scenes beyond the classical discharge field. The concomitant stimulation of the silent surround results in a selective increase in mutual information and spike-based efficiency.

Experimental evidence for sparse coding of natural stimuli has been found for other sensory modalities as well, as demonstrated in the auditory cortex. Here again, stimuli that have high probabilities of being encountered in the natural environment of the animal are optimally encoded by sparse activity. Evidence is much scarcer in the somatosensory modality, although neural activity in the upper layers of the barrel cortex is particularly low, consistent with the requirement of a sparse regime, where each neuron should be active only rarely. Furthermore, intracortical electrical micro-stimulation experiments, where only one or a very few neurons are selectively recruited, have been reported to bias the perceptual judgement of the observer during a behavioral recognition task

in a predictable way. This last set of data strongly suggests that each spike seems to count in the sparse mode regime. Consequently, when our sensory cortices operate in an activation state adapted to the most likely input statistics, any discrete activity change can dramatically influence our perception. This holds, whether the change is produced externally by a sudden discontinuity in the sensory input (that cannot be predicted by our own motor activity) or internally by top-down processes.

Top-Down Processing and Cortical Correlates of Expectancy

In previous instances, we have observed that evoked responses in primary sensory cortices can be modulated by concurrent activation of surround regions, beyond the limits of the classical receptive field. We also presented evidence indicative of strong correlates between cortical center–surround neural nonlinearities and biases or illusions in perceptual judgment. While these contextual effects originate partly from the underlying horizontal connectivity intrinsic to the primary sensory cortices, they also depend on the feedback connectivity of higher cortical areas onto the primary sensory cortex. Top-down influences in sensory processing take many different forms, for instance, cross-modal interactions produced in synesthesia, attention-related modulation, and belief propagation or expectancy. In the latter case, templates of sensory stimuli that occur in a highly reproducible way are continuously updated and constantly compared with incoming sensory information. Detection errors in assessing the presence of a sensory stimulus or incorrect identification might arise from the match or mismatch between bottom-up information from the sensory periphery and the top-down feedback arising from stored internal representations and working memory.

Expectancy can be viewed as a dynamic process which prepares the organism to react in an adapted way to sensory inputs which have been primed by the immediate history of the brain. In music cognition, melodic expectation is the tendency for the listener to predict what might come next in the stimulus train, a continuity-in-time feature predicted by the Gestalt theory. Expectancy allows the system to respond – or not – in due time by taking into account the recent presentations of a similar sensory context. It has been studied within two major frameworks. The first, linked to probability theory, posits that in uncertain situations (close to the absolute threshold), responses to highly probable elements are faster and/or more precise than those to rare ones. The second one is more concerned with the impact of the local sensory context and is less directly connected to long-term probabilistic assessments of events. Different dimensions of the stimulus pattern, for example, temporal, spatial, and

multimodal, can be critical in anticipating the timing as well as the identity of future sensory events.

Expectancy-related physiological signals have been observed in the case of the sudden omission (hence, unexpected absence) of a stimulus during a repetitive sequence of stimulation. This omitted stimulus induces characteristic signatures in human cortical evoked potentials in the visual and auditory modalities. Such a detection of a change within a sequence of temporally discrete events requires computing, representing, and retaining for some interval of time the temporal interval between the events, together with the event itself. Following this line of argument, the detection of a change or of a violation of expectancy occurs when there is a mismatch between the representation of the current event and the stored representation of the expected event. As illustrated in the case of the cortical mismatch negativity, the characteristic evoked potential associated with such a violation essentially reflects a pre-attentive, preconscious task-independent process that does not require directed attention.

The cortical imprint evoked by new stimuli interacts with spontaneous or attention-gated recall of internal templates stored for the most frequent sensory events. The neural processes through which these bottom-up and top-down representations interact might be supposed to have a distinctive electrophysiological signature. For example, a neural response to the expected event should be available to recording during the omission of a stimulus. Such expectancy processes have been studied in the rat vibrissal system. The array of specialized vibrissae is the input stage of an extremely sensitive tactile system, comparable in resolution to the human fingers. Moreover, the natural stimuli in this system are strongly conditioned by the arrangement of whiskers on the mystacial pad that follows a precise geometrical pattern in rostro-caudal rows and dorso-ventral arcs. During the whiskers' exploratory behavior, rats move their vibrissae rostro-caudally, creating a *de facto* functional asymmetry between rows and arcs: whiskers in the same row will tend to contact an object successively, whereas whiskers in the same arc either will contact the object nearly simultaneously, or will not contact the object at all. Therefore, the natural whisking movement generates a repetitive pattern of rostro-caudal stimulation that might generate an expectancy wave of activity in the cortex. By defining stimulus conditions as being either predictable or unpredictable, and by recording different neuronal responses across the two types of stimuli, one can infer whether expectancies of the organism account for the observed differential responses. A clear expectancy response can be detected in humans by measuring auditory evoked potentials during repetitive stimulation with a low rate of omission (around 10%). In the somatosensory barrel field, omitted stimuli

protocols reveal neural correlates which can be recorded even in anesthetized animals, consistent with the pre-attentive nature of the mismatch negativity reported in the auditory cortex. In conclusion, these results are in agreement with the idea that the somatosensory cortex is generating hypotheses about stimulus characteristics on the basis of stored representations which are compared with the ever-updated information stream coming from the sensory periphery.

Other forms of expectancy are used by the brain to filter out the sensory perturbation produced by the self-generated motor activity which serves to gather relevant sensory information. The best-known examples have been described in the visuo-oculomotor system in mammals and in the electrosensory system in the electric fish. Both provide examples, at the functional and structural level, of the existence within the brain of internal copies (outflow and efferent copy) of motor signals (saccades or electric discharge). These contextual signals prepare the sensorium to ignore the expected recalibration of the sensory space produced by the sudden shift in gaze fixation or the generated electric field.

Conclusion

From this overview, we conclude that low-level perception can be defined as a cortical-based computation, constantly building unconscious or self-generated inferences during the processing of sensory events. The outcome of the perceptual process is highly conditional on the context of predictive knowledge derived from the past sensorimotor experience. In many cases, perception departs from the physical reality, but this is what ultimately feeds and guides our interactions with the world.

Acknowledgments

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See also: Attention and Speed of Information Processing; Brain Imaging; Conscious and the Unconscious; Disorders of Face Processing; Hallucinations in Neuropsychiatry and Drug Abuse: From Phenomenology to Pathophysiology; Mirror Neuron Mechanism; Novelty; Peripersonal Space and Body Schema; Role of Neuronal Synchrony in Normal and Pathological Brain Functions; Vision.

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Peripersonal Space and Body Schema

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Glossary

Bimodal neurons – A particular class of neurons that presents receptive fields for stimulations coming from different sensory modalities. For instance, a visuotactile neuron is driven by a tactile stimulus on the tactile receptive field as well as by a visual stimulus presented in the visual receptive field close to the tactile one.

Deafferentation – The elimination or interruption of sensory nerve impulses by destruction of or injury to the sensory nerve fibers. The level at which the disruption happens defines the type of deafferentation: central if the lesion involves the central nervous system, and peripheral if the lesion involves the peripheral nervous system.

Double dissociation – Term that was introduced by H. L. Teuber in 1955. This is the demonstration that two experimental manipulations each have different effects on two dependent variables; if one manipulation affects the first variable and not the second, the other manipulation affects the second variable and not the first. If one can demonstrate not only that a lesion in brain structure A impairs function X but not Y, and further demonstrates that a lesion to brain structure B impairs function Y but spares function X, one can make more specific inferences about brain function and function localization. In cognitive neuroscience, double dissociation is an experimental technique by which two cerebral areas are functionally dissociated by two behavioral tests, each test being affected by a lesion in one zone and not the other. In a series of patients with traumatic brain injury, one might find two patients, A and B. Patient A has difficulty performing cognitive tests for, say, auditory memory but has no problem with visual memory. Patient B has the opposite problem. By using neuroimaging (or neuropathology post mortem) to identify the overlap and dissociation among lesioned areas of the brain, one can infer something about the localization of visual and auditory function in the normal brain.

Multisensory integration – The way information from the different sensory modalities, such as sight, sound, touch, smell, self-motion, and taste, may be integrated

by the nervous system. Such integration may result in unified perceptual experiences that are coherent across sensory modalities. Multisensory integration also concerns how different sensory modalities interact and alter each other’s processing.

Neuroplasticity – Neuroplasticity (also referred to as brain plasticity, cortical plasticity, or cortical remapping) is the changing of neurons and the organization of their networks and thus their function by experience. This idea was first proposed in 1892 by Santiago Ramón y Cajal – the proposer of the neuron doctrine though the idea was largely neglected for the next 50 years.

Proprioception – The sense of the relative position of neighbouring parts of the body. Unlike the six exteroceptive senses (sight, taste, smell, touch, hearing, and balance) by which we perceive the outside world, and interoceptive senses, by which we perceive the pain and the stretching of internal organs, proprioception is a third, distinct sensory modality that provides feedback solely on the status of the body internally. It is the sense that indicates whether the body is moving with required effort, as well as where the various parts of the body are located in relation to each other.

Receptive field – The receptive field of a sensory neuron is a region of space in which the presence of a stimulus will alter the firing of that neuron. Receptive fields have been identified for neurons of the auditory system, the somatosensory system, and the visual system. The concept of receptive fields can be extended to further up the neural system; if many sensory receptors all form synapses with a single cell further up, they collectively form the receptive field of that cell. In the somatosensory system, receptive fields are regions of the skin or of internal organs. Some types of mechanoreceptors have large receptive fields, while others have smaller ones. Large receptive fields allow the cell to detect changes over a wider area, but lead to a less precise perception. Thus, the fingers, which require the ability to detect fine detail, have many, densely packed mechanoreceptors with small receptive fields, while the back and legs, for example, have fewer

receptors with large receptive fields. Receptors with large receptive fields usually have a hot spot, an area within the receptive field (usually in the center, directly over the receptor) where stimulation produces the most intense response.

The Peripersonal Space

The peripersonal space consists of a region immediately surrounding the body, characterized by a high degree of multisensory integration among visual, tactile, and auditory information, which differs from farther regions of space. Although we perceive the space as something continuously defined and unitarily represented – as in the Descartes' geometrical definition we are used to – space derives from the perceptual space and it is composed of different neuronal representations, each built in relation to the behavior we can perform in the environment. The peripersonal space representation can thus be added to the classical triadic space taxonomy that can be described from a phenomenological point of view: The personal space, occupied by the body itself, whose representation is mainly built via proprioceptive and tactile information, but also with the contribution of visual input about body parts in the space; the extrapersonal space, principally based on visual and auditory inputs that convey information from the far space; the reaching space, within the extrapersonal space but proximal to the body, functionally defined according to the distance at which an object can be reached by the subject's hand without moving his/her trunk. The peripersonal space, mainly based on the integration of tactile and visual information coming from the body and the space immediately around the body, constitutes a privileged interface for the body to interact with nearby objects (see **Figure 1**).

Neurophysiological Basis of Peripersonal Space in Nonhuman Primates and the Human

One of the first scientists to formulate the concept of a special area of space around the body was Hediger, director of the Zurich Zoo from 1954 to 1973. In his formulation, this region of space was called the flight zone and corresponds to a margin of safety around the animal's body. When a threatening object enters this safety margin, the animal escapes. In a more psychological context, many researchers noted that the human behaves as if s/he had an invisible bubble of protective space surrounding her/his body. Whenever this proximal

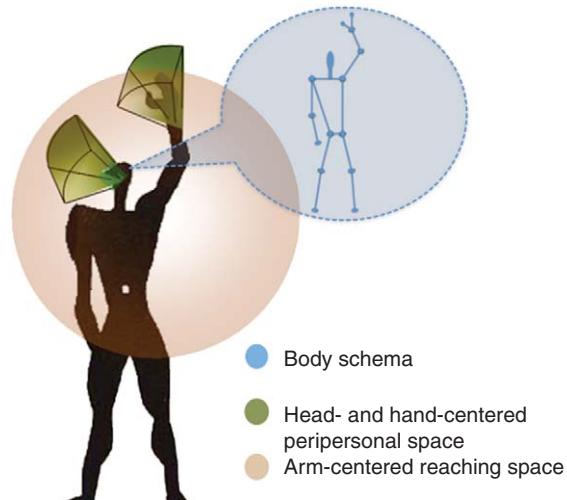


Figure 1 The figure depicts the body schema, the head- and hand-centered peripersonal space and the arm centered reaching space. The head- and hand-centered peripersonal space (green area) is mainly based on the integration of tactile information (from the skin) and visual information and it constitutes a privileged interface between the body and the external world. The body schema (in light blue) is a representation of the body parts dimensions and position the brain uses to plan and execute actions.

space boundary is violated, the person steps away to reinstate the safety margin. The size of this region of space is supposed to increase in a potentially threatening context with respect to friendly company.

With the discovery of bimodal visuotactile neurons in the monkey brain, the peripersonal space found both a more precise definition and its physiological basis. Hyvärinen and Poranen in the 1970s reported that some neurons in the parietal area 7 of nonanesthetized macaque monkeys were activated by a tactile stimulus delivered onto the tactile receptive field on a specific body part, as well as by a visual stimulus whenever presented close to the same body part. However, it is only in the 1980s that the systematic studies of Rizzolatti's group revealed the properties and the distribution of these neurons in an anterior region of the monkey brain, namely the ventral premotor cortex (area F4). Most of these neurons respond to stimuli in one or two sensory modalities. According to the particular modality activating the neurons, they were classified as somatosensory, visual, or bimodal (visual and somatosensory) neurons. Visual neurons are located rostral to the arcuate sulcus (area 8, or frontal eye field (FEF)), whereas somatosensory and bimodal neurons are found predominantly caudal to the sulcus (area F4). The parts of the body most represented are the hands and the mouth. According to the location of their visually responding region (i.e., their receptive field (RF)), bimodal neurons were subdivided into percutaneous (54%) and distant peripersonal neurons (46%). The former responded best to stimuli presented a few centimeters

from the skin, the latter to stimuli within the animal's reaching distance. The visual RFs were spatially related to the tactile ones. Therefore, an important property of these neurons, as with other cells in different multisensory areas (see below), is that the extent of their visual RF is limited in depth to a few centimeters (in most cases from ~5 to ~50 cm) out of the tactile ones. Moreover, when the arm is moved under the monkey's view, the visual RF follows the body part, being anchored to the tactile RF of that particular body part. A peripersonal region is similarly coded around the monkey's head. In particular, some neurons in the ventral intraparietal area (VIP) have visuotactile receptive fields mostly localized on the animal's face and head. As for other body parts, VIP neurons may thus build a multisensory representation of the head-centered peripersonal space. Through this interconnected network of bimodal areas, as Graziano pointed out,

the visual space near the animal is represented as if it were a gelatinous medium surrounding the body that deforms whenever the head rotates or the limbs move. Such a map would give the location of the visual stimulus with respect to the body surface, in somatotopic coordinates.

(see below for the functional role played by peripersonal space).

An important characteristic of the bimodal neurons is the dynamic property of their visual RFs. Iriki's group studied bimodal neurons of the postcentral parietal gyrus, somewhat extending into the intraparietal sulcus, that code for the peripersonal space of the hand-arm in monkeys. They showed their visual RF is not fixed, but can be expanded. Indeed, Iriki and colleagues trained monkeys to use a rake to reach for food pellets placed out of the animal's hand-reaching distance. Following this tool-training, the visual RF of some bimodal neurons coding for the hand peripersonal space was elongated toward the tool-tip, such that the tool appeared to be included within the visual RF. A few minutes after the training, the visually responsive area changed again, apparently shrinking back to its original size. These modifications were not observed if the rake was just passively held by the animal, suggesting that for such a change to occur, the tool has to be actively employed to perform an action. In other words, the dynamic aspect depends on the execution of a specific motor action. In a similar vein, Fogassi and colleagues also found that the visual RF of F4's visuotactile neurons expand when the visual stimulus velocity increases while approaching the cutaneous RF, a property that could be crucial for preparing and/or executing actions toward nearby objects.

Several studies support the existence of a similar representation of the space around the body in the

human. In this respect, the study of a neuropsychological condition called 'extinction' provided considerable insight into the behavioral characteristics of multimodal spatial representation in the human brain. Extinction is a pathological sign following brain damage whereby patients may fail to perceive contralesional stimuli only under conditions of double (contra- and ipsi-lesional) simultaneous stimulation, thus revealing the competitive nature of this phenomenon. A number of studies have shown that extinction can emerge when concurrent stimuli are presented in different sensory modalities: a visual stimulus close to the ipsi-lesional hand can extinguish a touch delivered on the contra-lesional hand. These studies reported the presence of stronger cross-modal visual-tactile extinction when visual stimuli were displayed in the near, as compared to the far, space, providing a neuropsychological support to the idea that the human brain represents peripersonal space through an integrated multisensory visuotactile system. Moreover, as described in monkey studies, even in the human the visual peripersonal space remains anchored to the hand when this is moved in another hemi-space, suggesting that peripersonal space is coded in a hand-centered coordinate system. As for the hand, a multisensory mechanism is involved in representing peripersonal space in relation to the human head. By showing stronger visual-tactile extinction for homologous (left and right cheek) than nonhomologous combinations of stimuli (e.g., left hand and right cheek) we demonstrated the modular organization of peripersonal space, different regions adjacent to different body parts being represented separately. Further support to this view has been recently provided by neuroimaging findings showing a human parietal face area representing head-centered visual and tactile maps. Finally, we have shown that human peripersonal space also features plastic properties, akin to those shown in the monkey. A similar re-coding of visual stimuli located in far space, as if they were closer to the participants' body, has been documented behaviorally in extinction patients following the use of a rake to retrieve distant objects. In this study, cross-modal visual-tactile extinction was assessed by presenting visual stimuli far from the patients' ipsi-lesional hand, at the distal edge of a 38-cm long rake passively held in their hand. The patients' performance was evaluated before tool-use, immediately following a 5-min period of tool-use, and after a further 5–10 min resting period. The authors found that far away visual stimuli induced more contra-lesional extinction immediately after tool-use, than before tool-use. Therefore, near and far space are separately represented and what is near or far is not aprioristically defined, but depends functionally upon movements that allow the body to interact with objects in space. Several authors have since suggested that tool-use-dependent changes in multisensory processing

may reflect changes occurring in another brain representation, namely the body schema.

The Body Schema

The body schema is a representation of dimension and position of body parts in the external space (see **Figure 1**) whose conception can be traced back to 1883, when Pierre Bonnier suggested the existence of an organized spatial representation (or ‘spatial sense’) of the body. However, is the ‘postural schemata’ introduced later by Head and Holmes (1911–12) to be universally considered as the first model of a plastic representation of the body. The main characteristics of this representation are to be finalized to action, to be dynamically updated, and strictly internally coherent.

Body Schema for Action (Executed and Imagined)

To accurately reach-to-grasp an object the brain needs to compute not only the position, shape, and dimension of the target, but also of one’s own body and, in particular, of the body part one wants to use to execute the action (the arm, in this example). The body schema is the representation of the body and its parts the brain uses to do this, among other aims. The spatial position and dimension of body parts are computed by combining information derived from different sensory modalities, such as proprioception, kinesthesia, touch and vision, in a sensory-motor schema. Head and Holmes suggested that the main function of the body schema is to appreciate the execution of active and passive movements, in contrast with another representation – a ‘Superficial Schemata’ – involved in tactile stimuli localization on the body surface. This dichotomy will remain in the subsequent literature, bringing to the fore more commonly used terms of body schema and body image (see below) that, however, do not unambiguously relate to the originally proposed two-faced representation. Several studies have been undertaken to provide evidence supporting this idea, and in particular showing the existence of a double dissociation, that is, the possibility of observing a deficit that is limited to one body representation in a (group of) patient, with the inverse pattern being observable in another (group of) patient. Deafferented patients, for example, have been shown to be able to localize a touch on their own hand despite a deficit in localizing the hand’s position in space, or vice versa. More recent work proposed the existence of three different levels in which the body is represented. In these models, the body schema is presented in contrast to other body representations such as the body image and the body structural description. The body image is a semantic and lexical representation of the body and its

relationship with external objects, while the body structural description is a topological map of locations derived primarily from visual information. Contrary to the body schema, these representations operate at a conscious level. Schwoebel and Coslett have recently tested this model’s validity on a large group of stroke patients. The authors developed a battery of tasks to examine the prevalence and anatomical substrates of the deficits of body representations. Patients with a deficit of the body schema succeeded in tests assessing the body image and body structural description, such as localizing isolated body parts and tactile inputs, matching body parts by location (a target body part was visually presented and subjects were asked to point among three pictures of body parts to the one that was closest on the body surface to the target body part), to match body parts by function (e.g., Is the knee more akin to the wrist or thumb? What body part wears the watch?). However, the same patients were impaired in performing tasks impinging in the body schema, such as imagining the execution of a series of hand movements with different levels of difficulty, and then actually executing those same movements. When response times for both imagined and executed movements were analyzed, the results showed a poor correlation between the two measures, thus suggesting a deficit of the body schema. In the same study, these patients were also unable to perform the hand-laterality task that requires a mental rotation of the hand. Patients were presented with a picture of a hand and asked to indicate if the stimulus was the right or the left hand. To solve the task the participant needs to mentally rotate his/her own hand until it matches the position of the stimulus picture, but this was not possible for patients with deficits of body schema. The lesion analysis suggested that the body schema is dependent on the dorsolateral frontal cortex and posterior parietal cortex.

Body Schema Representation is Plastic

As the body changes continuously in position and dimension throughout life, its cerebral representation needs to be updated for the brain to correctly plan and execute actions. Changes in body-part dimension develop relatively slowly, normally taking years, whereas postural changes are quicker and more frequent. Despite this difference in timescale, both need to be taken into account in the updating of the body schema. Actually, even abnormally fast changes in bodily dimensions are taken into account. Di Russo and colleagues showed a rapid cortical reorganization in the primary somatosensory cortex (SI) and in the associative parietal cortex after surgical extension of lower limbs. Acondroplastic dwarf subjects were tested before undergoing a progressive extension (PE) that increased the length of their legs by about 15 cm in 6 months. The authors observed an expansion and a shift

of the area responding to the foot-tactile stimulation in the SI 15 days after the PE, which disappeared at the follow-up (6 months later). Crucially, a change in activation was also observed in the superior parietal lobule (SPL) that was still present at the follow-up examination. SPL is thus suggested to be a crucial area in the parietal cortex involved in coding the relationship among body parts and between the body and the environment.

The notion that the body schema is plastic can be traced back to the seminal paper by Head and Holmes, where they wrote:

By means of perpetual alterations in position we are always building up a postural model of ourselves which constantly changes. Every new posture or movement is recorded on this plastic schema, and the activity of the cortex brings every fresh group of sensations evoked by altered posture into relation with it. Immediate postural recognition follows as soon as the relation is complete.

Two fundamental ideas are exposed here. First, the body schema is essentially a multisensory motor representation, as proprioceptive, kinesthetic, tactile, and visual information contribute in building it. Second, updating it takes place at an unconscious level, without needing an attentive effort. So, we do not need to think about the position of our feet at every step, or of our arm length to decide if we can reach for an object. Once the update is completed, we can consciously report the position of our body, verbally or by pointing to a body part. In the same paper, Head and Holmes added:

It is to the existence of these "schemata" that we owe the power of projecting our recognition of posture, movement and locality beyond the limits of our own bodies to the end of some instrument held in the hand. Without them we could not probe with a stick, nor use a spoon unless our eyes were fixed upon the plate. Anything which participates in the conscious movement of our bodies is added to the model of ourselves and becomes part of these schemata: a woman's power of localization may extend to the feather in her hat.

In this plastic feature of the body schema related to tool-use seems to reside the origin of the potential overlap with the concept of peripersonal space. As reported above, a large amount of studies relating skilful tool-use to the plasticity of the body schema actually refers to findings that pertain to the multisensory processing of peripersonal space. Irikis' findings in the monkey – showing enlarged visual RFs of bimodal neurons in the parietal cortex after training with a rake – as well as the human tool-use studies showing changes of multisensory interactions in the peripersonal space both of healthy subjects and neurological patients, have been taken as evidence that tool-use modifies the body schema. Similar instances

of such a conceptual overlap can be found in apparently remote domains, such as that of time perception. Yamamoto and Kitazawa asked a group of healthy subjects to perform a temporal order judgment task of two subsequent vibratory stimuli delivered on the tip of two hand-held sticks. When subjects were asked to cross the tips of the sticks without crossing their hands, they observed an alteration of the performance that was comparable to that obtained when subjects crossed their own hands. This finding provides evidence for a proprioceptively mediated referral of tactile stimuli to the tip of hand-held tools. It is unclear, however, to what extent the multisensory effects reported above can be ascribed to a change in the body schema and/or in the peripersonal space processing. We provided more direct evidence for a modification of the body schema following the use of a tool. We recorded, in healthy participants, the kinematic of freehand movements before and after training with a mechanical grabber – used to grasp objects. After the use of the tool, subjects performed the same freehand movement with a different kinematic profile. In particular, they took a longer time to achieve the maximal acceleration, velocity, and deceleration and the amplitude of these parameters was reduced. This particular kinematic pattern, involving only the transport component of the movement fits the kinematic difference that is naturally present in subjects on the basis of their morphology. Indeed, when a given movement is performed by subjects that have a different arm length, 'long-arm' subjects will show longer latencies and reduced amplitudes compared to 'short-arm' subjects. When we use a tool, the representation of our acting body changes so that the tool becomes a part of the body. This modification takes place rapidly, without requiring learning processes. However, the tool-use-dependent plasticity does not vanish immediately, the kinematic changes being present at least up to 15 min after the training with the mechanical grab. This direct measure of changes in the body schema may thus provide a new sensitive test to verify whether changes in the body schema invariably imply changes in the multisensory processing of peripersonal space, or they can be dissociated.

Internal Coherence

The body schema does not accept any incoherence. This means that when a conflict occurs between two inputs, the brain solves it in the direction of one of them. This mechanism is responsible for many perceptual illusions as, for example, the kinesthetic fusion illusion, the rubber-hand illusion, or the tendon-vibration illusion. The kinesthetic fusion illusion has been described by Craske and colleagues in 1978. Blindfolded subjects were seated with their arms stretched in front of them and separated by a plexiglas panel where a button and a probe were fixed.

Subjects had to press the button with their right index finger, which made the probe touch the left arm. In the experimental condition, the button and the probe positions were not coincident, so that pushing the button delivered a tactile stimulation through the probe that was displaced 12 cm away from the button. This paradigm induces a conflict between the proprioceptive and kinesthetic information (from the right-finger movement) and the tactile stimulus (on the left arm). The brain solves this conflict by initiating the feeling in the subject that the two spatial positions are coincident and, consequently, of the left arm being longer than it actually is. Similarly, in the rubber-hand illusion a conflict between visual and tactile inputs is solved in favor of the first one, ensuring that the subject feels the seen rubber hand as his/her own hand. The tendon-vibration illusion arises when a vibration is applied to the biceps or the triceps of the subject's arm. This vibration elicits a kinesthetic illusion of passive extension or flexion of the elbow, respectively. If the vibration is applied when the subject is holding with the vibrated hand the tip of his index finger of the opposite hand, an illusion of elongation, or shrinking, of the held finger is induced. de Vignemont and colleagues used this illusion and asked subjects to perform a perceptual judgment of the distance of two tactile stimuli delivered on the elongated/shrunk finger. They found that the tactile distance feels greater when the stimulated body part feels temporarily elongated. Interestingly, the contrary is not true as the perceptual judgment is not affected when the finger is perceived as shorter. The authors explained this result as caused by anisotropy of the body surface: ontogenetic changes are in the direction of a growing body and cannot normally be reversed. The body schema seems to have the ability to quickly change in the direction of a growing body (ontogenetic changes, rapid body-part elongation, tool-embodiment, etc.), but is resistant to modification in the opposite direction as they are not biologically plausible.

Space and Body for Action

Can the body schema and the peripersonal space be conceived of as the two faces of the same concept and cerebral representation? The former, classically action related, would be referred to the represented structure and position of the body used by the brain to perform an action; the latter is also action-oriented and refers to the multisensory space immediately surrounding the body, which could be used for performing freehand actions as well as using a functional tool. A large corpus of findings indeed supports the involvement of peripersonal space in the guidance of involuntary defensive movements. In the monkey, electrical stimulation of multisensory areas evokes complex pattern of hand-arm movements

compatible with avoidance or defensive reactions, such as withdrawal of the hand, turning of the head, or lifting the hand as if to defend the side of the head. It would thus be adaptive that responses possibly evoked by multisensory neurons are fast and mainly outside the control of top-down mechanisms. However, these multisensory interfaces might be adaptive, in addition, for producing voluntary actions toward objects, such as grasping a glass of water. Bimodal neuronal properties allow the brain to represent an object in a coordinate system centered on the body that can be continuously updated during bodily movements. Remarkably, some bimodal neurons also respond when the arm is voluntarily moved within the reaching space and have been proposed to code goal-directed actions. We provided evidence that voluntarily acting on objects triggers hand-centered remapping of multisensory perception by asking healthy participants to discriminate touches on the hand they used to grasp an object that contained task-irrelevant visual distractors. This provides a measure of how the visual-tactile interaction varies in real time with the action unfolding. Compared to a static condition, the start of the grasping action selectively increased the interference exerted by visual inputs originating from the far target object on tactile stimuli delivered to the grasping hand. This modulation reveals a re-mapping of the peripersonal space that, besides being time-locked with the action onset and regulated in real time with the action itself, does not require any tool-use to occur. The multisensory-motor neural machinery acting as an anticipatory interface between the body and nearby objects may thus have been selected throughout evolution to drive both involuntary avoidance reactions and voluntary approaching movements, with common adaptive advantages for defensive and manipulative actions.

Conclusions

The plastic feature of spatial and bodily representations, together with their involvement in motor control, have raised the possibility that the peripersonal space and the body schema are tightly related concepts, if not a unique one. The central point is to understand how the two concepts of peripersonal space and body schema are operationally separable. Although it is logically conceivable that a modification of one of them may occur leaving the other unchanged, no definitive evidence is yet available to support neither their dissociation nor their association. In this respect, future research is awaited to definitively answer the question of whether talking about body schema is the same as talking about peripersonal space.

See also: Behavioral Planning; Neurophysiological Approach of the Frontal Lobe Function in Primates; Brain Imaging; Mirror Neuron Mechanism; Neural Representations of Intended Movement; Orientation and Navigation; Voluntary Movement: Control, Learning and Memory.

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Taste Perception and Behavior in Rodents and Flies

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Glossary

Cibarial sense organ – cibarium – Food pocket of the insect's external mouth cavity between the base of the hypopharynx and the undersurface of the clypeus, in which food is chewed.

Labral sense organ - labrum – Upper lip, covering the base of the mandibles and forming the roof of the mouth. The labrum is a small sclerite articulating with the lower margin of the insect's face. It helps to hold food in place during chewing by the mandibles.

Papillae – foliate – (Papilla: small protuberance, Folium: leaf) Bilateral structures with the appearance of a leaf, irrigated by the lateral lingual glands. Foliate papillae are found on the posterior edges of the tongue and made of a succession of invaginations embedded in the epithelium. The lateral walls of these invaginations are filled with taste buds opening into the trench. The total number of taste buds found in human foliate papillae varies between 15 and 1500.

Papillae – fungiform – (Papilla: small protuberance, Fungus: mushroom) Small mushroom-shaped structures spread over the anterior two-thirds of the tongue and protruding slightly from the dorsal surface. Each papilla can contain up to 20 taste buds (human); however, it is estimated that up to 60% of fungiform papillae lack taste buds. There are around 200 fungiform papillae on the tongue with a higher density at the tip (150 taste buds per square centimeter).

Papillae – circumvallate – (Papilla: small protuberance, Circum: around, Vallum: rampart) Structures distributed along a chevron-shaped line in front of the sulcus terminalis on the dorsal-posterior surface of the human tongue. Each circumvallate papilla has the appearance of a horseshoe-shaped invagination opening a trench under the surface of the tongue. The trench is irrigated by the

ducts of the von Ebner gland, and the walls are covered with up to 800 taste buds opening into it. In mice, there is only one circumvallate papilla whereas in humans the total number varies between 3 and 13 per individual.

Pheromone – A chemical (or cocktail of chemicals) that triggers a natural behavioral response in another member of the same species. Their use among insects has been particularly well documented. In addition, some vertebrates and plants communicate by using pheromones. Pheromones are produced by various glands and their chemoreception can initiate social responses (e.g., sex, aggregation, alarm, and food-trail) and eventually endocrine changes.

Proboscis – Elongated appendage from the head of an animal, either a vertebrate or an invertebrate. In general, this refers to the tubular feeding and sucking organ of insects (e.g., moths, flies, and butterflies), worms, and gastropod mollusks. This composite organ is formed of the maxillae and labium brought together to form a tube through which liquids are drawn up to the mouth by the cibarial pump.

Sense organ – A structure, which is a receptor for external or internal stimulation. A sense organ is often referred to as a receptor organ. A specialized organ or structure, such as the eye, ear, tongue, nose, or skin for vertebrates and the sensory sensilla for invertebrates, where sensory neurons are concentrated and that functions as a receptor.

Taste sensilla – Sensilla (sg.= *sensillum*) are insect sensory organs protruding from the cuticle, or sometimes lie within or beneath it. They are divided into chemical, mechanical, thermal, and visual sensilla. A sensillum is a simple sense organ consisting of one or a few receptor cells at the end of a nerve connection generally responsive to a common modality.

The identification of food sources is a prerequisite to survival. This task involves the integration of information coming from several senses. Sight, hearing, and smell can all provide long-range cues about the putative location of a prey or food source, but somatosensation and taste will ultimately decide on the ingestion of the food. Because the main function of the gustatory system is to distinguish the good (nutrient rich) from the bad (toxic), taste information collected in the peripheral structures and processed in the higher brain centers is wired to evoke an appropriate behavioral response.

Anatomy of the Gustatory System

Peripheral Structures: Taste Buds and Taste Sensilla

In mammals, the structures monitoring the chemical composition of foods are called taste buds. Although taste buds are mostly concentrated on the dorsolateral surface of the tongue in the fungiform, foliate, and circumvallate papillae (**Figure 1**), others are judiciously distributed in the oral cavity on the palate, the epiglottis, the pharynx, and the larynx. Taste buds are small oval-shaped arrangements of 30–100 elongated cells of three distinguishable types. Type I cells operate as supporting cells. Taste receptors for sweet, umami, and bitter compounds reside on the apical microvilli of type II cells. Type III cells are thought to express a candidate sour receptor and have the distinct characteristic of making synaptic connections with afferent sensory fibers. The precise role of the various cell types in tastants detection is still under investigation. Two important features characterize the elongated cells composing the mammalian taste bud. Unlike in flies where taste receptors are found on primary sensory neurons, mammalian taste bud cells (TBCs) are polarized neuroepithelial cells with functionally distinct apical and basal regions. On the apical side, TBCs are in contact with the saliva by means of microvilli protruding through the taste pore. Below the pore, tight junctions create a selective intercellular barrier thereby isolating the basal region dedicated to cell–cell communication. Gap junctions, paracrine secretions, and synapses indicate that cells within the taste bud communicate with each other and with the cranial nerve fibers through the release of neurotransmitters (ATP); at the same time, sensory nerves are thought to provide TBCs with trophic factors necessary for their survival. It is estimated that taste cells are renewed every 2 weeks from a pool of progenitor cells found at the base of the bud.

In *Drosophila*, the gustatory system has been investigated in both larvae and adults. During these two developmental stages, insects display very different lifestyles: larvae develop directly on their food source whereas adults can fly over a large distance to find both an appropriate nutritive source and a mate. This can

explain the higher complexity for the organization of the adult taste system. The head of the adult carries about 4 times more gustatory receptor neurons (GRNs: 300) than that of the larva (80). However, this difference is less marked than for the olfactory system.

The larva carries three principal chemosensory organs on its head, each of which contains multiple sensilla. The dorsal organ (DO) consists of a multiporous dome housing 21 olfactory receptor neurons (ORNs) surrounded by a ring of six sensilla, mostly containing GRNs. The two other external organs, the terminal organ (TO) and the ventral organ (VO), contain predominantly GRNs but also some neurons likely involved in thermo-, mechano-, or hygrosensation. The pharynx is also lined with three paired organs: the dorsal, ventral, and posterior pharyngeal sense organs (DPS, VPS, PPS), which mostly contain taste sensilla.

In the adult, the peripheral structure dedicated to detecting tastants is called the taste sensillum. There are three types of sensilla according to their size: small (s-type), long (l-type), and intermediate (i-type). A large number of taste sensilla are found on the labial palps, at the tip of the proboscis, the equivalent of the tongue in mammals (62 taste hairs each housing two (i-type) or four GRNs (l- and s-types) and about 60 taste pegs housing one GRN each) (**Figure 1**). These organs allow the fly to sample tastants before their ingestion. Some other taste hairs are distributed on the outer edges of the wings and also on the legs and ovipositor. Three pairs of bilateral internal taste organs are symmetrically disposed around the pharynx: the labral sense organ (LSO), and the ventral and dorsal cibarial sense organs (VCSO, DCSO). These internal organs contain several sensilla housing GRNs. They allow the fly to sample the food after ingestion, but before it enters into the digestive tract. Taste sensilla have an opening or pore at their distal end allowing tastants to come into contact with the lymph that fills them. Each sensillum contains either two or four gustatory neurons depending on the type of sensilla (see above) and a mechanosensory neuron. Gustatory neurons extend a dendrite into the shaft of the sensillum lymph cavity. The lymph is produced by the supporting cells surrounding the gustatory neurons, including the thecogen, tormogen, and tricogen, which are also thought to play a role in mechanosensation. Electrophysiological recordings of gustatory neurons and immunohistological localization of taste receptors in the sensillum are beginning to provide a clear picture of the composition and sensitivity of receptor neurons in each type of sensillum, but substantial work remains to reach a complete and comprehensive map. Four main types of gustatory neurons are distinguished based on their electrophysiological features: gustatory neurons responding best to sugars (S cell), to water (w cell), to low salt (L1 cell), or high salt and bitter compounds (L2 cell).

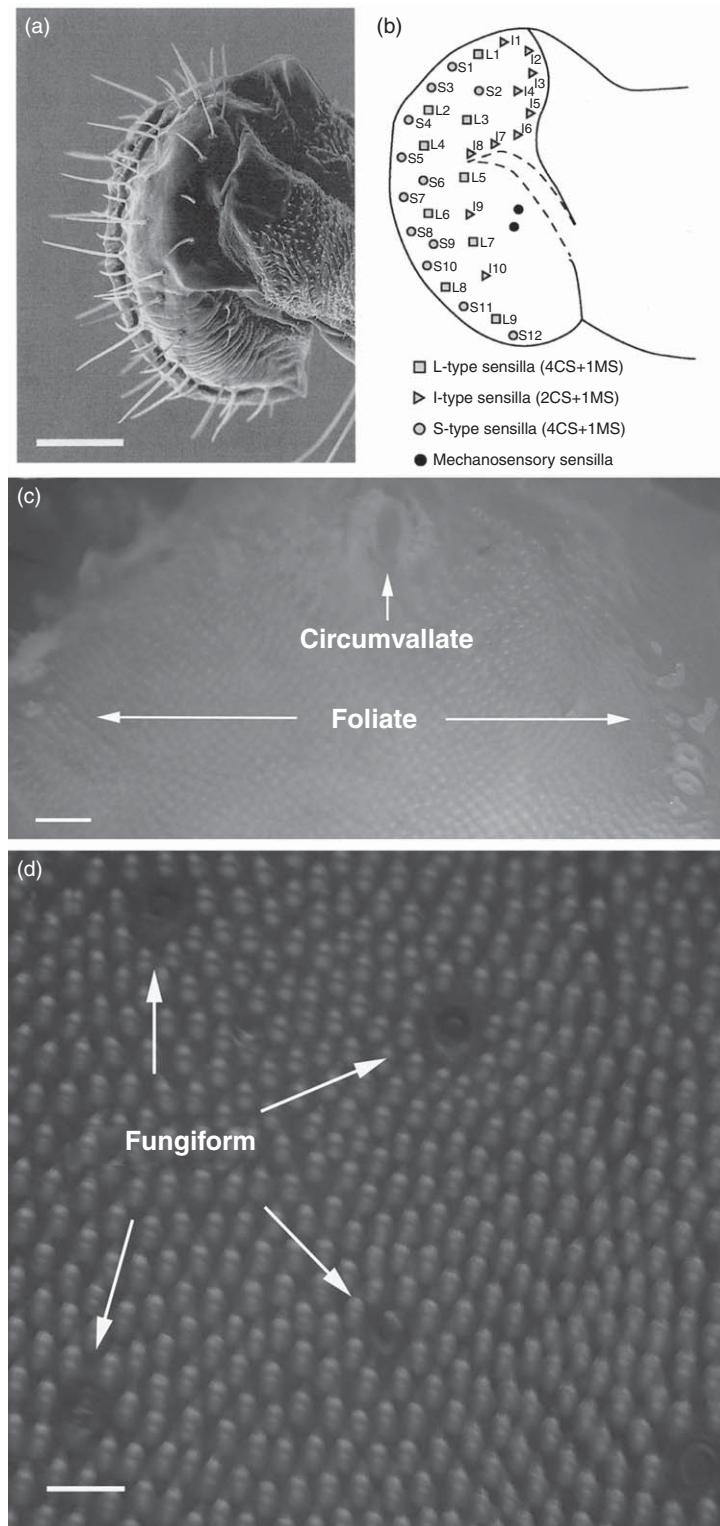


Figure 1 Location of the main taste sensitive organs in *Drosophila* and mouse. (a) Surface image of a left labellar lobe on the proboscis showing the distribution of the chemosensilla. Scale = 30 μ m. (b) Diagram illustrating the arrangement of sensilla on the lobe. Each lobe or palp contains 12 short bristles (S-type: open circles) each with four chemosensory (CS) + one mechanosensory (MS) neurons, nine long bristles (I-type: squares) with four CS + one MS, 10 intermediate bristles (I-type: triangles) each with two CS + one MS as well as two mechanosensory sensilla (filled circles). (c) Transmission image of an unfolded sheet of posterior mouse tongue epithelium showing the location of the unique circumvallate papilla and the two foliate papillae on both lateral edges. Posterior is top. Scale = 400 μ m. (d) Transmission image of a sheet of anterior mouse tongue epithelium showing the location of fungiform papillae surrounded by filiform papillae (pegs). Filiform papillae cover the entire dorsal surface of the tongue and do not contain taste buds. Posterior is top. Scale = 125 μ m. (a & b) Reproduced from Hiroi M, Marion-Poll F, and Tanimura T (2002) Differentiated response to sugars among labellar chemosensilla in *Drosophila*. *Zoological Science* 19: 1009–1018 with permission from The Zoological Society of Japan.

Projections to the Brain

In mammals, the gustatory information collected by the taste cells travels through primary sensory neurons from cranial nerves VIIth, IXth, and Xth via the geniculate, petrosal, and nodose ganglia, respectively, before forming a synapse with neurons in the rostral-lateral part of the nucleus of the solitary tract (rNST). Fibers carrying gustatory information from the chorda tympani branch of the cranial nerve VIIth innervate taste buds in the fungiform and anterior part of the foliate papillae, while the greater superior petrosal branch innervates the palate.

The posterior region of the foliate as well as the circumvallate papillae is innervated by the lingual branch of the glossopharyngeal nerve (IXth), whereas the superior laryngeal branch of the vagus nerve (Xth) contacts the taste buds in the larynx. It is thought that single nerve fibers from the cranial nerves contact a few taste buds and that each taste bud is in contact with several fibers.

In rodents, the taste information travels through the rNST to the medial parabrachial nucleus of the pons on the ipsilateral side, then via the ventroposteromedial nucleus of the thalamus before reaching the gustatory neocortex. Inputs from the parabrachial nucleus and rNST also reach the basal forebrain areas, such as the hypothalamus and the amygdala. This system is likely involved in the hedonic aspect and autonomic functions linked to feeding, whereas information processing in the thalamocortical areas determines perception of taste intensity and quality.

In *Drosophila*, the cell bodies of the sensory neurons housed in larval sense organs are collected in the ganglia located beneath each organ. The sensory inputs

originated in their GRNs are conveyed through four nerves, each of which projects into a distinct region of the subesophageal ganglia (SOG; located at the ventral side of the central brain complex). However, in the larval SOG, there is no spatial segregation by GRN type, indicating that the same tastant can induce different behavioral response according to the sensilla it has been processed by. In the SOG, a set of 20 interneurons expressing the *bugin* gene seems to modulate different aspects of larval feeding. These interneurons establish dendritic arborization with the GRNs and send processes to several neural and endocrine centers involved in feeding. Moreover, the genetic blocking of *bugin*-positive neurons in adults dramatically increases feeding.

In adults, all GRNs are targeted to the SOG. Although there is no morphologically apparent substructure dividing the SOG, the projection of gustatory afferents from different appendages project in distinct SOG regions: the GRNs from labial, tarsal, and internal taste organs, respectively, end in the central, posterior, and anterior-dorsal part of the SOG. Similarly to the taste system of larvae, adults show no segregation by type of GRN but rather by type of appendage, suggesting that the same tastant can induce distinct behavioral effects if it is detected by different gustatory organs. However, the two principal types of GRN, carrying either Gr66a or Gr5a receptors (see below), mediate, respectively, repulsive or attractive stimuli, and show nonoverlapping projection patterns in the SOG (Figure 2). Other putative bitter GRNs expressing Gr32a or Gr47a show a projection pattern that largely overlaps with that of Gr66a-expressing neurons. Therefore, the separation of repulsive and attractive stimuli seems to occur at the first neuronal level.

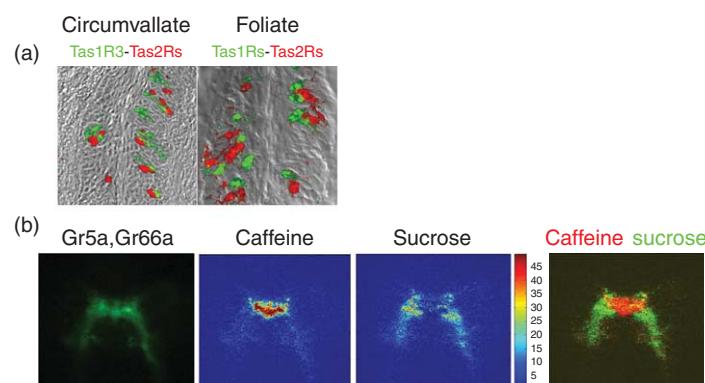


Figure 2 (a) Expression patterns of Tas1Rs and Tas2Rs on the mouse tongue define two subsets of taste cells. Double-label *in situ* hybridization on sections of mouse circumvallate papillae (right panel) with probes specific for Tas2Rs (red) or Tas1R3 (green) or foliate papillae (left panel) with a mix of probes specific for Tas2Rs (red) or Tas1Rs (green). (b) Central projections of the Gr5a and Gr66a GRN are spatially segregated. G-CaMP fluorescence imaging of sweet-responsive Gr5a and bitter-responsive Gr66a neurons in the SOG (first panel from the left) after stimulation with 100 mM caffeine (the ligand of the Gr66a receptor; second panel from the left) or 1 M sucrose (third panel from the left). Overlay of the caffeine- (red) or sucrose-induced (green) response. (a) Reprinted from Nelson G, Hoon M, Chandrashekhar J et al. (2001) Mammalian sweet taste receptors. *Cell* 106: 381–390 with permission from Elsevier. (b) Reprinted from Marella S, Fischler W, Kong P et al. (2006) Imaging taste responses in the fly brain reveals a functional map of taste category and behavior. *Neuron* 49: 285–295 with permission from Elsevier.

Despite some quantitative differences, there are many similarities between the larval and the adult taste systems, suggesting that many elements persist throughout the complete metamorphosis of this insect. Some stage difference can nevertheless be observed as for the projection pattern of Gr66a-expressing neurons, which is exclusively ipsilateral in the larval brain and bilateral in the adult SOG.

Tastants Detection and Behavioral Output

Taste Modalities and Associated Behaviors

Taste cells harbor receptors recognizing a wide variety of soluble chemicals with extremely variable structures, including ions, carbohydrates, alkaloids, small proteins, and amino acids. In humans, the taste sensations evoked by these compounds can be categorized into five primary modalities known as bitter, salty, sour, sweet, and umami. Behavioral responses associated with each of these modalities are related to the biological relevance of the compounds connected with each category.

Several behavioral assays are available to assess and quantify the response of rodents to soluble chemicals. The two-bottle choice test is a simple and robust assay to evaluate attraction or aversion to chemicals in solution. In this test, after a period of training and starvation designed to promote absorption and reduce apprehension, rodents are offered a pair of bottles containing the solutions to be tested. If the test period is short (<10 min), the total quantity sampled and the ratio of solutions consumed is considered to be reflecting palatability and orosensory factors while longer testing period also reflects postigestive effects. An alternative strategy involves presenting food-deprived animals with a choice of solutions and computing the number of licks produced over a very short period of time (10–60 s). By allowing such transient access to the solution, the brief-access test is thought to completely exclude postigestive events known to influence intake and preference; therefore, it is suitable to determine the contribution of oral factors and intensity thresholds.

Perceptual discrimination can be assessed with conditioning paradigms during which an animal is trained to perform a specific task after tasting one of two solutions. Typically, only solutions that can be discriminated will allow the animal to learn the task. Another paradigm involves pairing a given taste stimulus with a gastric malaise induced by the simultaneous intraperitoneal injection of LiCl. The very robust resulting conditioned taste aversion is applied to the study of perceptual categorization of taste compounds.

Using these tests and the others more elaborate in rodents, it was determined that salty, sour, sweet, and umami compounds tested at or just above detection thresholds are preferred over water, while bitter-tasting

compounds are strongly aversive. Detection thresholds for bitter compounds are generally lower than that of preferred compounds. At high concentration, salty, sour, sweet, and umami compounds elicit an aversive response.

Behavioral tests carried out with larvae or adult flies aim to measure not only the attractive or repulsive effect of varied food molecules but also that of adult contact pheromones mostly perceived by the gustatory system. Behavioral tests performed with larvae are designed to measure individual or group responses exclusively to food compounds. Basically, starved larvae taken at the end of their second instar (period during which the maximum feeding response is shown) are placed on a Petri dish at the frontier between two media. These media always consist of a choice that can involve either an attractive (sugar) or a repulsive substance (high salt, bitter) or one of these substance combined with a neutral medium (agar). The precise behavior (individual) or the distribution on each medium (group) is measured during a test period generally lasting between 10 and 30 min.

Adults can be tested for three major behavioral paradigms to measure their feeding or sexual responses. For feeding tests, they are kept fasting during a few hours; for mating tests, they are kept in isolation during all adult development to prevent inter-individual experience. Feeding response can be measured with groups of flies kept, in darkness, in a small box containing a choice of two types of food colored with different dyes. The preference is subsequently assessed by looking at the transparent abdomen of the fly and scoring the tint of the food ingested. The second feeding assay takes advantage of the proboscis extension reflex, which individually measures the aversive or appetitive effect of a stimulus deposited on the fly leg. Typically, feeding tests have shown that bitter and high salt concentration are aversive while sweet and water stimuli are attractive to both larvae and adults.

Pheromonal gustatory perception is measured by the intensity of courtship and the willingness to mate that individual male direct to various target flies either alone or paired. These tests, which involve target flies carrying various quality and quantity of contact pheromones, can last between 5 and 60 min.

Detection and Transduction of Sweeteners and Amino Acids

In mice, a small family of GPCRs comprising three members (Tas1R1, Tas1R2, Tas1R3) mediates the detection of most of the sweeteners and amino acids. These GPCRs assemble as pairs to produce functional receptors for sweet-tasting compounds (Tas1R2–Tas1R3) or L-amino acids (Tas1R1–Tas1R3). Their expression pattern defines two subsets (Tas1R1–Tas1R3- or Tas1R2–Tas1R3-expressing cells) of type II taste bud cells.

The gene coding for Tas1R3 is found at a genetic locus (*Sac* locus) influencing behavioral responses to sucrose and saccharine in several mouse strains; consequently, naturally occurring polymorphisms in the amino-terminal extracellular region of the receptor have been associated with saccharine preference. Tas1R2–Tas1R3 is broadly tuned to a large range of sweet-tasting compounds, including D-amino acids, peptides, natural sugars, and artificial sweeteners, whereas Tas1R1–Tas1R3 is activated by L-amino acids and potentiated by purine nucleotides, a distinctive feature of umami taste (Table 1).

The coupling of these receptors to the transduction machinery appears to involve a common signaling pathway whereby G $\beta\gamma$ subunits released from the heterotrimeric G protein coupled to the receptors leads to the activation of phospholipase C beta-2, which, in turn, hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP2) into inositol 3-phosphate (IP3) and diacylglycerol (DAG). IP3 then causes the release of Ca²⁺ from intracellular stores, subsequently leading to the activation of TRPM5, a member of the TRP family of ion channels. Gating of TRPM5 by intracellular Ca²⁺ allows entry of monovalent cations into the cell and subsequent depolarization. The role of other second messengers, such as cAMP, thought to be important for sugar signal transduction remains unknown.

In *Drosophila*, a family of 60 GPCRs called Grs for gustatory receptors is thought to be responsible for tastant detection. Grs were found to be expressed in larvae's terminal taste organ as well as in GRNs of the labial palp and legs. Gr5a, a member of this family found in about 50% of the labial palp's GRNs, is activated by the sugar trehalose but not maltose or sucrose. Neurons carrying Gr5a mediate responses to appetitive stimuli such as trehalose as well as low salt concentration. Mutant flies without a functional Gr5a are able to detect sucrose, suggesting that other yet unidentified receptors are involved in sugar detection. It has been proposed that the Gr64 subfamily of receptors of which Gr64f is present in sugar-detecting sensilla (long and small) are such candidates.

Detection and Transduction of Bitter

A family of about 30 divergent GPCRs called Tas2Rs has been tied to bitter detection in mammals. The genes coding for the Tas2Rs are located at genetic loci associated with bitter sensitivity in human and mice. In fact, genetically inherited modifications in the human Tas2R38 and mouse Tas2R5 receptor proteins are linked with variation in sensitivity to the bitter compounds phenylthiocarbamide (PTC) and cycloheximide, respectively. The divergence in sequence identity among members of the Tas2R family is expected to reflect the

structural diversity of bitter-tasting compounds with which they interact. Tas2Rs characterized so far display high selectivity to bitter-tasting ligands (Table 1).

In rodents, TBCs expressing Tas2Rs are found in all tongue papillae as well as on the palate. They define a subset of type II cells separate from that expressing Tas1R2–Tas1R3 or Tas1R1–Tas1R3 (Figure 2). Multiple intracellular signaling components have been detected in Tas2Rs expressing cells, including the G-protein alpha-subunit gustducin as well as the receptor for inositol triphosphate type 3. Behavioral evidence emanating from knock-out mice supports a role for gustducin, phospholipase C beta-2, and TRPM5 in bitter detection but evidence exists for additional signaling pathways.

Drosophila small and intermediate sensilla are thought to contain bitter receptors. More than 20 Grs have been reported as expressed in bitter sensilla, including Gr66a a receptor activated by the bitter compound caffeine. Gr66a-expressing GRNs in the labial palp emanate from s-type and i-type sensilla and represent a subset of neurons distinct from that expressing Gr5a. Gr66a-expressing neurons seem to respond to a wide variety of tastant molecules, including bitter molecules, wasabi, and, more surprisingly, 7-tricosene, which is a long chain hydrocarbon known to act as a male inhibitory pheromone. While it has been shown that caffeine binds to the Gr66a receptor, the receptor for the male pheromone has yet to be identified. Both substances induce similar repulsive effect on feeding and sexual behavior. They can also act additively and cross-stimulate the same taste neuron (which corresponds to the L2 cell). This indicates that taste neurons inducing a typical behavior (repulsion) can respond to very different molecules. The signaling pathways downstream of Gr66a are presently unknown.

Detection of Salts and Acids

Sour and salty stimuli are thought to be detected by ion channels in mammals. A heteromer composed of two members of the transient receptor potential (TRP) family of ion channels (PKD2L1 and PKD1L3) has emerged as a strong candidate sensor for sour stimuli in mice. PKD2L1 is confined to type III taste bud cells.

Salty sensation is thought to be mediated, at least in part by an amiloride-sensitive sodium channel (ASSC), which belongs to the epithelial sodium channel (ENaC) family of ion channels.

In *Drosophila*, two members of the DEG/ENaC ion channel family, pk11 and pk19, are likely involved in the response of flies to high salt concentration that are normally repulsive. Intriguingly, another ion channel of the same family (ppk25) seems to be also involved in the response to putative female contact pheromones, which elicit an attractive response in males.

Table 1 Receptor–ligand relationships for taste receptor

Taste quality	Receptor	Species	Ligand	Assay
Bitter	Gr66a	<i>Drosophila</i>	Caffeine Theophylline Denatonium Cycloheximide	Behavioral response
	Tas2R5	Mouse		Calcium imaging GTP γ S
	Tas2R8	Mouse	Denatonium PROP	Behavioral response Calcium imaging
	Tas2R9	Rat	Cycloheximide	Calcium imaging
	Tas2R4	Human	Denatonium PROP	Calcium imaging
	Tas2R7	Human	Chloroquine Quinacrine Strychnine Papaverine	GTP γ S
	Tas2R10	Human	Strychnine	Calcium imaging
	Tas2R14	Human	Aristolochic acid 1,8-naphthaldehydic acid 1-naphthoic acid 1-nitrolnaphthalene Sodium benzoate Picrotin PicROTOxin Piperonylic acid -(α)-thujone	GTP γ S Calcium imaging
	Tas2R16	Human	Salicin	Calcium imaging
	Tas2R38	Human	PTC	Calcium imaging
Sweet	Tas2R43	Human	Aloin Aristolochic acid Saccharin	Psychophysics Calcium imaging GTP γ S
	Tas2R44	Human	Denatonium Aristolochic acid Saccharin Acesulfame K	Calcium imaging GTP γ S
	Tas2R47	Human	Denatonium	GTP γ S
	Tas2R61	Human	6-nitrosaccharin	GTP γ S

(Continued)

Table 1 (Continued)

<i>Taste quality</i>	<i>Receptor</i>	<i>Species</i>	<i>Ligand</i>	<i>Assay</i>
Umami	Tas1R1–Tas1R3	Mouse	L-Amino acids	Calcium imaging Nerve recordings Behavioral response
	Tas1R1–Tas1R3	Rat	L-Glutamate L-Aspartate	Calcium imaging
	Tas1R1–Tas1R3	Human	L-Glutamate L-Aspartate L-AP4	Calcium imaging
Sour	PKD2L1–PKD1L2	Mouse	Citric acid Malic Acid Tartaric acid Phosphoric acid Hydrochloric acid	Calcium imaging Electrophysiological recordings
Sweet	Gr5a	<i>Drosophila</i>	Trehalose	Behavioral response Electrophysiological recordings Calcium imaging
	Tas1R2–Tas1R3	Mouse	Sucrose Fructose Glucose Maltose Saccharin Acesulfame k D-Phenylalanine D-Tryptophane D-Alanine	Calcium imaging Calcium imaging Nerve recordings Behavioral response
	Tas1R2–Tas1R3	Rat	Sucrose Fructose Glucose Maltose Saccharin Acesulfame k D-Tryptophane	Calcium imaging
	Tas1R2–Tas1R3	Human	Sucrose Fructose Glucose Maltose Saccharin Acesulfame k D-Phenylalanine Cyclamate Aspartame Monellin Thaumatin	Calcium imaging

Additional Taste Modalities

The mechanism of water detection and the identity of the receptor of the water cell found in *Drosophila* sensilla remain largely unknown, although a genetic marker (NP1017) specific to the W cell has been identified. Interestingly, in the hamster, the superior laryngeal branch of the vagus nerve innervating chemosensory receptor cells within the laryngeal epithelium present the particularity to be responsive to water.

In flies, Gr21 and Gr63 are important for the detection of CO₂, a gustatory stimulus signaling the presence of fermentation. In larvae, the response to CO₂ depends on Gr21a-expressing neurons, which project in a specific SOG region distinct from the four subregions described above. In adults, Gr21a and Gr63a should be simultaneously present to allow an acute response to CO₂, suggesting that both receptors function together as a membrane-bound CO₂ receptor.

Taste Information Coding

Over the years, tremendous progress in our understanding of the functional organization of the gustatory system has been achieved. The expression patterns of the rodent receptors involved in the detection of tastants strongly

suggest that, within the taste bud, distinct subsets of TBCs are specialized for the detection of bitter-, sweet-, sour-, and umami-tasting compounds. Whether the signal is processed in the taste buds or specific connections with afferent nerve fibers are established remains a debated issue. Nonetheless, gustatory nerve recordings indicate that single fibers carry the information from several taste qualities, although their sensitivities vary according to the taste stimulus. In the rat, fibers of the chorda tympani and glossopharyngeal nerve can be classified into NaCl best, quinine best, sucrose best, and HCl best based on their relative sensitivity to four types of taste stimuli. Glossopharyngeal taste fibers are predominantly responsive to HCl and quinine, while those of the chorda tympani nerve are mainly responsive to sucrose and NaCl. In adult *Drosophila*, the two principal types of taste receptors are located in nonoverlapping sets of GRNs and project to different areas much like GRN emanating from different peripheral taste organs do. Thus, both quality and position are represented in the fly SOG. In rodents, a clear map of taste quality in the NST remains to be seen. Nevertheless, behavioral evidence emanating from genetically modified mice expressing a bitter taste receptor in sweet taste receptor-expressing cells supports a linear system for the detection and associated behavioral response of attractive and aversive compounds as that apparent in *Drosophila* (Figure 3).

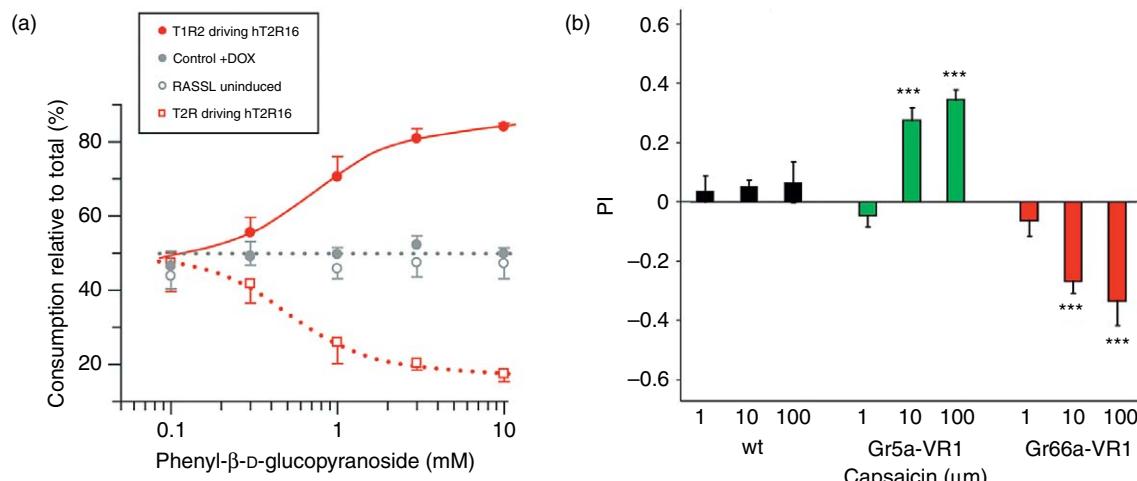


Figure 3 Specific sets of taste receptor cells in mice or GRNs in *Drosophila* mediate attractive or aversive behaviors. (a) Targeted inducible expression of the human Tas2R16 receptor in Tas2R19 expressing taste bud cells elicits a dose-dependent aversive response to phenyl-β-D-glucopyranoside, the ligand of the Tas2R16 receptor (red circles) as measured by standard two-bottle preference assay. By opposition, expression of the human Tas2R16 in Tas1R2 expressing taste bud cells produces mice attracted by phenyl-β-D-glucopyranoside (red squares). Control mice without the Tas2R16 receptor display no behavioral response to the same molecule (gray circles). (b) In *Drosophila*, a similar experiment was conducted with VR1, an ion channel activated by capsaicin and expressed in Gr5a- or Gr66a-expressing GRNs. Behavioral taste preference assays in these genetically engineered flies show that capsaicin does not elicit a significant behavioral response in control flies (black bars) whereas expression of VR1 in Gr5a neurons induced a robust dose-dependent attraction to capsaicin (green bars). On the contrary, VR1 expression in Gr66a neurons made flies strongly aversive to capsaicin (red bars). (a) Reprinted from Mueller K L, Hoon M A, Erlenbach I et al. (2005) The receptors and coding logic for bitter taste. *Nature* 434: 225–229 with permission from Macmillan Publishers Ltd. (b) Reprinted from Marella S, Fischler W, Kong P et al. (2006) Neuron, 49, Marella et al., Imaging taste responses in the fly brain reveals a functional map of taste category and behavior. *Neuron* 49: 285–295 with permission from Elsevier.

One can envision that such a system providing straight information about the quality and position of nutrients might be beneficial for the efficient control of the ingestive behavior, while integration of multisensory information and perhaps processing of temporal patterns are required for behaviors linked with reward, appetite, and emotion. Future research investigating the topography of projection to the NST and beyond and processing of the information in various brain regions will undoubtedly provide valuable insight into taste-guided behaviors.

In conclusion, although sucrose or denatonium elicit similar behaviors in *Drosophila* and mice, their sweet and bitter taste receptors have no sequence similarity. Nevertheless, in these two species, aversive or attractive behaviors appear guided by neuronal or neuroepithelial chemosensory receptor cells specialized in the detection of palatable or unpalatable compounds. Further research into the central mechanisms of taste processing in these species will address whether evolutionary conserved mechanisms take place at this level as well.

See also: Feeding; From Sensation to Perception; Genes and Behavior: Animal Models; Mating Behavior; Obesity and Binge Eating Disorder; Stress and Energy Homeostasis.

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Tinnitus: Processing of Auditory Phantom Sound

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Glossary

Audiometry – Behavioral measurement of hearing thresholds at octave-spaced frequencies between 125 and 8 kHz.

Hair cells – Receptor cells in the cochlea (inner ear) comprising inner hair cells (true receptor cells) that act like microphones and outer hair cells that have an amplifying function on the mechanical vibrations in the inner ear. Outer hair cells are especially susceptible to damage by excessive noise and certain drugs, resulting in hearing loss.

Ménière's disease – A clinical disorder characterized by the combination of (low-)frequency hearing loss, roaring tinnitus, fullness in the ear, and vertigo.

Neural synchrony – More or less coincident firing of nerve cells, resulting in enhanced evoked potentials and efficient stimulation of other neurons downstream that are innervated by the synchronously firing cells.

Neuropathic pain – Initiated or caused by a primary lesion or dysfunction in the nervous system that cannot be explained by a single disease process or a single specific location of damage.

Recruitment – A phenomenon of abnormal loudness (or evoked potential amplitude) growth in ears with outer hair cell loss; despite a hearing loss the perceived loudness (or the evoked potential amplitude) is comparable to (or even larger than) those in normal-hearing people at high intensity levels. The result is a reduced dynamic range for processing sound.

More common is subjective tinnitus: a sound that is heard in the absence of any external or internal physical sound source. A benign form of subjective tinnitus is the short (<30 s) experience of a (usually) tonal ringing in the ear, sometimes accompanied by fullness in the ear and a slight transient hearing loss. Its cause is largely unknown, but it is likely not related to the death of a cochlear hair cell as sometimes suggested.

About 10–15% of adults experience tinnitus, and most commonly describe it as ringing, buzzing, cricket-like, hissing, whistling, and humming, and less frequently as a roaring sound. It is largely unknown why about 80% of the people with chronic tinnitus suffer little or do not seek treatment for their tinnitus, whereas about 20% manifest a clinically significant condition. When patients visit a doctor because of annoying tinnitus, a majority of them think that they have a serious problem and might become deaf. This is not the case in the vast majority of cases. It is usually the other way around: a mild-to-moderate high-frequency hearing loss that people do not find too bothersome in most cases is usually the cause of tinnitus. Typically, about 20% of people report that the tinnitus sounds were equal in both ears, about 35% report having tinnitus only in one ear, and most of the others report tinnitus to be stronger in one ear.

Where is Tinnitus?

Is tinnitus in the ear or in the brain? Tinnitus sensations associated with hearing loss are nearly always localized toward the affected ear(s). Does this mean that tinnitus is generated in the ear? Early on, in cases of truly debilitating tinnitus, a common surgical procedure was to cut the auditory nerve from the tinnitus ear in order to abolish tinnitus. However, in the majority of cases, the tinnitus sensation persisted after resection of the auditory nerve. This suggests that localizing the source of tinnitus is not so simple. In most cases tinnitus is indeed initiated by processes in the ear and, in some, its source may be in the ear as in the case for the very short transient forms of tinnitus and also potentially in some temporary forms that last less than a few weeks. However, most chronic tinnitus is of central origin and its source has shifted to the brain.

The localization of tinnitus to one or both ears is most likely attributable to a phantom sensation and is not unlike that related to sensations or pain experienced

What is Tinnitus?

In common English, tinnitus stands for ‘ringing in the ears,’ but hissing and roaring sounds also fall under the rubric of tinnitus. A more comprehensive definition is: “tinnitus is the conscious perception of a sound that is not generated by any source outside the body.” This definition comprises two forms of tinnitus: objective and subjective.

Objective tinnitus can be traced to defective or malfunctioning parts in the body, and results from internal sound sources such as abnormally strong pulsating blood vessels and spasms of the middle ear muscles. Most of these defects can be treated surgically and often result in the disappearance of tinnitus.

after losing a digit or, more severely, a limb. Itch or pain in a nonexistent part of the body is truly annoying and so is tinnitus. The pitch of tinnitus corresponds, when there is a hearing loss, to the frequency of that hearing loss. In case of low-frequency hearing loss (as in Ménière's disease) the tinnitus is low pitched ('roaring'), but in noise-induced high-frequency hearing loss the pitch of the tinnitus corresponds to a high-pitched ringing or hissing sound. The brain 'hears' the sound of the missing frequencies in the ear: a phantom sensation (**Figure 1**).

Not all persons with hearing problems resulting from hair cell loss have tinnitus or complain about tinnitus. In other words, cochlear damage does not always result in tinnitus. It is thus likely that differences in central processing are also important in the generation of tinnitus. Conversely, not all persons with tinnitus have a hearing loss. This suggests that the idea of a phantom sound does not universally work and that tinnitus is not a unitary concept.

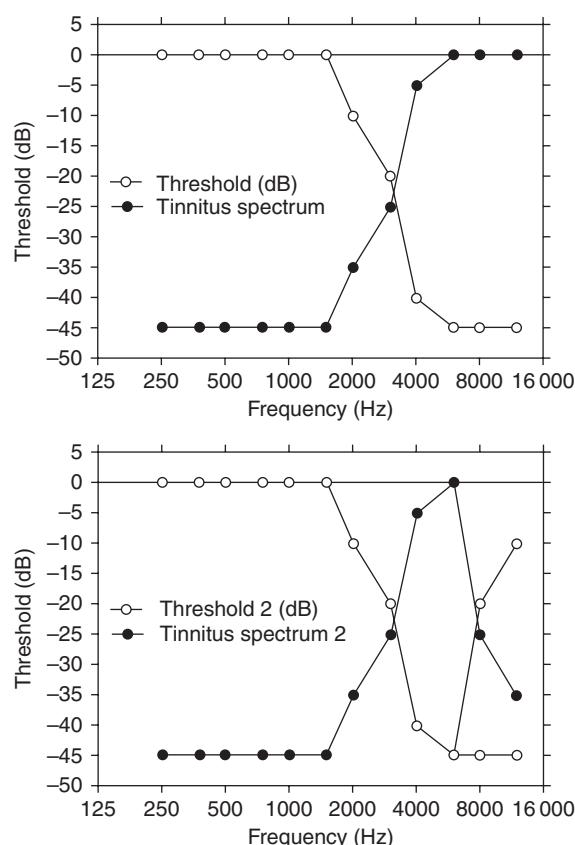


Figure 1 The tinnitus spectrum, that is, the range of frequencies making up the tinnitus, is closely related to the inverse of the hearing loss. This is shown here schematically. The top example with a relatively broad frequency loss likely would represent a hissing tinnitus, whereas the bottom example with a limited frequency range of loss could represent a more tonal tinnitus. A strict one-to-one correspondence of hearing loss and tinnitus pitch does not hold however.

Tinnitus without audiometric hearing loss can still be attributed to damage in the inner ear. Dead regions in the cochlea that are too small to be detected with standard octave spaced audiograms or hearing loss at frequencies above the 8-kHz upper limit of standard clinical measurement can be a contributor.

Somatic Tinnitus

Tinnitus can also result from head and neck injury and typically localizes to the ipsilateral ear, but generally there is no resulting hearing loss in that ear. There are at least two entrance points for somatosensory information in the auditory system: the dorsal cochlear nucleus (DCN) in the brainstem and the inferior colliculus (IC) in the midbrain. The input to the DCN granule cells originates among others from the trigeminal nuclei and the dorsal column nuclei. These represent both facial and upper spinal cord information, reflecting the position of the head and pinnae that are relevant for localization of sound. The output of the granule cells via the horizontal fibers is typically excitatory to the fusiform cells (the principal output cells of the DCN) that also receive excitatory input from auditory nerve fibers. However, the horizontal fibers also excite cartwheel cells that in turn inhibit the fusiform cells. Thus the increase or decrease in the output of the somatic nuclei as a result of injury could disrupt the balance between excitation and inhibition in the DCN and affect the spontaneous input to the IC. This could account for sources of tinnitus as diverse as whiplash, temporo-mandibular joint problems, orofacial movements, and the like.

Listening to Tinnitus

Listening to one's own tinnitus and matching it to externally generated sounds aim to objectively quantify tinnitus. This area of research uses psychoacoustic methods that can assess pitch and loudness, and measure the effects of masking sounds on tinnitus.

Pitch

Pitch matches often occur in the frequency region of maximum hearing loss or occasionally at the edge frequency of the hearing loss. Pitch-matching reliability varies widely across patients. Pitch matches can also vary from day to day or within a day. This may represent subtle shifts in the dominant frequencies in the tinnitus spectrum. The tinnitus percept can often be synthesized by combining pure tones into a tinnitus spectrum (**Figure 1**).

Loudness

Loudness is usually measured by a rating procedure or by matching it to the level of external sounds. The loudness of tinnitus is typically matched to sound levels that are only a few decibels above the hearing threshold. However, because of the recruitment type of hearing loss that underlies tinnitus this could still represent a fairly loud sound. The loudness level of tinnitus fluctuates, and this could be due to test-retest variability, actual fluctuation of the tinnitus loudness, and changes in tinnitus pitch or loudness produced by the measurement stimulus if presented to the tinnitus ear. Presenting a matching stimulus to the contralateral ear might reduce potential interference with tinnitus loudness, but because of central interactions it might not completely eliminate them. One way to avoid this sound-tinnitus interaction is to use cross-modal loudness matching.

Masking

Masking is based on two mechanisms: (1) a so-called ‘line-busy’ effect where the masking sound activates the neurons and prevents them from firing to a probe sound (e.g., tinnitus) and (2) a suppression effect where the masker interferes with the mechanical activity pattern of the probe sound in the cochlea. Although pure tones can mask tinnitus completely in the majority of patients, it does not follow the standard effects that a masker has on an external probe sound. It appears that the cochlear suppression mechanism is impaired in tinnitus patients likely because of the hearing loss (based on the measurements of psychoacoustic tuning curves). This finding again points to central effects that are different compared to controls. If the changes induced by the masker, and the generation site of tinnitus, were at the cochlear level, the masking of an external pure tone would be similar to the masking of tinnitus.

Residual Inhibition

This is a postmasking effect that, because of its long duration (usually seconds, but can last for minutes to hours), is again a central effect: in controls, such forward masking on external tones always lasts less than half a second. The residual inhibition is generally the largest when using masking sounds in the range of hearing loss and that resemble the tinnitus spectrum. The results suggest that cortical map reorganization induced by noise-induced hearing loss, which results in an overrepresentation of the edge frequency in the audiogram, is not the principal source of the tinnitus sensation. Because, in that case, one would expect the tinnitus pitch to match the edge frequency and that edge-frequency sounds would result in the largest residual inhibition.

Measuring Tinnitus Annoyance

Results of psychoacoustic-loudness estimates of tinnitus have been repeatedly shown to have little, if any, correlation with the degree of tinnitus severity or annoyance. The latter is generally assessed using tinnitus questionnaires. There are at least a dozen published outcome instruments that are used to obtain tinnitus-severity ratings; however, there is no consensus regarding their use across tinnitus treatment centers. In some ways, tinnitus resembles certain forms of pain, specifically neuropathic pain. Pain and tinnitus both may cause emotional and psychological distress that is out of proportion to the magnitude of the injury. Moreover, both pain and tinnitus are often associated with dysfunctional, inappropriate coping strategies. So treatments applicable to pain may be relevant to consider in the case of tinnitus.

Objective Measures in the Brains of People with Tinnitus

Objective methods to assess ongoing and spontaneous brain function in humans include recording the electroencephalogram (EEG) or the magneto-encephalogram (MEG). Stimulus-evoked changes therein are known as auditory evoked potentials (AEPs) or evoked magnetic fields (EMFs). The AEPs ([Figure 2](#)) are typically recorded from the scalp and reflect compound action potential activity in the auditory nerve and certain brain-stem centers (the auditory brainstem response), or compound postsynaptic potentials in the auditory cortex (the middle- and long-latency cortical potentials). In order to be detectable at the scalp, the neural activity needs to be tightly synchronized in time and the neural sources need to be oriented parallel in space.

Positron Emission Tomography and Functional Magnetic Resonance Imaging

Neural activity requires energy, which has to be provided by the blood flow in the brain regions that need it. Changes in the blood flow can be much localized and can be indirectly measured using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). The advantage of PET is that it is a silent technique whereas fMRI suffers from a high level of background noise related to the scanning cycle that could mask the tinnitus effects one wants to measure. The disadvantage of PET is that it has a poor spatial resolution compared to fMRI and that it requires injection of a radioactive tracer. High-resolution frequency-place (tonotopic) maps in auditory cortex have been obtained from fMRI by using high-strength magnetic fields (7 T).

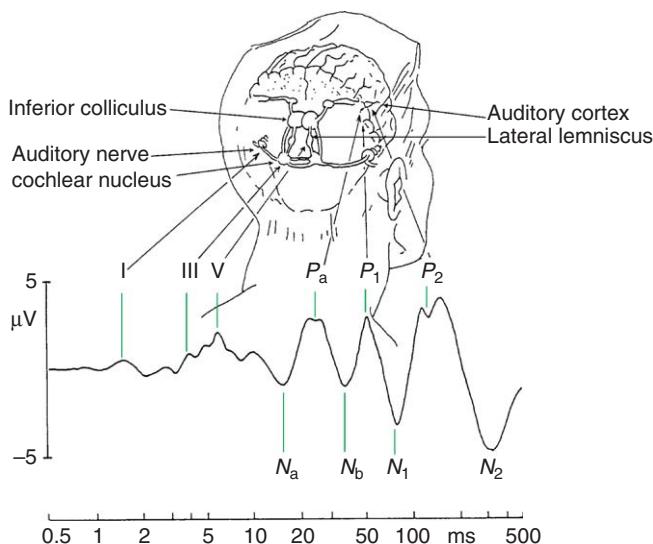


Figure 2 Schematic of auditory evoked potentials and putative generation sites. The waves labeled I, III, and V represent components of the auditory brainstem response (arrows indicate origin of these waves and the names of the structures). The components N_a , P_a , and N_b form the middle-latency response, and the P_1 , N_1 , P_2 , and N_2 represent the late cortical potentials. Note the logarithmic time axis. Reproduced from Picton TW, Hillyard SA, Krausz HI, and Galambos R (1974) Human auditory evoked potentials. I. Evaluation of components. *Electroencephalography and Clinical Neurophysiology* 36: 179–190 with permission from Elsevier.

Imaging tinnitus in humans needs to demonstrate increased neural activity during silence (generally seen as the crucial substrate linked to tinnitus). This latter problem cannot be readily solved at the group-comparison level between tinnitus patients and normal controls because of high variability in, and poor control of, resting-activity levels among people. Imaging tinnitus has been addressed indirectly by focusing on a group of persons, who can modulate the strength of their tinnitus by either changing eye-gaze direction or by making orofacial movements. An alternative is to temporarily abolish the tinnitus by intravenous lidocaine injection. For these groups, it has been demonstrated with PET scans that increased tinnitus loudness corresponds to increased neural activity in several auditory and nonauditory areas. The group data typically showed significant tinnitus-related hyperactivation in auditory cortex, but not in the primary areas (**Figure 3**).

In most cases, the presence of tinnitus can be linked to hearing loss. Various sources of evidence indicate that reduction of primary sensory input to the brain leads to an enhanced power in the delta frequency range (<4 Hz). The spontaneous cortical neuronal activity was characterized by a marked reduction in alpha (8–12 Hz) power together with an enhancement in delta (1.5–4 Hz) as compared to a normal-hearing control group. This pattern was especially pronounced for temporal regions of the brain. Moreover, tinnitus-related distress showed strong associations with this abnormal spontaneous activity pattern. There is also evidence that in persons with tinnitus spontaneous gamma-band activity correlates both with the annoyance of tinnitus and the ear to which it was localized.

By comparing human tinnitus subjects with normal controls and using tonal edge-frequency stimuli and tonal stimuli with a one-octave-lower frequency to elicit EMFs, it was found that the source location for the edge-frequency N_1 dipole (**Figure 2**) was abnormal. However, the map abnormalities that this study indicated did not relate to the strength of the tinnitus percept. This suggests that tonotopic map changes might be an epiphenomenon. In the auditory brainstem response, no differences in amplitude compared to controls were found but exceptionally large middle-latency response waveforms in the problem tinnitus group were found compared to normal-hearing controls, and a hearing-loss group without tinnitus. This could point to increased neural synchrony in auditory structures from the midbrain up.

All in all, these findings suggest that changes in the spontaneous EEG or MEG spectrum, increased evoked-potential or evoked-magnetic-field amplitude suggesting increased neural synchrony, tonotopic map changes in auditory cortex, and increased spontaneous firing rates in the auditory cortex are potential neural correlates of tinnitus in humans.

Objective Measures in the Auditory System of Animals

Do Animals Have Tinnitus?

The neural substrate of tinnitus can only be adequately studied in animal models that show behavioral evidence of tinnitus induced by agents similar to those causing

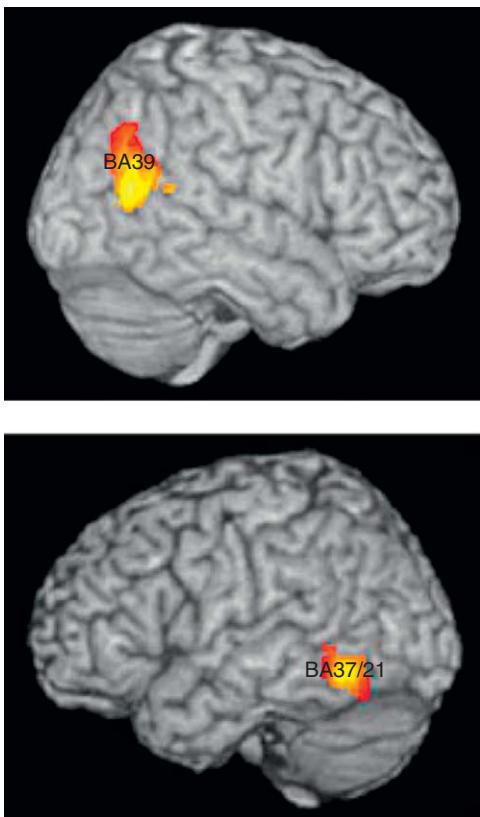


Figure 3 The PET-recorded activity related to tinnitus is seen in the right angular gyrus (BA39) and the left lower temporal cortex (BA37/21). Yellow colors indicate the highest activity levels. Here the significant differences between activity during tinnitus and that following intravenous injection of lidocaine, which temporarily abolishes tinnitus, has been plotted. This is an indirect way of quantifying the activity related to tinnitus. Reprinted from Plewnia C, Reimold M, Najib A, et al. (2007) Dose-dependent attenuation of auditory phantom perception (tinnitus) by PET-guided repetitive transcranial magnetic stimulation. *Human Brain Mapping* 28: 238–246 with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

tinnitus in humans. Behavioral test models have been devised for several species. The findings are evidence that the conditions causing tinnitus in humans are also the same for common experimental animals.

Tinnitus and Changes in Spontaneous Firing Rate

A generally accepted neural correlate of tinnitus is increased spontaneous firing rate (SFR), and this is supported by objective measurements in humans as reviewed above. The fact that tinnitus is audible and ‘normal’ spontaneous activity in the auditory system is not (or only under extreme conditions such as being for some time in a completely soundproof room) indicates that (1) either the SFR is higher than a certain threshold set by evolution and lifetime experience (a

type of memory) or (2) the spontaneous firing activity in persons with tinnitus is different from that in people without tinnitus. These differences could be in temporal properties, such as burst firing (demonstrated to occur in the IC) or other changes in the serial correlation between the firings. It could be changes in the interspike interval distribution or, conversely, changes in the spectrum of spontaneous activity. It is also possible that changes occur in higher-order statistics of the population firing activity. However, most commonly, the changes observed in animal models are in the SFR as shown in the next two paragraphs.

Noise trauma. After noise trauma, the SFR in auditory nerve fibers (ANFs) was generally significantly reduced, but was significantly enhanced *in vitro* in chinchilla DCN. The *in vivo* recordings in hamster DCN indicated massive increases in SFR of superficial layer cells 5–180 days after noise exposure but not 2 days after. Complete or nearly complete section of ascending or descending inputs did not significantly affect the magnitude of DCN hyperactivity. The SFR increase correlated significantly with the strength of the behavioral index of tinnitus in the same animals. In the IC of mice, noise trauma significantly increased the SFR. In cat auditory cortex (AC), significant increases in SFR occurred after 2 h following the trauma, but not immediately (<15 min) following it. At least 3 weeks after the trauma, the SFR was significantly larger than in controls at all recording sites tested and not in the region of the hearing loss alone, although that region showed more pronounced changes.

Salicylate. Chronic salicylate application in doses that did not produce hearing loss did increase the spontaneous activity of the auditory nerve. Such low doses of salicylate in humans can result in tinnitus. Larger, acute doses (200 mg kg^{-1}) of salicylate failed to increase SFR in cat and gerbil ANF, whereas a still higher dose of 400 mg kg^{-1} did. In rats, moderate doses ($>200 \text{ mg kg}^{-1}$) of salicylate resulted in decreased mean interspike intervals (suggestive for increased SFR or burst firing) in IC neurons. In the IC of guinea pigs, 200 mg kg^{-1} of sodium salicylate increased the mean SFR threefold at 100 min after application. In mice, however, a dose of $200\text{--}300 \text{ mg kg}^{-1}$ decreased SFR significantly in IC. In anesthetized cats, salicylate at a dose of 200 mg kg^{-1} did produce increased SFRs for high characteristic frequencies (CFs) in secondary auditory cortex, but did not have an effect in primary auditory cortex. In awake rats, salicylate levels of 150 mg kg^{-1} that induced behavioral signs of tinnitus decreased SFRs in primary auditory cortex significantly. Therefore, salicylate results appear to be variable and more species dependent than those found for noise trauma.

Changes in Neural Synchrony

The degree to which the action potentials from two different neurons are time locked or, in other words, fire in synchrony may be important for tinnitus, as also suggested by the increased AEPs in people with tinnitus. Neural synchrony under spontaneous firing conditions was studied in the same single-neuron pairs before and after a 1-h exposure to a 5- or 6-kHz tone presented at a level of 115–120 dB SPL. The average hearing loss 6 h after the trauma was about 40 dB in the range of 6–32 kHz. Recordings from the primary auditory cortex were done using two multi-electrode arrays of eight electrodes each in each cat before and up to 6 h after exposure to the trauma tone while leaving the electrodes in place. The increase in peak synchrony was significant within 15 min after the trauma, and increased to an average of 54% increase over the pre-exposure values at 2 h after the trauma. Thus, increased neural synchrony may be a more direct contributor to tinnitus than increased SFR, because the latter takes 2 h to significantly increase, and tinnitus is generally experienced immediately after noise trauma. Chronic effects of noise trauma showed that the peak synchrony for neuron pairs in reorganized cortical areas were significantly higher than for pairs in nonreorganized areas and also significantly higher than for controls. Thus, cortical tonotopic map reorganization (see below) and increase in peak cross-correlation coefficients are closely linked.

Changes in Cortical Tonotopic Maps

Local mechanical damage to the cochlea, ototoxic-drug damage to the cochlea, and noise-induced hearing loss, all cause tonotopic map changes in primary

auditory cortex (**Figure 4**). The map changes are not causally related to the hearing loss but are always accompanied by increased SFR and increased neural synchrony. Map changes do not occur, if immediately after noise trauma, a compensatory complex sound that mimics the expected hearing loss in bandwidth and level is presented for several weeks. When this happens, the downregulation of inhibition that usually follows noise-induced hearing loss likely does not occur and the unmasking of new excitatory inputs does not happen or is reversed. When this ‘unmasking’ trigger for tonotopic map reorganization is absent, map changes do not occur despite a remaining hearing loss. If the enhanced acoustic environment does not match the hearing-loss frequencies, the effect thereof is lost.

Population Neural Activity

Despite a reduction in the compound action potential amplitude of the auditory nerve and the local field potential (LFP, an intracranially measured AEP) in the cochlear nucleus following noise trauma, the LFP amplitude in the auditory midbrain was typically enhanced at high intensity levels. After salicylate application, single-unit firing rates in the AC of awake rats were reduced, but the LFP was enhanced at 5, 8, and 16 kHz, suggesting increased neural synchrony or reduced lateral inhibition, resulting in unmasking of new excitatory units. These changes are thus comparable to the absence of changes in the auditory brainstem response and the increased cortical evoked potentials as measured in humans (see above).

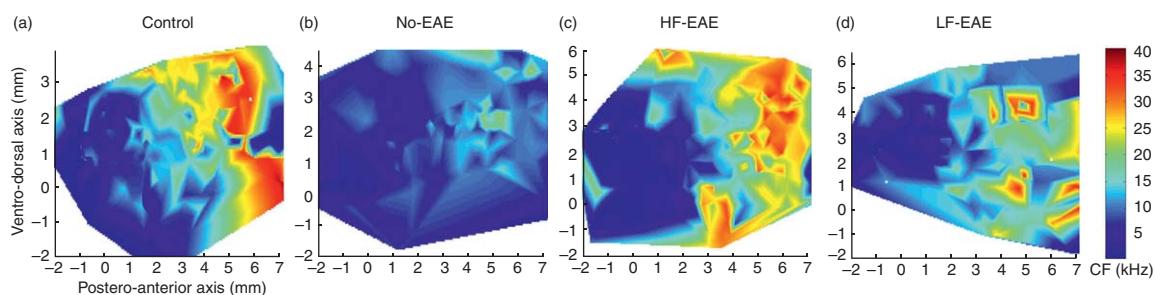


Figure 4 (a) Cortical tonotopic maps in control cats; (b) in cats following noise trauma and rearing in a quiet environment; (c) in cats following noise trauma and rearing in an enhanced acoustic environment that produced increased activation in the hearing loss range; and (d) in an enhanced acoustic environment that emphasized the normal hearing range. Along the horizontal axis (parallel to the midline) distance from the tip of the posterior ectosylvian sulcus (PES) is plotted, and along the vertical axis the distance in the ventral-medial axis again from the tip of the PES. The color bar indicates the characteristic frequency of each recording site with blue colors indicating low frequencies and red colors high frequencies. One notices the absence of high frequencies in the tonotopic map, and thus reorganization of the map, following noise trauma and rearing in quiet. These changes do not occur if an enhanced acoustic stimulation of the hearing loss frequencies occurs (c), but do occur when the enhanced acoustic stimulation does not match the hearing loss frequencies.

Homeostatic Mechanisms as a Cause for Tinnitus?

Homeostatic mechanisms are assumed to stabilize the mean activity of a neuron around a certain target level. The changes occur over timescales on the order of days and typically are effected by scaling the efficacy of all synapses of a neuron. Homeostatic plasticity can be modeled by a change in a gain factor that is proportional to the deviation of the mean activity from a certain target rate. In such a model, homeostatic plasticity restores the mean firing rate of a neuron after hearing loss. This mean firing rate includes both stimulus-driven and spontaneous parts, and both will be scaled upward to the target level. This applies to all neurons along the auditory pathway. Restoring the mean rate, therefore, likely increases the spontaneous rate throughout the auditory system. Interestingly, such a model also allows the evaluation of how the pathologic changes could be reversed through additional sensory stimulation. It is obvious that hyperactivity could be reversed in the model by restoring the regular spatial distribution of firing rates of auditory nerve fibers. As reviewed above, the effectiveness of stimulation that restores this spatial distribution of firing rates in the auditory nerve fibers has been demonstrated (**Figure 4**).

Conclusion

In the search for bottom-up (i.e., sense-organ driven) mechanisms of tinnitus, increased SFR and neural synchrony have attracted most of the attention. It is not clear whether increased SFRs in subcortical structures alone will lead to increased SFR in cortex, despite the action of homeostatic mechanisms. It is far more likely that increased neural synchrony in subcortical structures will propagate effectively along the auditory pathway and ultimately result in increased synchrony and/or increased SFRs in cortex. Increased neural synchrony combined with increased SFR would be very powerful indeed. Human studies using fMRI that find increased activation in certain brain regions potentially implicate an increased SFR in those regions. Enlarged evoked potential or magnetic fields in addition imply increased neural synchrony as its underlying mechanism. The fMRI data point to increased SFR in midbrain, thalamus, and cortex, whereas evoked potential data point to increased synchrony in these same structures. It is likely that both conditions co-occur as both may result from downregulated

inhibition levels. The laterality of the strongest activity in the EEG gamma band corresponded clearly with the laterality of the tinnitus percept. Gamma activity being a consequence of enhanced spontaneous neural synchrony was more prominent in tinnitus subjects than in controls. In that sense, gamma activity may play a similar role as it has been posited to do in normal auditory perception.

See also: Brain Imaging; Cognition: Learning and Memory: Pavlovian; Emotion–Cognition Interactions; Human Fear and Anxiety; Plasticity in the Primary Auditory Cortex: Substrate of Specific Long-Term Memory Traces; Regulation of the HPA Axis by Acute and Chronic Stress; Role of Neuronal Synchrony in Normal and Pathological Brain Functions.

Further Reading

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Vision

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Glossary

Binocular rivalry – In this paradigm, two different images are presented separately to the two eyes. Under some conditions, the two images are fused to a combined perception, but this occurs only rarely. Most of the time one of the two images is perceived and the other image is suppressed. Which of the two images is perceived alternates, similarly as in bistable stimuli such as the Necker cube, for instance.

Functional magnetic resonance imaging (fMRI) – Using the different magnetic properties of oxygenated and deoxygenated hemoglobin, this imaging technique measures the blood-oxygen-level-dependent (BOLD) signal. A high BOLD signal is caused by a high oxygen level, which is in turn caused by a high cerebral blood flow. Cerebral blood flow increases if the consumption of oxygen is high. Neurophysiological studies showed that the BOLD signal is related to the input and the internal processing of a given area rather than its output.

Motion detector or Reichardt detector – A neural circuit for the detection of visual motion proposed by German biologists Werner Reichardt and Bernhard Hassenstein. It is based on the simple idea that represented movement as a position change from location A to location B, occurring within a certain time period t . A neuron N_c receives input from two neurons N_a and N_b whose receptive fields are at locations A and B. The connection from N_b to N_c is delayed by time t , and the neuron N_c only fires if the signal from N_a and N_b reaches N_c at the same time: it multiplies, so to speak, the incoming signals. In other words, the neuron N_c only fires if N_a is stimulated at time t later than N_b . Thus, neuron N_c is a detector of motion from location B to A. To disentangle flickering stimuli from true motion, two Reichardt detectors with opposite directions have to be combined.

Primary visual cortex (V1) – V1 is located in the occipital cortex and is crossed by a white stripe of axons; therefore, it is also called striate cortex. It is the first cortical area that processes visual information transmitted by the optic radiation. A lesion of V1 leads to blindness in the affected visual field and to a loss of conscious visual perception. However, V1 is not sufficient for conscious visual experience because lesions in other parts of the brain can lead to blindness for selective visual attributes such as motion or faces.

Random-dot kinematogram – Such displays are often used to study motion perception. Dots are distributed randomly in the display. In each new frame, a few randomly selected dots (i.e., the signal dots) are replaced by dots in the same direction and with the same step size, while the remaining dots (i.e., the noise dots) are replaced randomly. The advantage of this kind of stimuli is that they allow to vary the strength of the motion signal by varying the proportion of signal dots. Most importantly, motion cannot be recovered by following a single dot. Instead, information has to be integrated across the visual field.

Stereogram – In a stereogram, two nearly identical pictures are presented to the two eyes. By introducing spatial offsets to objects between the two pictures, a perception of depth is introduced. In vision research, stereograms are often made up of random arrangements of black and white dots, with some spatial offset between the pictures for the two eyes. In a special version, the anticorrelated random-dot stereogram, one random-dot pattern is the contrast-inverted version of the other pattern.

The significance of visual perception for the daily life of humans and other primates can be seen by the number and size of cortical brain areas associated with visual processing and the preparation of vision-related behavior. Next to the primary visual cortex (V1) and the adjacent area V2, which occupy around 13% and 10% of the cortex, respectively, more than 40 anatomically and/or functionally distinct subdivisions of visual responsive cortex were identified. In total, around 60% of the primate cerebral cortex is involved in the perception, interpretation, and the reaction to visual stimuli.

Overview

Information processing in the visual system begins with the photoreceptors in the eye. In the retina, there is a large degree of convergence from photoreceptors to ganglion cells. In the primary visual pathway, the different ganglion cells send their axons to the cells in the different layers of lateral geniculus in the thalamus, which project mainly to the primary visual cortex. In the cortex, there is

an enormous amount of divergence. Every part of the visual field has to be examined along several properties such as color, orientation, texture, movement, and depth. After this initial stage of processing in V1, processing of visual information splits into two distinct pathways, a dorsal processing stream toward the parietal cortex and a ventral processing stream toward the lower temporal cortex.

Despite our knowledge about where different aspects of visual stimuli are processed, our understanding of how they are processed is rather limited. The initial stages of cortical processing seem to deal with the extraction of single stimulus features. In V1, neurons are capable of signaling the orientation and color of stimuli. Enormous progress has been made in identifying the selectivity of higher levels of processing for specific visual stimuli (e.g., hands, faces, or movements), but the computations underlying this selectivity are largely unknown. The processing stages from simple edge primitives to whole objects are still a big mystery for scientists, and the fact that the visual system can solve this in less than 100 ms does not make its solution any easier!

Retinal Processing

The human retina is densely packed with photoreceptors that transform light into electrical signals. Humans possess four different types of retinal photoreceptors: rods and three types of cones. Whereas rods have a high sensitivity and are important for perception under low illumination, cones are the basis of perception during daylight. The three different types of cones differ in their spectral absorption curves and thus enable the perception of color. Information from the cones is conveyed to retinal ganglion cells through a complicated network of horizontal and vertical connections that achieve a highly efficient coding process.

Retinal ganglion cell are always stimulated from the same photoreceptors, which react to stimuli in a certain part of the visual field. This area in the visual field, where visual stimulation causes a physiological response, can be accurately mapped, and is called the receptive field. Ganglion cells that are positioned adjacent to one another have overlapping receptive fields, and they also project to the neighboring neurons in the next, higher processing stage. This spatial order is maintained from the receptor level in the retina to higher levels of processing in the cortex. At each stage, the size of the receptive fields increases.

Almost all (90%) retinal ganglion cells send their signals through their axons (the optic nerve) to the lateral geniculate nucleus (LGN), which is located in the thalamus. The LGN is the most important subcortical relay station between eye and cortex. It is made up of six layers:

the two inner layers (1 and 2), which contain relatively large (magno-)neurons (about 10% of the LGN population) with large receptive fields and fast, transient responses. The four outer layers contain smaller (parvo-)neurons (about 80% of the LGN population) with small receptive fields and slow, sustained responses. The distinction between a magno- and a parvocellular pathway starts at the first synapse in the retina with diffuse bipolar cells and parasol ganglion cells for the magno- and midget bipolar and midget ganglion cells for the parvocellular pathway. Although there are differences in the functional properties of these neurons, our visual system could work with only one of these pathways. The biggest difference is that magnocellular cells of the LGN are almost completely colorblind. They process information only about the brightness of the visual stimuli, have a higher contrast gain at all luminance levels, and are involved in the processing of rapid picture sequences and flickering stimuli. The parvocellular layers of the LGN process information about the brightness and red–green differences. Damage to parvocellular layers of the LGN leads to a loss of color vision, and also results in a reduction of visual acuity. The information regarding blue–yellow differences is passed on to the koniocellular layers of the LGN. These are thin in-between layers and were discovered more recently.

Through the preservation of spatial order of the retinal ganglion cells, also the LGN is retinotopically organized. In the optic chiasm, axons of nasal–retinal ganglion cells cross the side, so that each LGN receives only input from the contralateral visual field. However, the separation of the signals of the two eyes is still preserved as the layers 1, 4, and 6 receive input from the contralateral eye and the layers 2, 3, and 5 from the ipsilateral eye. The visual maps of the six layers sit above one another, and the receptive field of vertically aligned neurons are located at the same retinal position. The LGN is not only a relay station for incoming signals from the eye to the V1, but it also receives input from the cortex and the brainstem, which modulates the retinal information stream into the LGN.

Retinal ganglion cells have small circular receptive fields, which show an antagonistic center-surround organization. In the fovea, the location of greatest acuity, the receptive field centers are only a few minutes in diameter, whereas in the periphery they reach 3°–5° of visual angle. Approximately half of the ganglion cells are the so-called on-center cells: they are excited by light projected into their center and inhibited by light illuminating their surround. The remaining are off-center cells, and they are inhibited by central light and excited by surround light. If the entire receptive field of either ganglion cell-type is stimulated with light, they respond only weakly. Because of this antagonistic center-surround mechanism, ganglion cells are very sensitive to spatial contrast. If ganglion cells are stimulated by, for example, black

and white patterns, the simultaneous inhibition and excitation of center and surround causes a response behavior, which can lead to increased differences in brightness perception (Mach bands; Figure 1).

Lateral inhibition intensifies the signals at the edges and blurs the regions of slower-intensity transition. A statistical analysis of natural scenes shows that a large amount of our visual environment consists of stimuli of slow-intensity transition. If ganglion cells did not encode the relative intensities of an image, many neighboring ganglion cells would forward the same signal, which would be very inefficient for the analysis of image information. Instead, because of the lateral inhibition, the bandwidth is reserved for accentuating edges.

A similar problem of redundancy, which is inefficient encoding, arises from color vision. Color vision results from the three different types of cones in the retina. They

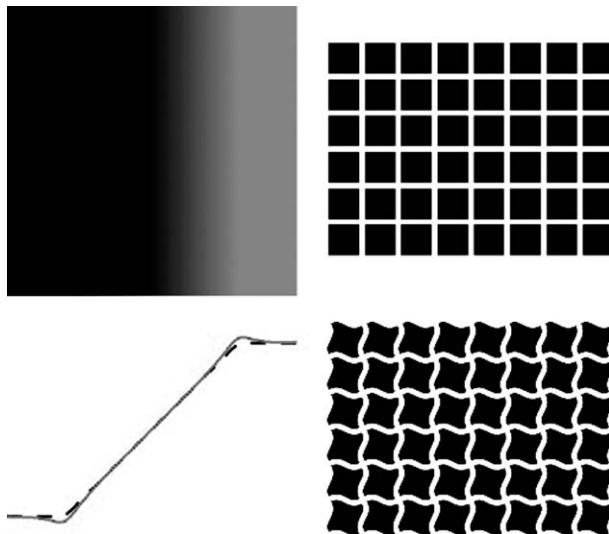


Figure 1 Lateral inhibition. (Top, left) Mach bands. In the middle of the nineteenth century, the physician Ernst Mach discovered that in the perception of a stimulus with a luminance profile, dark and light bands appear at the points where the contrast begins to change physically. (Bottom, left) Stimulus intensity (black) and the resulting neuronal activity of an on-center ganglion cell (red). (Top, right) Hermann grid. When looking at the Hermann grid, illusory small gray disks appear at the white alley crossings situated away from the fixated crossing. These illusory spots may be explained by the centre-surround antagonism or lateral inhibition within the concentric receptive fields of retinal and/or geniculate ganglion cells. The illusion is strongest when the bar width matches the size of receptive-field centers. The smaller diameters in the fovea explain why the illusion tends to be absent in foveal vision. (Bottom, right) Grid modification. When the grid edges are curved, the illusion is reduced, suggesting that also orientation-sensitive cells in the LGN and visual cortex contribute to this illusion. The straight and the curved Hermann grid were taken with permission from Geier J, László B, Hudák M and Séra L (2008) Straightness as the main factor of the Hermann grid illusion. *Perception* 37(5): 651–665 published by Pion Limited, London.

differ from one another in terms of their spectral absorption of light, as shown in Figure 2.

One cone-type, the s-cone, receptor absorbs light mostly in a short-wavelength area between 350 and 450 nm with a peak of 420 nm. The absorption spectrums of the other two cone types peak in the middle- and long-wavelength part of the spectrum and have largely overlapping sensitivities. The reason for this similarity in absorption is that these cones evolved from the same ancestor rather than recent (approximately 35 million years ago). This means that for the eye, the signals of long- (L-) and middle-wavelength-sensitive (M-) cones are highly correlated. The redundancy can be cleaned up by encoding the signals differently. The calculated sum total of the L- and M-cones corresponds to luminance. At the same time, the difference between the signals from the L- and M-cones is calculated, and the difference between the signals from the S-cones and the sum of the L- and M-cones. These difference mechanisms are called color-opponent mechanisms, and their signals are passed on to the parvocellular and the koniocellular layers of the LGN. The color-sensitive neurons in the parvocellular LGN layers show a preference in the red–green cardinal color direction and those in the koniocellular layers, in blue–yellow. Neurons that react strongest to mixed colors, for example, orange, first appear in area V1.

Cortical Visual Processing

Retinotopy and Maps

When we fixate a small object, its image is projected onto a small position in the center of the retina, the fovea centralis, which occupies around 2° of the vision field. Foveal vision has the greatest acuity because here the density of photoreceptors is highest, and also that of the retinal ganglion cells; and the proportionally largest area of visual cortex is devoted to it. It is only in the fovea that we have a high resolution of detailed vision, which is essential for activities such as reading this text. The fovea contains approximately 50 000 ganglion cells per square millimeter compared with 1000 in the periphery. In addition, in contrast to other retinal areas, photoreceptors in the fovea have a 1:1 connection with ganglion cells. Because only a few ganglion cells project to a single neuron in the LGN, the fovea, which only takes up 0.01% of the whole retina, and its immediate surrounding, is represented by approximately half of the neurons in the LGN. The V1 maintains these proportions. Half of the neurons in V1 represent the fovea and its directly connected regions (Figure 3).

Recent investigations suggest that the increased representation of the fovea in the cortex is not only due the density of foveal ganglion cells, but it is also the result of assigning additional area for foveal input. A ganglion cell

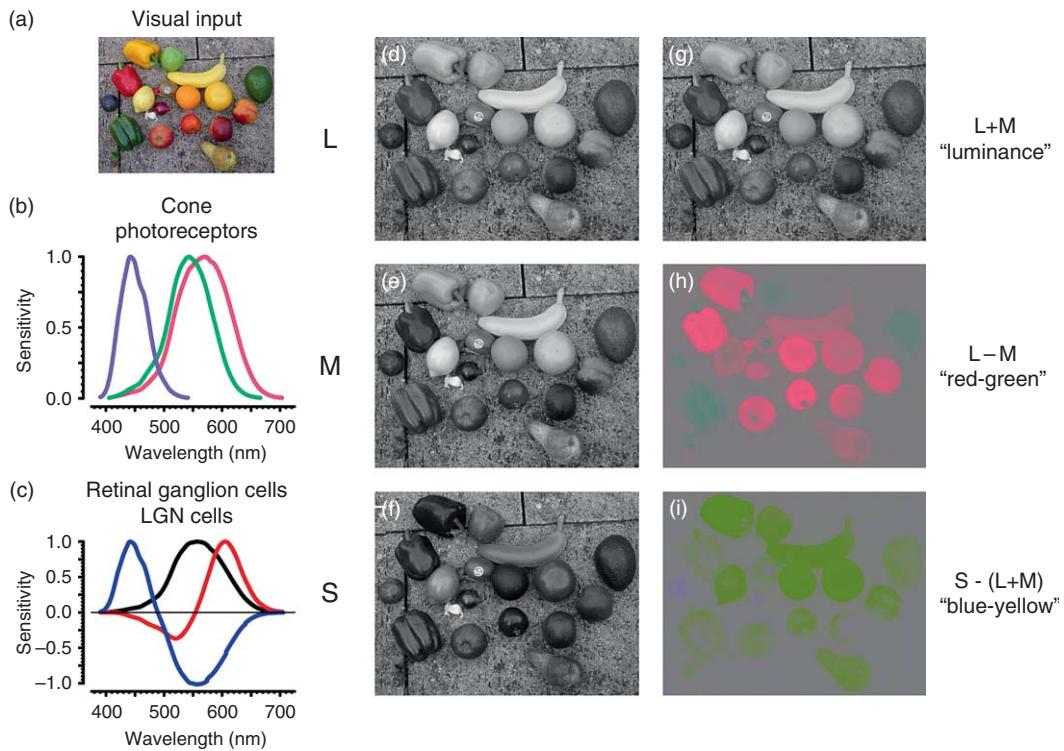


Figure 2 Opponent colors. The transformation of cone signals in color-opponent coordinates. Color vision (e.g., of the picture shown in (a)) starts with the absorption of light by three types of cone photoreceptor (L, M, and S) in the retina (b). The three black and white pictures ((d)–(f)) show how the three cone types are excited by the image in (a). Please note that the L- and M-cone images are similar. The electrical signals generated by these photoreceptors go through complicated circuitry (c) that transforms the signals into three channels: one carrying luminance and the other two being color opponent, red-green and blue-yellow ((g)–(i)). These color-opponent signals are sent to the visual cortex through the thalamic lateral geniculate nucleus (LGN).

close to the fovea is connected to 3–6 times as many cortical cells than a ganglion cell in the retinal periphery. The maximum available processing capacity is concentrated on a relatively small foveal region at the center of our visual field. Because the eyes are movable and any object of interest can thus be brought to project onto the fovea, a large field of vision (180° horizontally) and a precise recognition of details can be achieved at the same time with rather limited resources.

It is known since long that the LGN and V1 are organized in visual field maps, which means that the receptive fields of neighboring neurons cover adjacent areas in the retinal image. Recent advances in functional magnetic resonance imaging (fMRI) have made it possible to investigate these visual maps in higher cortical visual areas as well. Visual maps have been found in various visual areas in the occipital, temporal, and parietal cortices, and these visual maps are arranged in clusters of several maps with a confluent representation of the fovea. As most of these different visual areas are sensitive to specific aspects of the stimuli, the visual scene is sampled several times in the human brain in a spatial orderly fashion. Such topographically organized maps might be especially useful to combine information from different

modalities. For instance, neurons in the parietal cortex have overlapping receptive fields for visual and auditory stimuli and might contribute to the integration of visual and auditory signals.

The Primary Visual Cortex (V1)

David Hubel and Torsten Wiesel examined the response behavior of neurons in V1 to visual stimuli, and they received the Nobel prize in 1981 for this work. In contrast to neurons in the retina or the LGN, V1 neurons respond only slightly or not at all to point-light stimuli, but respond very strongly to short light stripes or lines. Depending on the type of visual stimuli, several types of neurons can be distinguished.

Simple cells respond to stripes or lines of a certain orientation. The elongated receptive fields of simple cells are also distinguished by an excitatory and inhibitory region lying next to one another and oriented in a certain direction (Figure 4). Following the work of Hubel and Wiesel, these long excitatory and inhibitory regions in simple cells are created through the convergence of a few concentric organized cells. Lateral connections between oriented cells lead to a further sharpening of the orientation tuning.

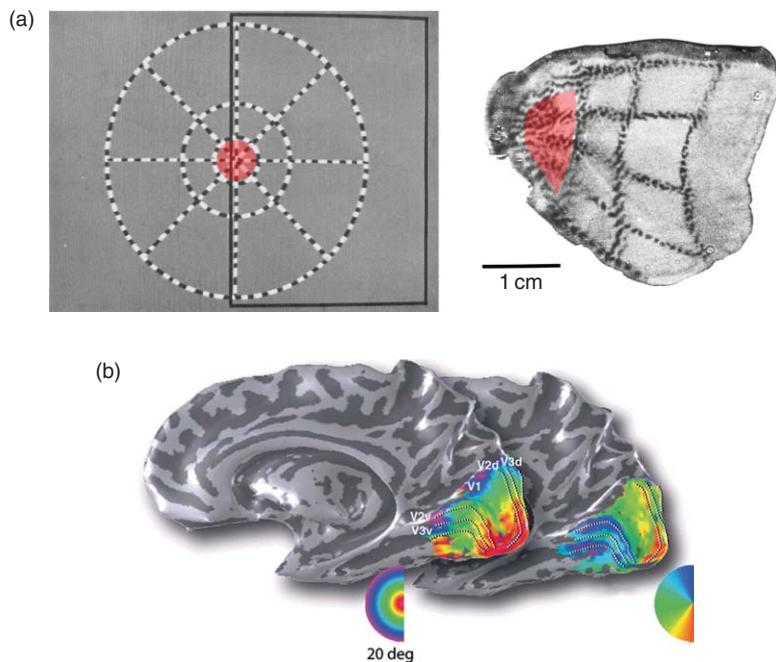


Figure 3 Representation of the fovea in the visual cortex. Although the fovea only takes up an area of 2° in the visual field, it uses a large proportion of the neurons in the primary visual cortex (V1) and the extrastriate visual areas (V2) and (V3). (a) Staining. The stimulus for the mapping of the receptive fields is shown left and the resulting mapping on the right. The foveal region in the stimulus and the cortical tissue is shaded in red. (b) Retinotopic organization of visual areas V1, V2, and V3 measured by fMRI in the human brain with a series of expanding concentric annuli (left) or rotating sectors (right) (a) (modified from Tootell RB, Silverman MS, Hamilton SL, Switkes E, and De Valois RL (1988) Functional anatomy of macaque striate cortex. V. Spatial frequency. *Journal of Neuroscience* 8: 1610–1624. (b) From Wandell BA, Dumoulin SO, and Brewer AA (2007) Visual field maps in human cortex. *Neuron* 56(2): 366–383.

Accordingly, the strongest response results from an elongated stimulus presented in the same orientation and width as the excitatory region of the cell. The selective sensitivity for a particular orientation is measured by comparing the cell's response to stimuli of different orientations (orientation-tuning curve).

Complex cells do not show a clear spatial division between excitatory and inhibitory regions in their receptive fields. However, they do answer selectively to the orientation of elongated stimuli, but independent of the exact position of the stimuli within the receptive field. The properties of the receptive fields of complex cells can also be explained in terms of convergent excitatory input from simple cells with similar receptive fields.

Hypercomplex (end-stopped) cells respond to edges or lines of a specific length. If the stimulus exceeds what has been termed the ‘classical receptive field’ or ‘minimum response field,’ activation of the nonclassical surround region modulates the firing of the cells.

By placing microelectrodes into the cortex, it was discovered that the center of the receptive fields from overlapping neurons are at the same location in the visual field and that these neurons also prefer the same stimulus orientation. By examining the orientation preference of neurons tangentially along a certain cortical layer, we find that the optimal stimulus orientation shifts in continuous

steps through all angles, as we measure from consecutive, adjacent, column-shaped regions. These were conveniently named orientation columns, and each column has a width of approximately $30\text{--}100\ \mu\text{m}$. With the help of a radioactive substance, the activity of neurons can be directly imaged on the cortical surface. Using this method, it was possible to prove the organized structure of these columns in V1. We find the organized orientation columns along one dimension and the so-called ocular-dominance columns along the other dimension, which show a preference for the left or right eye, consecutively alternating as we measure from one column to the next.

To summarize, V1 seems to be made out of a column system, which is characterized by three features with respect to (1) the position of the receptive fields (all neurons within approximately 1 mm^2 receive their input from the same location on the retina); (2) the ocular-dominance dimension (along this dimension, the columns of the left and right eyes alternate systematically; so within a 1-mm^2 section, there are essentially two ocular-dominance columns, one for each eye); and (3) the orientation dimension (every ocular-dominance column contains a complete set of orientation columns from 0° to 360°). Hence a 1-mm^2 section contains the two eye-dominance columns and a series of orientation columns and is called a

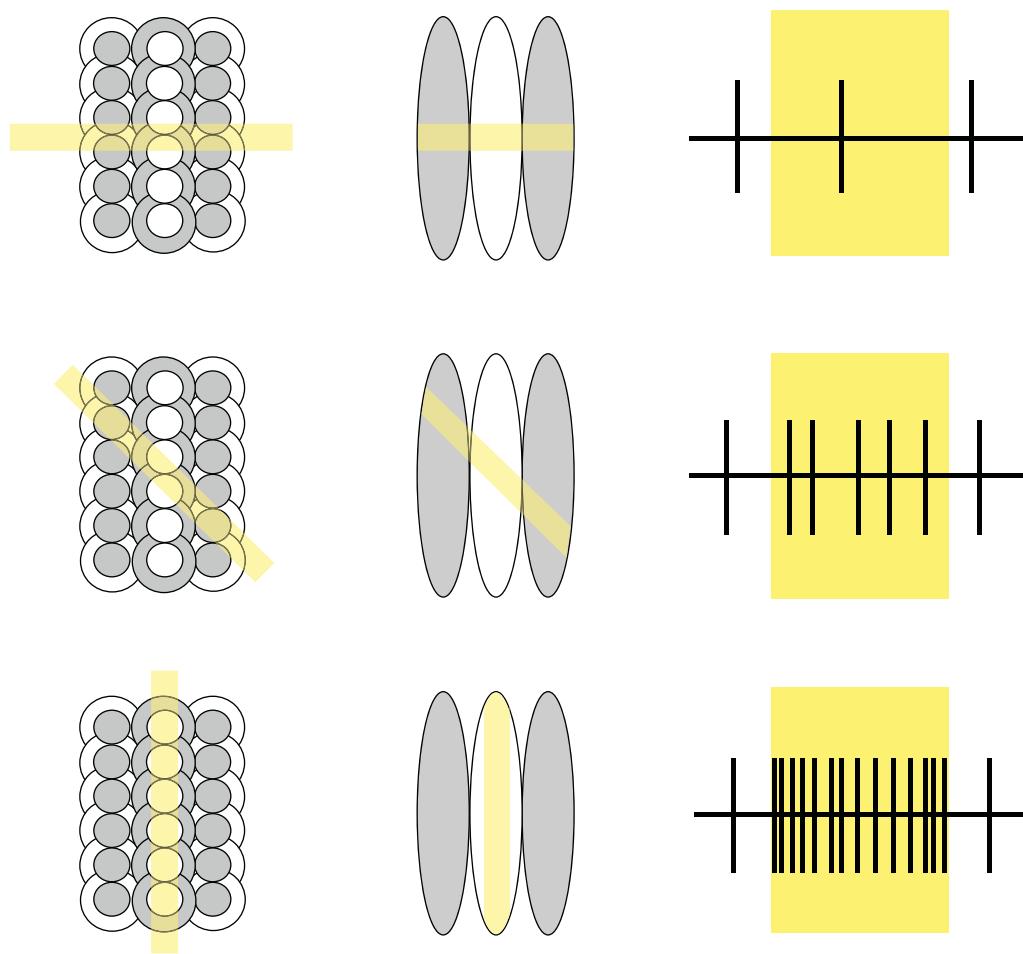


Figure 4 Orientation sensitivity and response behavior of neurons in V1. The left side shows how oriented receptive fields can be combined from concentric receptive fields in the input layer. Excitatory and inhibitory areas are shown in white and gray, respectively. In the middle is the larger orientation sensitive receptive field of simple cells. The right side demonstrates the simple cell's response to light bars (shown in yellow) of various orientations. Every vertical line corresponds to one action potential. This neuron reacts best to vertical light bars.

hypercolumn. The surface of the primary visual cortex is made out of regular ordered hypercolumns, which represent the necessary requirements for the detailed analysis of visual stimuli.

Parallel Processing Pathways

In the 1980s, the first parallel-processing computers appeared and some scientific theories of brain processing have been modelled on this technology. The functional column system in V1 shows that different aspects of visual stimuli are processed distinct from each other. Every image point is analyzed in terms of its orientation, color, movement, and depth. It has been proposed that the brain computes all these local analyses at the same time in parallel. There is some empirical support for this hypothesis. At the level of anatomy, V1 and V2 show the largest number of distinct connections. The enzyme cytochrome oxidase (CO) makes those areas rich in this substance

visible when stained. Within layers 2 and 3 of area V1, there are CO-rich blob regions in the centers of the hypercolumns, which result in a characteristic spotted appearance. In area V2, we also see a distinct pattern when stained with CO, in which thick and thin stripes alternate with pale stripes. It has been hypothesized that these anatomical differences also have functional significance. The idea is that color is processed in the blob regions of V1 and the thin stripes in that of V2. A similar pattern was postulated for the analyses of movement (in the thick stripes of V2) and of forms (in the pale stripes of V2). However, several in-depth studies found only a slight tendency for the postulated division. In general, most neurons from for example V2 simultaneously react to several visual attributes. A strict division of visual information in parallel-processing pathways could not be confirmed. In addition, psychophysically, it has been shown that form and movement can also be perceived by stimuli exclusively defined by color. For further

evaluation of visual signals, simultaneous, multidimensional analyses have an important advantage. The information of different properties of an object (e.g., color, form, and movement) does not have to be combined again at an uncertain higher-processing level.

Cortical Computations

Several investigations have been carried out to identify the areas in the visual cortex containing those neurons capable of coding for a particular stimulus property. However, knowing where a calculation might be performed is not nearly as interesting as knowing which computation is performed. Over the years, there has been progress with respect to the computations made by visual cortex. A few of these features are considered separately.

Motion

Motion perception plays a central role in visual perception. Not only is it used to compute the speed and direction of moving objects, but it is also quite important for the control of one's own body and eye movements. Research in this area has succeeded in determining a cortical neuronal circuit whose properties are also consistent with several behavioral experiments. Around 1970, the small middle temporal area (MT, see Figure 5) was discovered in monkeys, which seems to be a very important area for local motion processing. It primarily contains neurons sensitive to the direction of motion stimuli, and acts similar to the Reichardt's model. The specific motion disorders of patient L.M., who suffered from bilateral strokes in the temporoparietooccipital cortex, confirmed that area MT is very important for motion perception in humans. In monkeys, bilateral lesions of area MT led to deficits in motion perception; however, if these lesions are small, monkeys recover within a week.

The combination of single-cell recordings, microstimulation experiments, and psychophysical studies in alert behaving monkeys provided direct evidence that neurons in area MT are involved in the process of determining the direction of motion of a stimulus. In these experiments, random-dot kinematograms were used and alert monkeys were trained to signal the direction of motion. At the same time, the activity of single cells in MT was measured. This way, the activity of single cells predicted quite precisely the direction of motion seen by the monkey observer. On average, neurons showed the same motion sensitivity as the monkey, with some neurons being slightly better and others slightly worse. To determine the behavioral response, the activity of a large number of neurons is averaged. Further work used microstimulation and found that stimulating a single-direction column in

area MT systematically affected the behavioral judgments of the monkey, as if a visual stimulus moving in the direction corresponding to the stimulated column had been presented. This result impressively demonstrates the significance of neurons in area MT for the perception of the direction of motion.

However, area MT is not the sole physiological basis for all kinds of perceived motion. For many different types of motion, such as biological motion, certain types of second-order motion or optic flow, cortical activity in several other areas has been observed in neuroimaging studies. Neurons in area MT signal the local motion of stimuli, even when human observers perceive a global motion in the opposite direction, indicating additional neural substrates for the perception of motion.

Color

Initial studies of the functional architecture of primate visual cortex found only a small percentage of about 10% of all neurons devoted to the analysis of color signals. In these studies, the quest was for neurons that would respond to stimuli defined by color only, and not to luminance. According to a view promoting a functional segregation, it was thought that these neurons would analyze the color of objects, while other specialized neurons would deal with form, motion, and depth. In contrast to these results, later neuroimaging studies observed a strong and vigorous response to color in V1 and many extrastriate visual areas that exceeded the response to luminance stimuli. The solution to this seeming contradiction was found when the color properties of single neurons in V1 were investigated in more detail. There is a large proportion of neurons that responds well both to color and to luminance. This is in line with anatomical findings that the three streams (Figure 2) that arrive in the visual cortex from the retina do not remain segregated but do get mixed to a large degree. Along these lines, many recent studies failed to find a strong segregation of functional properties in V1 and V2. Rather, it seems that the tuning of each cell for different visual stimulus attributes is more or less statistically independent. For example, knowing that a cell is selective for color does not allow a prediction about the orientation selectivity of that cell.

Although it was initially thought that the chromatic properties of cortical cells in V1 are quite similar to those of the retinal cells, more details have been discovered in recent years. In the retina, responses of a neuron to all colors can be predicted just by the knowledge of the cells' preferred color, since these neurons sum their cone inputs in linearly. Furthermore, the preferred colors fall into two clusters of L-M and S-(L+M). While a majority of the cells in V1 still follow the linear model, the color preferences are more widely distributed. These cells still show quite a broad tuning to color, responding to many

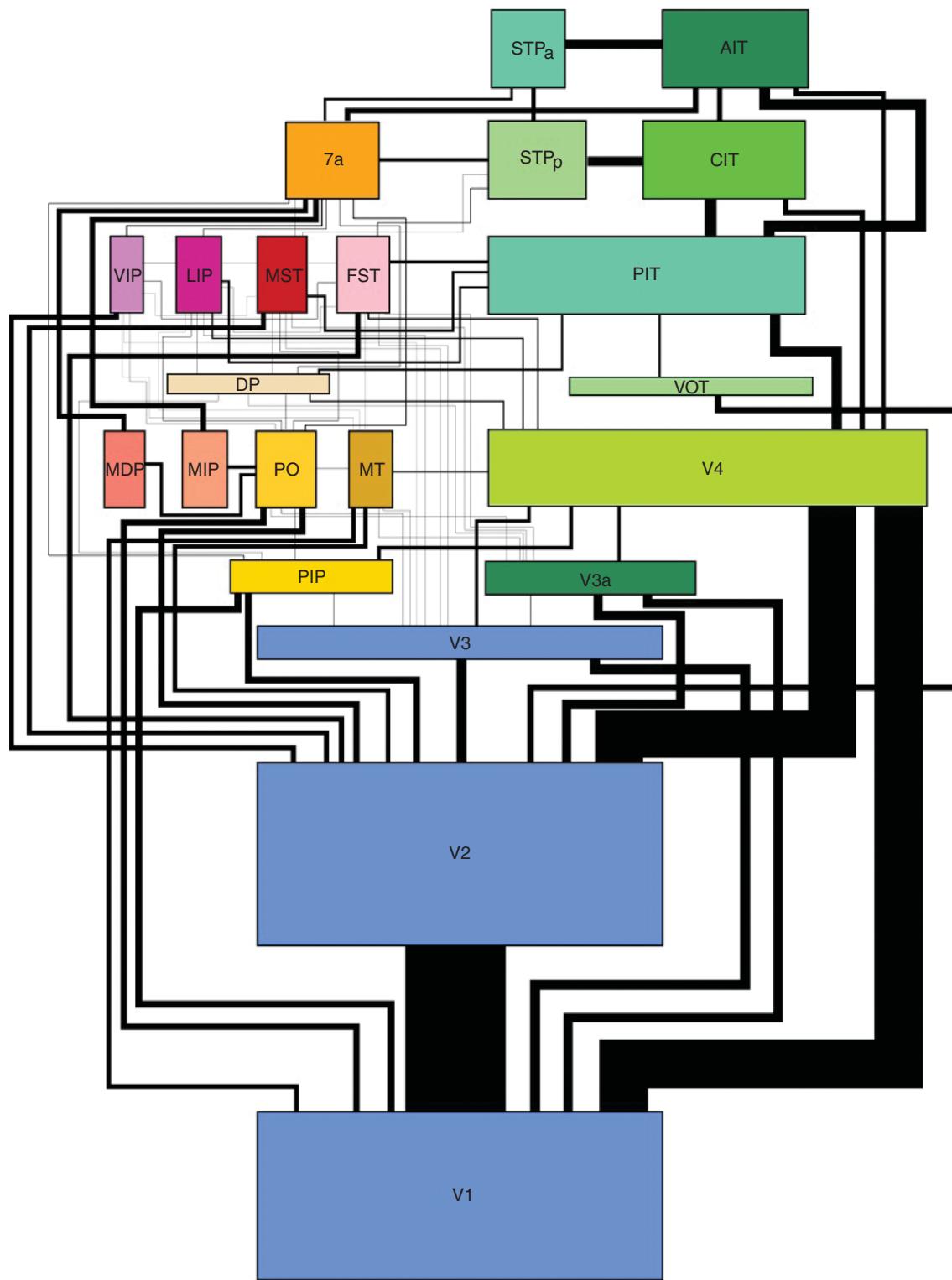


Figure 5 Cortical visual pathways. Schematic representation of the main anatomical connections between different cortical areas of the human vision system. The size of the boxes corresponds roughly to the relative size of the regions, and the thickness of the connections to the strengths of the anatomical connections between them. Frontal areas are not shown. From Walisch P and Movshon JA (2008) Structure and Function Come Unglued in the Visual Cortex. *Neuron* 60: 195–197. The original version of this figure was prepared in 1998 by John Maunsell. Names and definitions of visual areas are from Felleman DJ and Van Essen DC (1991) Distributed Hierarchical Processing in the Primate Cerebral Cortex. *Cerebral Cortex* 1: 1–47. Box size proportional to cortical surface area and line thickness proportional to number of fibers is from Lennie P (1998) Single units and visual cortical organization. *Perception* 27: 889–935.

different hues. In V2, a subpopulation of neurons that has more restrictive responses limited to a rather narrow range of hues was found, mimicking the behavior of the so-called higher-order color mechanisms observed psychophysically. Similar effects have been reported in V4, and there seems to be a progression from V1 through V2 to V4 and inferotemporal (IT) cortex in terms of the specificity of the chromatic response.

Another important characteristic of color cells that emerges in V1 is double opponency. As mentioned above, the single-opponent cells in the retina and LGN cannot signal spatial and chromatic properties at the same time. They respond best to the color of large unstructured stimuli. Double-opponent cells are both spatially and chromatically opponent and are therefore able to signal chromatic boundaries, for example, a red fruit next to a green leaf. Since they effectively compute color contrast – the difference between two neighboring colors – their response would be independent of the average color in the scene, which is mostly determined by the illumination color. Therefore, this would be an ideal mechanism to achieve color constancy, which is the ability of the primate visual system to assign a stable color to an object, independently of how it is illuminated. These double-opponent cells have been recently found in the primate visual cortex. These are members of the class of cells that respond equally well to luminance and color.

Depth

The perception of depth is a critical ability to act accurately in a three-dimensional (3D) environment. The reconstruction of depth is also a quite fundamental ability of the visual system, since information about depth is lost in the 2D projection on the retina and has to be inferred indirectly by a multitude of monocular and binocular depth cues.

After the invention of random-dot stereograms (RDSs) by Bela Julesz, it became possible to study binocular disparity without possible intrusions of monocular depth cues. Disparity is the horizontal offset between the retinal images in the two eyes. This offset allows to calculate the depth of objects relative to the fixated object. Corresponding image features in the two retinal images have to be identified to make use of this disparity. Therefore, one needs disparity-sensitive neurons, which receive input from both eyes at some spatial offset. Although disparity-sensitive neurons have been found a long time ago in several cortical visual areas, including V1, it was recently shown that the corresponding problem is not solved at the level of V1. Bruce Cumming and Andrew Parker measured neuronal responses to anticorrelated RDSs. In such a display, a random-dot pattern is shown to one eye and the contrast-inverse pattern with some spatial

offset to the other eye. Although these anticorrelated stereograms do not lead to depth perception, they activate the disparity neurons in V1. Therefore, neurons in V1 are not the neural substrate of depth perception. It is also known that neurons in V1 respond to absolute disparity, that is, to the amount of disparity between the two retinal images. More useful for the calculation of depth, however, is the relative disparity between two objects at different distances. There is evidence that neurons in V2 can encode relative disparity.

Through the widespread research on RDS, the effect of monocular depth cues such as linear perspective, texture gradients, relative size, and occlusion had been neglected quite dramatically. In contrast to binocular depth cues, monocular depth cues are perceivable with one eye and are completely contained in the visual scene. More recent work has shown the importance of monocular depth cues and how these different cues are combined with each other and with disparity for a single, optimally reliable depth estimate. It has been shown recently that monocular depth cues have a major effect on the retinotopic mapping in early visual areas. Scott Murray and colleagues presented pictures of 3D scenes that contained a sphere either at a near or a far perceived distance. Using fMRI, they were able to show that the size of activation in the retinotopic map of V1 depends on the perceived size of the sphere and not on the angular size of its retinal projection. This indicates that some monocular depth cues are incorporated in early visual processing. For instance, to use occlusion information for depth perception, it has to be determined to which surface a border that separates two surfaces belongs. This computation of border ownership is present as early as in V2.

Object Recognition

In the temporal cortex, areas respond specifically to certain object categories. For example, some cells in the infero-temporal cortex of primates respond selectively to hands or faces. Within the subset of face-specific cells, there are some that respond best to a frontal view of a face, whereas the removal of certain features, or changing the viewpoint (e.g., a profile view), results in a significant response decrease. Other neurons have been shown to respond best to profiles or to certain facial expressions or single facial parts or features. Sometimes, basic features, such as a line and two points, are enough to cause a response. It appears that there is a division in neuron populations for recognizing common features of faces versus whole faces. There are clusters of neurons with various response properties in the recognition of faces. The identification of an individual face probably occurs because of specific activation patterns of such a neuronal cluster. So there is no single

grandmother-neuron, which is special for recognizing the face of your grandmother. Nonetheless, it seems that there are other such specific areas for representing and recognizing various object categories in the temporal cortex of the human.

Given the high relevance of faces for human perception, it is not surprising to find a visual area that responds most vigorously to faces. However, there are also specialized areas for other types of stimuli: the parahippocampal place area (PPA), which responds best to pictures of scenes and landscapes and the extrastriate body area (EBA), which is activated especially well by pictures of bodies. The PPA seems to be specifically responsive to the spatial layout, which is contained in pictures of visual scenes, and seems also to play a role in navigation.

It is puzzling how neurons in the temporal lobes can have such complex response properties. This process becomes even more puzzling when we consider that these neurons are located only 5–10 processing stages away from V1. Neurons in V1 show high sensitivity to the orientation and length of edge-segments, hence for parts of objects. These neurons must be connected in such a way that whole objects can be recognized, in fact, with only a few processing stages. This was impressively demonstrated in experiments by Simon Thorpe and colleagues where observers had to detect the presence of an animal in a natural scene. The task of the subject was to hold a response key down and to release it only when an animal appeared in the image. It took on average 300 ms for subjects to release to button. This includes the processing time of the stimulus and the motor response. An analysis of evoked visual potentials showed that after only 150 ms, there were differences in the brain activity for the two kinds of pictures (animals vs. no animals). It takes about 50–80 ms for visual stimuli to reach V1, so this leaves only 70–100 ms for cortical processing of this complex task. This short time period allows only 5–10 additional cortical-processing stages.

Conclusion

Major advances in visual neuroscience were achieved in the past 40 years since the breakthrough work of Hubel and Wiesel, although it is clear that the most important processes are still unknown. Until now we have some rough ideas how humans recognize objects and perceive motion, but the visual system and the complexity of neural connections and interactions are still a mystery. The biggest advances are likely to come from the combination of single-cell measures and psychophysics.

See also: Agnosia; Attention and Speed of Information Processing; Brain Imaging; Conscious and the Unconscious; Disorders of Face Processing; From Sensation to Perception; Gaze Stabilization and the VOR; Hallucinations in Neuropsychiatry and Drug Abuse: From Phenomenology to Pathophysiology; Hemispheric Specialization: Language, Space, and Sexual Differentiation; Neural Basis of Recognition Memory in Nonhuman Primates; Neural Representations of Direction (Head Direction Cells); Orientation and Navigation; Place Cells; Short-Term Memory: Psychological and Neural Aspects; Temporal Lobe and Object Recognition.

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Bioenergetics of Sleep

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Glossary

Body mass index (BMI) – A measure obtained by dividing an individual's body weight by the square of their height. BMI provides an index to assess the normal range of an individual's body weight.

Basal metabolic rate (BMR) – The energy utilized by a person in calories, at rest, in a thermo-neutral environment and while being in a postabsorptive state (fasting for at least 12 h). BMR is a measure of the energy expended by the body for the normal functioning of vital internal organs.

Electroencephalogram (EEG) – The electrical activity of the brain measured by placing highly sensitive electrodes on the scalp.

Maximal oxygen consumption ($\text{VO}_2 \text{ max}$) – Also known as aerobic capacity, a measure of maximal oxygen consumption by an individual during maximal exertion. Increasing ability to transport and metabolize oxygen during high levels of exertion corresponds to the cardiorespiratory and physical fitness of an individual.

Metabolic equivalent (MET) – The amount of oxygen consumed while sitting at rest and is equal to 3.5 mL kg⁻¹ body weight per minute. The intensity of any aerobic physical activity may be gauged by the increase in oxygen consumption measured in multiples of MET units.

Non-rapid-eye-movement sleep (NREM) – It comprises stages 1 through 4 of normal sleep. NREM, unlike REM sleep, is characterized by little or absent eye movements and minimal muscle atonia and autonomic disruption. NREM sleep accounts for 75–80% of total sleep time in normal adult humans. Transition from wakefulness to stage 1 NREM sleep is characterized by the absence of alpha waves (8–13 Hz) usually seen in awake individuals. EEG of stages 1 and 2 of NREM sleep is characterized by theta waves (4–8 Hz), sleep spindles (burst of 12–16 Hz waves occurring for 0.5–1.5 s), and K complexes (single short high-voltage (greater than 100 µV) wave lasting longer than 0.5 s). Stages 3 and 4 of NREM sleep (SWS) produce predominantly low-frequency delta waves (2–4 Hz).

Rapid-eye-movement sleep (REM) – It comprises a stage of normal sleep characterized by rapid movements of the eye, muscle atonia, and autonomic disruption. Adult humans spend about 20–25% of their

total normal sleep duration in REM sleep. EEG during REM sleep is characterized by high-frequency, low-voltage waves distinct from other stages of sleep. A distinct cycling of the different sleep stages including REM sleep occurs in adult humans with each cycle lasting between 90 and 110 min.

Slow-wave sleep (SWS) – Also known as deep sleep, restful sleep, SWS comprises the deeper stages of NREM sleep (stages 3 and 4). The EEG during SWS is characterized by large-voltage (75 µV), low-frequency waves (2–4 Hz) also known as delta waves.

Energy Consumption during Sleep

Humans spend roughly about one-third of their lives in sleep, yet the many functions of sleep remain incompletely understood (Figure 1). In 1983, Horne suggested that sleep serves a restorative function in the brain. In 1995, Benington and Heller proposed a model postulating that the restorative function of sleep involves replenishing brain glycogen. Cerebral glycogen stores are depleted progressively during waking and thereby decreases the availability of a key energy source that supplements brain glucose. The transient reduction in cellular metabolism due to depleting energy sources results in the increased synthesis of adenosine in the brain. The stimulation of adenosine receptors on neurons in the cortex and thalamus results in electroencephalogram (EEG) manifestations similar to those seen during sleep deprivation. This model of sleep regulation as a restorative phenomenon assumes that the restoration takes place primarily during the slow-wave sleep (SWS) segments of non-rapid-eye-movement (NREM) sleep rather than rapid-eye-movement (REM) sleep. Interspecies and interstrain differences may be accounted for by the differences in the sleep-deprivation-induced increase in glucocorticoids. Regional differences in metabolic rate and glycogen metabolism may explain the diverse responses observed in different brain structures during sleep deprivation. Maintaining the brain glycogen energy store does not seem to be functionally related to sleep on a whole brain basis (Figure 2).

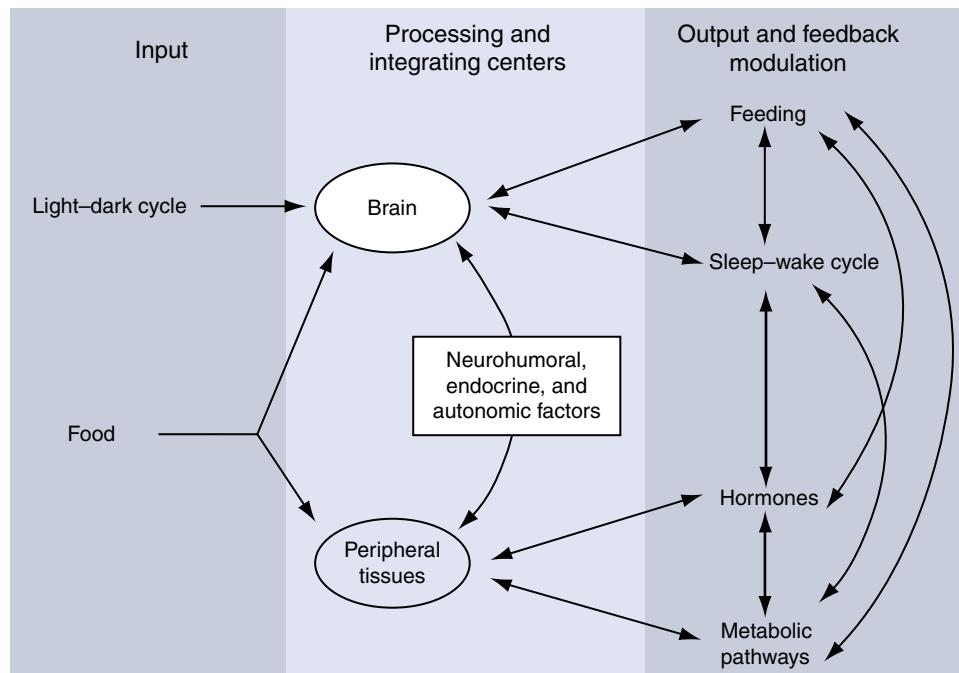


Figure 1 Interactions between the various components involved in the modulation of sleep and metabolism. Reprinted with permission from Ramsey KM, Marcheva B, Kohsaka A, and Bass J (2007) The clockwork of metabolism. *Annual Review of Nutrition* 27: 219–240.

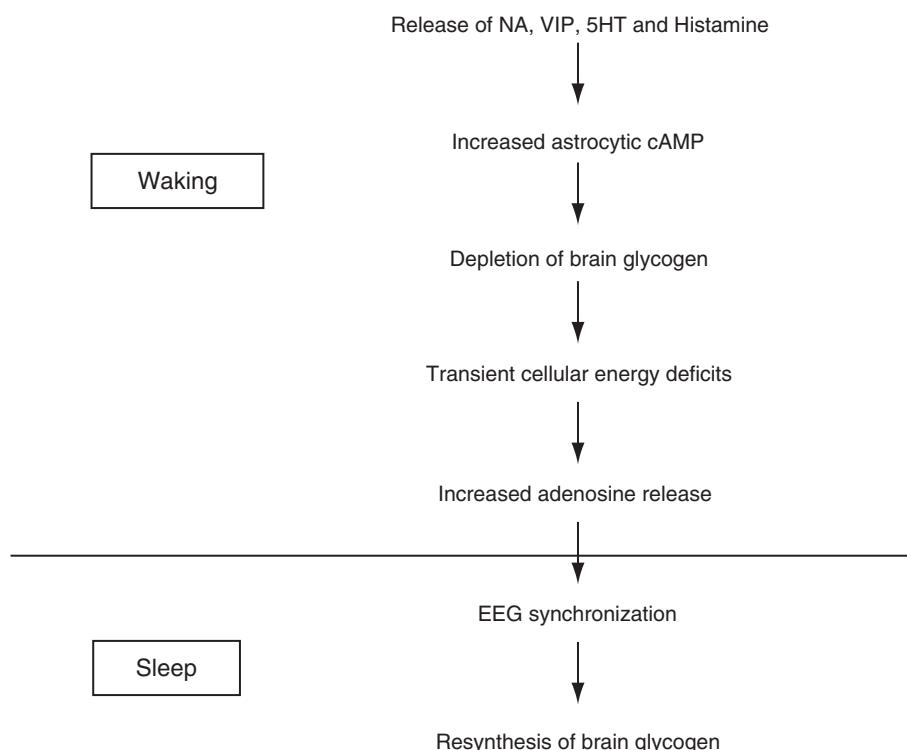


Figure 2 Neurochemical processes involved in the glycogen depletion hypothesis for the accumulation of sleep need during waking and its discharge during sleep. NA, noradrenaline; VIP, vaso-intestinal polypeptide; 5HT, 5-hydroxytryptamine (serotonin); cAMP, 3'-5'cyclic adenosine monophosphate; EEG, electroencephalogram. Reprinted with permission from Benington JH and Heller HC (1995) Restoration of brain energy metabolism as the function of sleep. *Progress in Neurobiology* 45: 347–360.

Temperature Regulation during Sleep

Evolutionary perspectives reveal that temperature regulation and sleep are interrelated and developed in response to similar physiological needs. Most species sleep during the circadian trough of their core body temperature rhythm. In humans, there is a close temporal relationship between sleep onset and the body temperature rhythm. Sleep is initiated on the declining portion of the temperature curve at a time when its rate of change, and body heat loss, is maximal (Figure 3). In the morning when heat production is dominant over heat loss, body temperature increases, as does the propensity to wake up. These findings indicate that the drive to sleep and the duration of sleep are tightly coupled with the thermoregulatory system.

The hypothalamus initiates thermoregulatory responses upon activation by inputs from thermoreceptors in the skin and internal thermoreceptors in the brain and blood vessels. The preoptic area of the hypothalamus, which contains a high density of thermoreceptors, regulates body temperature by altering the activity of the autonomic nervous system and by modulating sleep mechanisms. Temperature regulation in the human body varies according to the different stages of sleep, and sleep is altered in response to changes in temperature of the environment. The thermoregulatory efficiency during REM and SWS sleep in human adults is less prominent than in other mammals. In addition, the thermoregulatory process during REM sleep is less efficient than during SWS. Thermoregulation during REM sleep in neonates, in contrast to adults, is well maintained or

even enhanced. Transition between different stages of sleep has been proposed to change the hierarchical and functional control of the central nervous system (CNS) structures involved in thermoregulation.

A thermoneutral environment is defined as the range of air temperatures in the environment within which the metabolic rate is minimal and regulation of body temperature can be achieved by non evaporative physical processes alone. Changes from a thermoneutral environment disturb both the efficiency and structure of sleep. Sleeping in a nonthermoneutral environment results in a conflict between maintenance of homeothermia and sleep pressure. Thus, animals sleeping in cold or warm environments show alterations in sleep patterns. Different thermoregulatory responses are at play depending on the sleep stage and alterations in sleep observed outside of the thermoneutral environment.

Hibernation and Torpor

Sleep, shallow torpor, and hibernation appear to be homologous processes that evolved in response to the need for energy conservation. These three states comprise a continuum of decreasing metabolism and body temperature. In 1984, Berger observed that hibernation and torpor are regulated based on periodic environmental factors that affect food intake and energy balance. Mammals and birds are endotherms and homeotherms generating body heat from metabolism, thus maintaining a relatively constant body temperature across a wide range of environmental temperatures. Shallow torpor

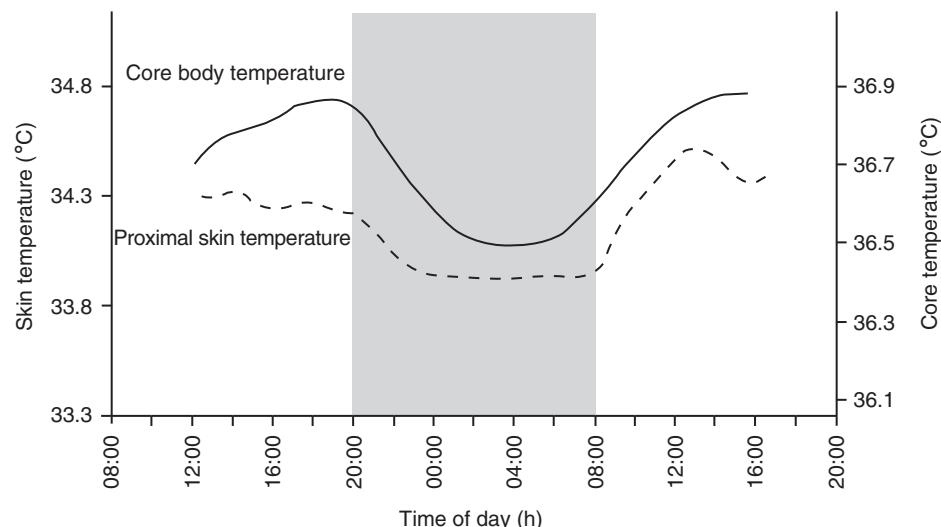


Figure 3 Smoothed average human core body and skin temperature curves under constant routine conditions showing declining body temperature during periods of increased propensity for sleep (gray shaded area depicts normal sleep period). Modified with permission from Van Someren EJ (2006) Mechanisms and functions of coupling between sleep and temperature rhythms. *Progress in Brain Research* 153: 309–324. Original source: Krauchi K and Wirz-Justice A (1994) Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditions in men. *American Journal of Physiology* 267: R819–R829.

and hibernation developed in order to maintain the high rates of metabolism and body heat during seasonal environmental changes and limitations in energy supplies. Decreases in body temperature and metabolism are seen uniformly in all species during sleep, although these changes are subtle and less profound than decreases observed during shallow torpor and hibernation. Endotherms adjust metabolic rates during inactivity so as to balance predation pressures against energy shortages. Shallow torpor is characterized by a decrease in body temperature, diminished responsiveness to environmental stimuli, and a quiescent posture. Shallow torpor may occur as part of the daily circadian rhythm during the normal sleep period or may extend over multiple days. Shallow torpor occurring during the drier and hotter seasons is termed ‘estivation.’ Hibernation is not as commonly seen as shallow torpor, and only a few species exhibit this behavioral state.

Similarities in the EEG patterns of sleep and hibernation suggest that hibernation may be an extension of sleep. Most mammals exhibiting hibernation enter this state of deep inactivity through sleep. The initial decrease in body temperature and metabolism that occurs during sleep is further enhanced as animals enter hibernation. An EEG characteristic of SWS is the predominant EEG pattern seen during hibernation. The decrease in REM sleep during hibernation is also linearly correlated with the decrease in body temperature. Body temperature is regulated continuously, although there is a decreased body temperature during hibernation.

Brain Energy Metabolism

The CNS is characterized by high metabolic demand. Although CNS tissue comprises about 2% of the body mass, CNS function uses about 20% of the total oxygen consumed. Glucose is the most important substrate utilized by the brain to maintain its function. Since glycogen, the storage form of glucose is found in very limited quantities in the brain, a continuous supply of glucose is essential for normal brain function. Oxidative metabolism of other sources of energy such as fats and ketone bodies in the brain is minimal. High plasma glucose levels and efficient transporters that shuttle glucose across the blood-brain barrier are important factors that have made glucose the preferred substrate for the brain. Under resting conditions, the ratio between the cerebral oxygen consumption (CMR_{O_2}) and cerebral glucose utilization (CMR_{glc}) is about 5.5, suggesting an oxidative efficiency of more than 90% for the consumed glucose. This implies that there is a direct relationship between oxidative metabolism and brain activity.

Energy metabolism in neurons is linked with glial cells. Astrocytes modulate blood flow in microvessels and

shuttle nutrients to the neuron. Metabolism in astrocytes accounts for a large proportion of glucose consumption during activated states of the brain. The energy derived from this process is utilized for accumulating the transmitter glutamate and its amidation to glutamine. Lactate produced during this process is transferred to neurons where it is then oxidatively metabolized. Astrocytes also utilize glucose for synthesis of glycogen (glycogenesis) (Figure 4). Neurons do not have the enzymes required for glycogenesis and therefore cannot store glucose as glycogen. Glycogenolysis occurring in the astrocytes in response to different stimuli provides an additional source of energy that may be utilized by neurons. Brain glucose plays an important role in regulating cerebral glycogenolysis. Brain glucose levels remain remarkably constant during sleep and vary to a much lesser extent than glycogen even in sleep deprivation.

Approximately 85–90% of glucose consumed in the brain is metabolized via the glycolytic pathway and oxidative tricarboxylic acid (TCA) cycle for the synthesis of ATP. Smaller amounts of glucose are utilized for other synthetic purposes such as glycogen (0–2%), nucleotides and lipids (5–10%), and proteins (0.5–2%). A large portion of the ATP produced from glucose is used for signaling and synaptic activity in the brain (75%). About 80% of brain energy is used for action potentials and postsynaptic potentials. Neuronal and glial resting potential maintenance take up about 13% and postsynaptic calcium entry and neurotransmitter cycling consume about 3% of the signaling-related expenditure of energy in the brain. Therefore, in resting conditions, approximately 10–15% of cerebral glucose is utilized for structural metabolism and 85–90% for functional needs.

Maximal glucose uptake occurs in regions rich in synapses, dendrites, and axons, while areas with cell bodies of neurons have low glucose utilization. Oxidative metabolism takes place in the mitochondria within cells where one of the important enzymes catalyzing the production of ATP is cytochrome oxidase. Large numbers of mitochondria and high concentrations of cytochrome oxidase in both the presynaptic and postsynaptic regions of neurons suggest that neuronal synapses require intense oxidative metabolism.

Regional Cerebral Glucose Metabolism during Sleep

Energy metabolism in the brain progressively declines during SWS and reaches its minimum level during the deepest stages of NREM. Cerebral glucose metabolism has been shown to decline by 30% during SWS from those during wakefulness. Although cerebral glucose metabolism does not differ significantly from waking levels during stage 2 NREM sleep, it decreases by 40%

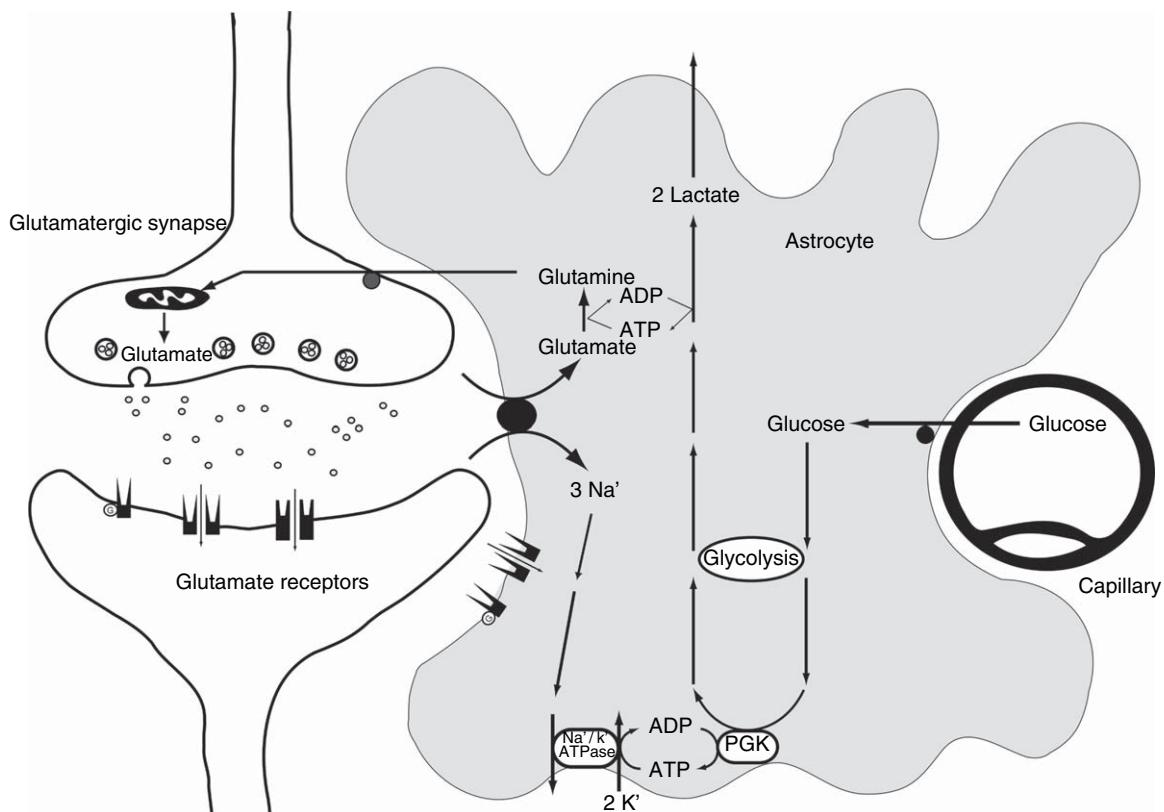


Figure 4 Glutamine–glutamate cycling between neurons and astrocytes is an important metabolic process seen during the activated states of the brain. Lactate production by astrocytes and its uptake and oxidative metabolism by neurons is also seen when the brain is active. ADP, adenosine diphosphate; ATP, adenosine triphosphate; PGK, phosphoglycerokinase. Modified with permission from Magistretti PJ and Pellerin L (1999) Cellular mechanisms of brain energy metabolism and their relevance to functional brain imaging. *Philosophical Transactions of the Royal Society B: Biological Sciences* 354: 1155–1163.

during stages 3 and 4 of NREM sleep (SWS) in humans. REM sleep is characterized by intense brain activity similar to that seen during waking. Direct measurements of metabolic parameters during REM sleep are difficult due to the short durations of REM sleep episodes in animals. Autoradiographic studies by Lydic *et al.* in 1991 revealed significant increases in glucose metabolism in regions comprising the thalamus, limbic system, and pontine reticular formation during REM sleep in cats. These regions have either cholinergic neurons or have significant cholinergic input from other areas, consistent with a key role for cholinergic and cholinoreceptive mechanisms in REM sleep regulation. Human studies during REM sleep have demonstrated similar levels of increase in cerebral metabolism. Increased cerebral blood flow is seen in the anterior cingulate, frontal, and extrastriate cortical areas besides the brainstem regions suggestive of activated cortical regions of the brain during REM sleep (**Figure 5**).

Inferences of metabolic activity from neuroimaging techniques measuring cerebral blood flow assume that cerebral blood flow correlates with neuronal activity

which in turn correlates with metabolic activity. There is a global decrease in cerebral blood flow during NREM sleep. Decreased blood flow during NREM sleep is seen in the dorsal pons, mesencephalon, thalamus, basal ganglia, basal forebrain, anterior hypothalamus, prefrontal cortex, anterior cingulate cortex, and precuneus. The regional decreases in blood flow may be grouped into three main cerebral areas – subcortical structures, cortical structures, and other areas.

Intense neuronal activity, high-energy metabolism, and increased blood flow to the brain characterize REM sleep. When compared to wakefulness, increased blood flow is seen in the cingulate cortex, temporo-occipital areas, basal forebrain, cerebellum, and caudate nucleus during REM sleep. Decreased regional blood flow is seen in the dorsolateral prefrontal cortex (DLPF), posterior cingulate gyrus, precuneus, and the inferior parietal cortex. The pedunculopontine tegmental (PPT) and the laterodorsal tegmental (LDT) nuclei project cholinergic neurons dorsally to the thalamus and ventrally to the basal forebrain where they mediate cortical activation.

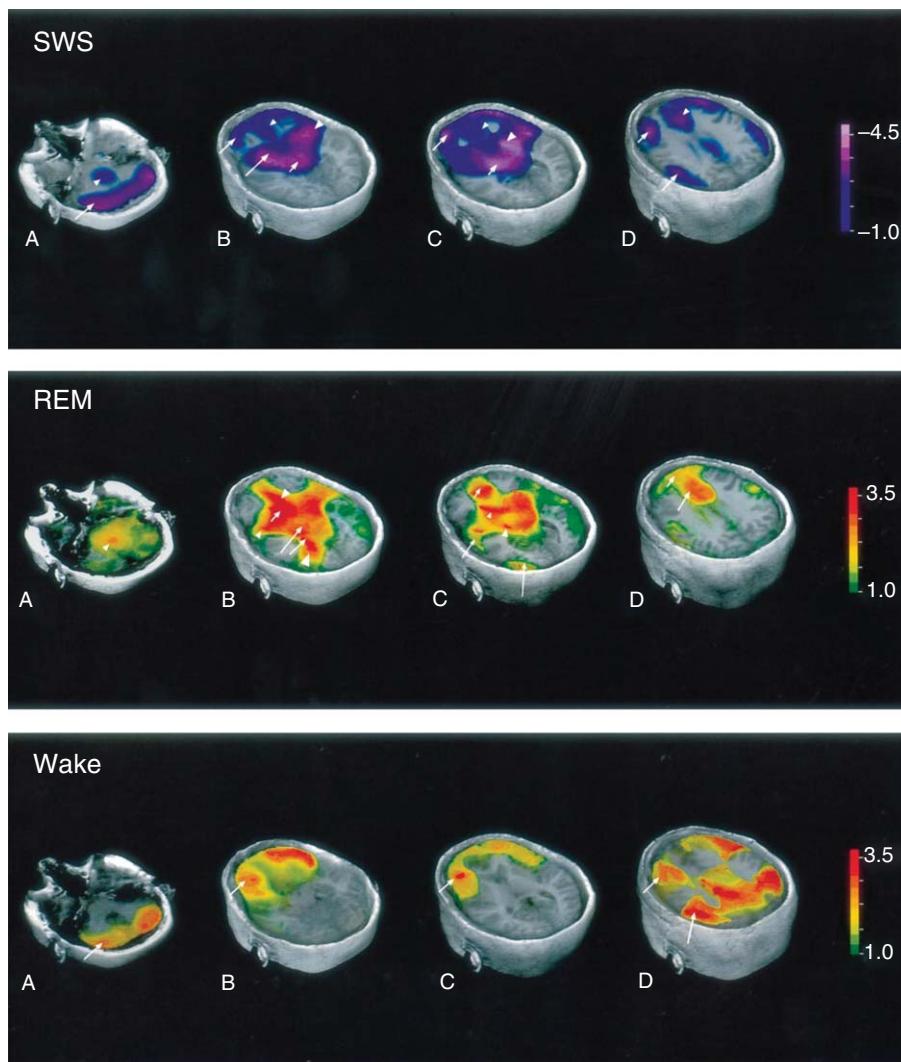


Figure 5 Brain maps illustrating changes in regional cerebral blood flow (rCBF) during different stages of sleep and wakefulness. Modified with permission from Braun AR, Balkin TJ, Wesensten NJ, et al. (1997) Regional cerebral blood flow throughout the sleep-wake cycle. An H₂(15)O PET study. *Brain* 120 (Pt 7): 1173–1197.

Hormones Regulating Sleep and Metabolism

Hormones that regulate feeding and sleep strengthen the coupling between sleep and energy metabolism. Both the normal sleep-wake cycle and altered sleep patterns show sizeable effects on the hormones of the hypothalamic-pituitary axis and other hormones which control carbohydrate metabolism, water, and electrolyte balance.

Growth hormone (GH) is a single-chain polypeptide hormone synthesized, stored, and secreted by the somatotroph cells of the anterior pituitary gland. The major secretory control of GH is exerted by two hypothalamic peptides, GH-releasing hormone (GHRH), which stimulates GH secretion, and somatostatin, which inhibits GH. Ghrelin, secreted from the stomach and pancreas, also

stimulates the release of GH from the anterior pituitary. Physiological factors affecting this balance at the level of the hypothalamus include stimulators of GH secretion such as exercise, hypoglycemia, protein intake, and sleep and inhibitors such as free fatty acids, hyperglycemia, and glucocorticoids. GH has profound anabolic effects on the tissues of the body. By acting on specific receptors, GH directly stimulates cell multiplication and growth involving a number of cell types especially cartilage, bone, and muscle. GH promotes lipolysis, increases protein synthesis, decreases the uptake of glucose by the liver, and stimulates gluconeogenesis. Although the secretion of GH occurs in several large pulses, the largest and the most predictable peak in secretion occurs shortly after the onset of sleep. GH release is stimulated by SWS and is proportional to the delta power of sleep.

Ghrelin is a peptide produced in response to fasting by the cells lining the fundus of the stomach and epsilon cells of the pancreas. Plasma ghrelin levels fall rapidly after food intake and rebound during hunger. Nocturnal ghrelin levels rise during the first half of the night and decline during the second half. Ghrelin by its action in the hypothalamic arcuate nucleus acts as a powerful GH secretagogue. Ghrelin also promotes the secretion of orexigenic neuropeptides, neuropeptide Y (NPY), and agouti-related peptide (AgRP) in the hypothalamic arcuate nucleus, resulting in increased feeding. It has been shown to cause a positive energy balance, increase adiposity, and reduce oxidation of fat. It has been suggested that ghrelin acts as an endogenous sleep-promoting factor. Exogenous ghrelin increases SWS and enhances delta activity during sleep while REM sleep is reduced during the later parts of the night. Exogenous ghrelin increases GH release as can be expected. Ghrelin administration increases cortisol levels during the early part of sleep in contrast to the late increase in cortisol seen during normal sleep. This effect differs from that of GHRH, which decreases cortisol secretion during sleep.

The thyroid hormones thyroxine and triiodothyronine regulate the rate of cellular growth and metabolism by regulating DNA transcription. They increase protein synthesis and oxygen consumption by increasing metabolism. Thyroid hormones also have modulatory effects on immune function, cognition, and emotions. In the fetus, thyroid hormones are crucial for the development of the brain. Thyrotropin-releasing hormone (TRH), secreted by the hypothalamus, stimulates the release of thyroid-stimulating hormone (TSH) from the anterior pituitary, which in turn stimulates the release of thyroid hormones from the thyroid gland. TSH secretion is related to the circadian rhythm; daytime levels are minimal and rise during the night to reach a maximum by the middle of the night before declining toward the morning. Thyroid hormone levels are high during the day and low at night. Alterations in sleep are common in thyroid disorders.

Corticotropin-releasing hormone (CRH), corticotropin (ACTH), and cortisol of the hypothalamic–pituitary–adrenocortical (HPA) system mediate body reactions to acute physical and psychological stress. A typical reaction to stress initiates a cascade of activity beginning with the release of CRH from the hypothalamus, which stimulates the release of ACTH from the pituitary. ACTH in turn facilitates the release of cortisol from the adrenal cortex. Sleep and the HPA system interact with each other in both directions. All cells in the body have receptors for cortisol, and therefore cortisol can be expected to have wide-ranging effects on the body. The overall effect of cortisol on metabolism is to ensure an adequate supply of glucose to vital organs including the brain at times of stress. Cortisol increases the synthesis of glucose in the liver from noncarbohydrate sources (gluconeogenesis) by mobilizing

substrates such as amino acids. Glucose uptake in the muscles and adipose tissue is inhibited and channeled into the blood for utilization by the brain. Cortisol stimulates the breakdown of fat in adipose tissue, providing an additional source of energy. Besides its enormous effects on glucose metabolism, cortisol also exerts important effects on development, cognitive, and immune functions (**Figure 6**).

A major proportion of ACTH and cortisol secretion occurs during sleep. The first pulse of ACTH and cortisol occurs a few hours after the onset of sleep, followed by further pulses of secretion until awakening. Cortisol is secreted during the second half of nightly sleep when the time spent in REM sleep is maximal. It is during the first few hours of sleep that the majority of SWS and the peak of GH secretion occur, at which time there is relatively no cortisol secretion. Predictably, in sleep deprivation, cortisol secretion is accentuated. In contrast to CRH, ACTH and cortisol increases SWS and reduces REM sleep.

Hypocretin (also known as orexin) is a neuropeptide secreted by the posterolateral hypothalamus that promotes increased feeding behavior and arousal. This dual function of hypocretin illustrates the close connection between the regulation of hunger and satiety and the regulation of sleep. The orexinergic neurons interact with arousal centers in the brainstem and basal forebrain, and important feeding centers in the hypothalamus. Activity of arousal-related monoaminergic neurons in the brainstem parallel that of the hypocretin neurons in the hypothalamus, suggesting a regulatory role of hypocretins on these neurons. Cholinergic neurons in the LDT/PPT which are active during wakefulness are stimulated by hypocretin. Hypocretin neurons strongly activate cholinergic neurons in the basal forebrain, which play an important role in behavior and cortical arousal. Activation of histaminergic neurons in the tubulo-mammillary nucleus (TMN) by hypocretin plays an important role in the regulation of arousal. Cerebrospinal fluid (CSF) levels of hypocretin are the highest during the active periods of the day. The hypocretinergic system communicates with the suprachiasmatic nucleus integrating the circadian rhythm with feeding behavior and sleep.

Leptin, a peptide synthesized and secreted by adipocytes, suppresses food intake and stimulates energy expenditure by its action on the hypothalamus. Although obese individuals have high circulating levels of leptin, they may be resistant to its effects (leptin resistance). Under normal conditions, there is a marked nocturnal rise in plasma leptin levels, which is partly dependent on food intake. Systemic administration of leptin in rats increases SWS and decreases REM sleep, an effect that is abolished by food deprivation. Leptin inhibits the activity of neuropeptide Y and hypocretin, both of which are peptides that increase appetite and wakefulness.

NPY is a peptide hormone/neurotransmitter secreted by the hypothalamus and associated with energy balance,

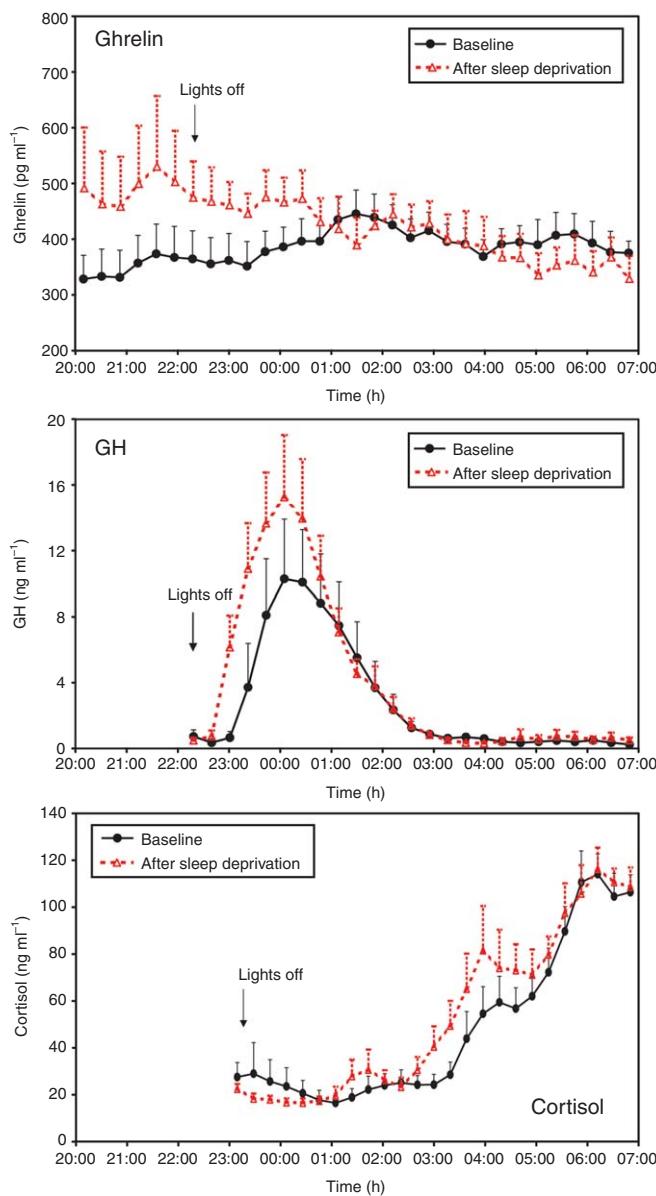


Figure 6 Nocturnal secretion of ghrelin, growth hormone (GH), and cortisol during normal sleep (black solid line) and after one night of total sleep deprivation (red dashed line) in eight human subjects. Modified with permission from Schüssler P, Uhr M, Ising M, et al. (2006) Nocturnal ghrelin, ACTH, GH and cortisol secretion after sleep deprivation in humans. *Psychoneuroendocrinology* 31: 915–923.

learning, and memory. It is a potent stimulator of food intake and facilitates the storage of energy, especially as fat. The effects of hypocretin on appetite may in part be modulated by NPY. Leptin can antagonize the effects of NPY.

Sleep Deprivation, Sleep Restriction, and Metabolic Disturbances

In 2004, it was estimated that more than 30% of adults and more than 17% of children and adolescents in the United States were obese. The prevalence of obesity in

adults doubled between 1980 and 2002, exponentially increasing the healthcare costs associated with obesity. Interestingly, over the same period, chronic voluntary sleep curtailment developed as a societal by-product of rapid economic and industrial expansion. More than 30% of adults in the United States reported sleeping less than 6 h per night in 2004. Recognizing the metabolic and neurohumoral links between obesity and altered sleep may provide opportunities for novel treatment modalities for both groups of disorders.

Sleep deprivation causes profound disturbances in glucose metabolism, the understanding of which may

provide insights into the pathogenesis of diabetes, one of the major risk factors of cardiovascular disease. Chronic sleep restriction, through its effects on a variety of neurohumoral factors, increases appetite and decreases energy expenditure, resulting in weight gain. Voluntary sleep restriction also modifies exogenous factors that affect metabolism such as choices of food and increased time to eat.

Pioneering studies of chronic partial sleep deprivation in humans have demonstrated significant alterations in the parameters of glucose metabolism. Sleep-deprived individuals show lower glucose tolerance (glucose clearance after exogenous glucose administration) and glucose effectiveness (a measure of non-insulin-dependent glucose clearance) (Figure 7). The mechanisms by which chronic sleep deprivation disrupts glucose metabolism may include increased sympathetic autonomic activity, altered secretion of counterregulatory hormones such as GH and cortisol, and increased activity of inflammatory cytokines.

Appetite regulation involves complex interactions between metabolic and hormonal signals and neural mechanisms. It is widely accepted that the appetite-control center in the brain is the arcuate nucleus of the hypothalamus, which integrates the peripheral signals. Appetite-regulating mechanisms have been shown to have strong links with neuronal systems regulating sleep. Chronic sleep deprivation causes an overall decrease in the levels of leptin, the satiety-promoting hormone secreted by the adipose tissue. Chronic sleep deficit advances the time of maximal secretion of leptin which occurs earlier in the night when compared to normal secretory activity while decreasing the amplitude of daily variation. Acute total sleep deprivation has the opposite effect of leptin levels. Ghrelin, the appetite-promoting peptide secreted by the stomach, has been shown to increase in sleep-deprived states. An increased ghrelin:leptin ratio during sleep restriction enhances the subjective feeling of hunger.

Energy expenditure is an important component of normal metabolic balance and plays a significant role in the control of body weight (Figure 8). Decreasing locomotor activity and a sedentary lifestyle result in lower energy expenditure. In endothermic animals, energy is required for basal cellular activity (BMR) at rest, food-related activity for the absorption, digestion, metabolism, and storage of nutrients, and physical-activity-related expenditures. Activity-related utilization of stored energy is the most variable component of the total energy expenditure. Increased activity corresponding to increased food intake can play a role in the control of body weight. Chronically sleep-deprived individuals report reduced physical activity, and therefore it may be assumed that energy expenditure is reduced. Leptin may increase energy expenditure by its thermogenic activity in brown adipose tissues. Ghrelin has been shown to decrease locomotor activity in rodents. Increased ghrelin and reduced leptin seen during chronic sleep deprivation may therefore negatively affect energy expenditure.

Maximal Oxygen Consumption, Bioenergetics, and Health

Sleep forms an important part of the restorative process in animals and adequate sleep is essential for optimal cognitive functioning. Understanding the relationship between sleep quality and exercise capacity may provide unique insights into how sleep affects metabolism and improves well-being. Vigorous exercise increases the need for oxygen in the body in order to maintain the high rates of metabolism. Maximal oxygen uptake ($\text{VO}_2 \text{ max}$) is a measure of the maximum oxygen consumption during maximal exertion. It is a good measure of exercise capacity and reflects the ability of an individual's body to transport and metabolize oxygen during incremental exercise. $\text{VO}_2 \text{ max}$ has been used as a measure of cardiopulmonary and physical fitness in athletes participating in

	Fully rested	After 5 days of sleep restriction	P level
K_G (% per minute)	2.40 ± 0.41	1.45 ± 0.31	<0.04
AIRg ($\mu\text{U mL}^{-1} \text{ min}$)	548 ± 158	378 ± 136	0.05
S_G (% / min)	2.6 ± 0.2	1.7 ± 0.2	<0.0005
SI ($10^4 \text{ min}^{-1} (\mu\text{U / mL})^{-1}$)	6.73 ± 1.24	5.41 ± 0.60	0.28
DI	2897 ± 404	1726 ± 395	0.0006

Figure 7 Results of intravenous glucose tolerance tests in healthy subjects after 5 days of sleep restriction (4 h in bed). KG, glucose tolerance; AIRg, acute insulin response to glucose; SG, glucose effectiveness; SI, insulin sensitivity; DI, disposition index. Modified with permission from Knutson KL and Van Cauter E (2008) Associations between sleep loss and increased risk of obesity and diabetes. *Annals of the New York Academy of Sciences* 1129: 287–304.

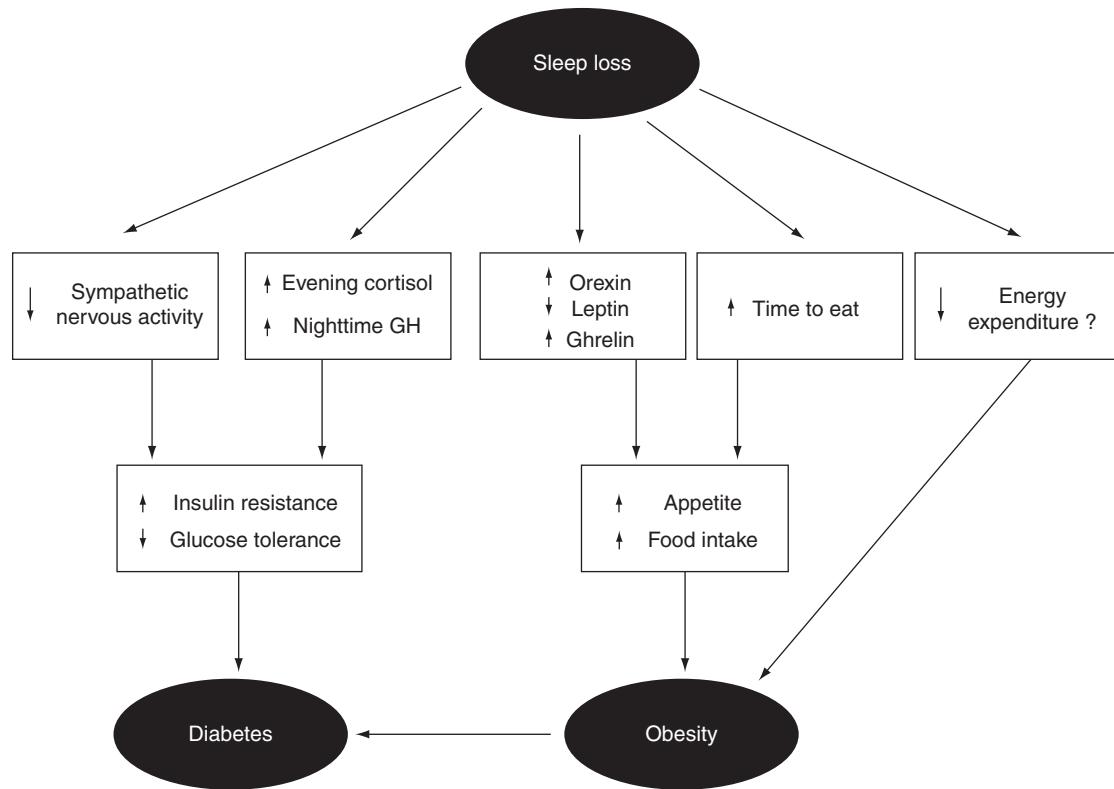


Figure 8 Possible pathways by which sleep loss leads to diabetes and obesity. Modified with permission from Knutson KL and Van Cauter E (2008) Associations between sleep loss and increased risk of obesity and diabetes. *Annals of the New York Academy of Sciences* 1129: 287–304.

high-endurance sports. Another measure of aerobic exercise capacity that is used more commonly is the metabolic equivalent (MET). One MET is defined as the amount of oxygen consumed while sitting at rest and is equal to 3.5 mL kg^{-1} body weight per minute. MET provides a useful tool to describe intensity of exercise activity in simple units.

A recent study by Kokkinos *et al.* in 2008 demonstrated a graded reduction in all causes of mortality with increasing exercise capacity. The risk for mortality was found to be 13% lower for every 1 MET increase in exercise capacity. Individuals with moderate exercise capacity (5–7 MET) had a 50–70% lowered risk of mortality compared to individuals with low exercise capacity (<5 MET). The enormous impact of maintaining even a moderate exercise capacity underlines the importance of aerobic fitness. Similar studies by Myers *et al.* have shown identical results; every 1 MET increase in exercise capacity resulted in a 12% improvement in survival. This study compared a number of other risk factors, including smoking, hypertension, diabetes, hyperlipidemia, and cardiac arrhythmias, with exercise capacity in predicting mortality. Compared to other risk factors, exercise capacity was identified as the measure with one of the best prognostic values for predicting mortality.

Sleep, Obesity, and Metabolic Syndrome

Metabolic derangements have a large impact on sleep. Individuals with obesity and metabolic syndrome are known to have disordered sleep. Decreased total sleep time, fragmentation of sleep, and altered sleep architecture have been described in such individuals. Obese individuals report significantly less total sleep time than individuals with normal body weight. Sleep apnea and excessive daytime sleepiness in obese patients may be manifestations of metabolic syndrome. Large abdominal circumference, hypertension, and diabetes, which are components of metabolic syndrome, have all been implicated in the heightened risk of sleep-disordered breathing.

Patients with obstructive sleep apnea syndrome (OSAS) have repetitive collapse of pharyngeal airways during sleep, leading to oxygen desaturation, sleep fragmentation, and increased daytime somnolence. There is a significant correlation between the severity of OSAS and increasing BMI. Recurrent apneic episodes in individuals with OSAS lead to intermittent hypoxia and sleep fragmentation, which in turn trigger sympathetic activation, cellular oxidative stress, and systemic inflammation. Inflammatory cytokines, tumor necrosis factor-alpha

(TNF- α), interleukin-1 beta (IL-1 β), and IL6, are involved in physiological sleep regulation. High levels of these cytokines have been demonstrated in obese patients with OSAS.

Chronic sympathetic activation along with oxidative stress and systemic inflammation in individuals with OSAS may cause insulin resistance and glucose intolerance. Epidemiological and cross-sectional studies have demonstrated a strong link between hypertension and OSAS, independent of other risk factors. Similar factors that link insulin resistance with OSAS are also thought to play a role in the pathogenesis of OSAS in hypertension. Abnormal lipid metabolism manifested as low high-density lipoprotein (HDL) levels and high triglycerides have also been reported in patients with OSAS. Reducing body weight and controlling other metabolic parameters may significantly improve apnea and oxygen desaturation in patients with OSAS. Sleep restriction causes a number of hormonal and metabolic peripheral effects that may cause obesity, and in turn leads to further sleep disruption.

See also: Conscious and the Unconscious; Feeding; Neuropeptides and Regulation of Water Intake; Sleep: Medical Disorders; Thermoregulation; Value of Animal Models for Predicting CNS Therapeutic Action.

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Circadian and Ultradian Clocks/Rhythms

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Glossary

Chronotherapeutics – The treatment protocol for cancer whereby the time of day when chemotherapy is administered is chosen in order to be maximally effective in treating the tumor, but have minimal effects on the patient.

Circadian – Biological rhythms approximately 24 h in duration.

Clock – A device used to measure time.

Entrainment – The process by which an environmental stimulus regulates the period and phase of an oscillator.

Infradian – Rhythms with a period of days, weeks, or years.

NREM – Nonrapid eye movement sleep, consisting of several sleep stages that are defined based on the frequency of brain electrical activity.

Oscillator – A series of components with a combined action that varies between extreme values over a precise period of time.

Period – The duration of a single oscillation measured from peak to peak or from one reference point to the next, e.g., from midnight to midnight on a 24-h clock.

Phase – The momentary state of an oscillation.

REM – Rapid eye movement sleep.

Ultradian – Rhythms with a period of less than 1 day.

weeks, months, or even years. Examples include the human menstrual cycle, hibernation, and the 17-year cycle of certain North American cicadas, respectively (see [Figure 1](#)).

The coordination of parallel and serial processes necessitates rhythmicity at all levels of biological organization. Ultradian rhythms likely arose in order to separate in time those mutually incompatible chemical reactions that could not be physically separated within the space of a single cell. In more complex systems, ultradian rhythmicity provides a metabolically inexpensive mechanism for efficient signal transmission. For example, it may be energetically expensive or even toxic for an organism to have constantly high levels of a hormone in circulation, when the same signal can be transmitted by pulsatile hormone release. Temporal separation of incompatible chemical reactions is also observed at a circadian timescale, such as the daily rhythm of photosynthesis. Circadian rhythmicity is also critical for the synchronization of multiple biological processes with the external environment and allows the organism to anticipate the environmental demands of the solar cycle.

Properties of Biological Clocks

In their most basic form, biological rhythms consist of a negative-feedback loop with a time delay. For example, there are numerous proteins that repress transcription of their own genes, such as the period (PER) and cryptochromes (CRY) proteins, essential components of the mammalian circadian clock (described in more detail below; see [Figure 2](#)). At least one negative-feedback loop is necessary for an oscillation. Most biological clocks are made up of a series of weakly coupled oscillators, which make them more robust with respect to perturbations by outside stimuli. Greater complexity also tends to allow for timing of longer intervals.

The most important characteristic, and hallmark of a true biological clock, is temperature compensation: the period of the oscillation must be very similar over a wide range of biological temperatures. This has been studied extensively in circadian rhythms, but is also a characteristic of ultradian clocks. Intrinsic rhythmicity and a free-running period under constant conditions are also necessary properties for biological clocks.

Introduction

The term ‘biological rhythm’ can be used to describe any molecular, physiological, or behavioral event or process that is recurring, and is a defining characteristic of all living organisms. Rhythmicity can be observed over the entire range of timescales from fractions of a second to years, but can be broadly grouped into three categories: ultradian, circadian, and infradian. Ultradian rhythms, such as cell division or the human rapid eye movement (REM)–non-REM (NREM) sleep cycle, can have a period of seconds, minutes, or hours, and occur multiple times per day. Circadian rhythms, including photosynthesis, the rest–activity cycle, and the plasma concentration of numerous circulating hormones, repeat approximately every 24 h. Infradian rhythms have periods of longer than 24 h, ranging from days to

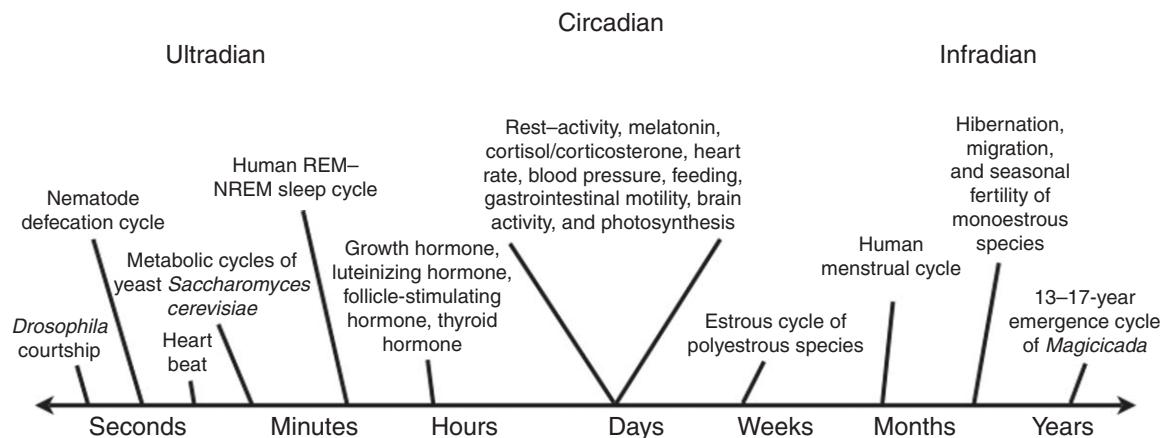


Figure 1 Timescale of biological rhythmicity with examples.

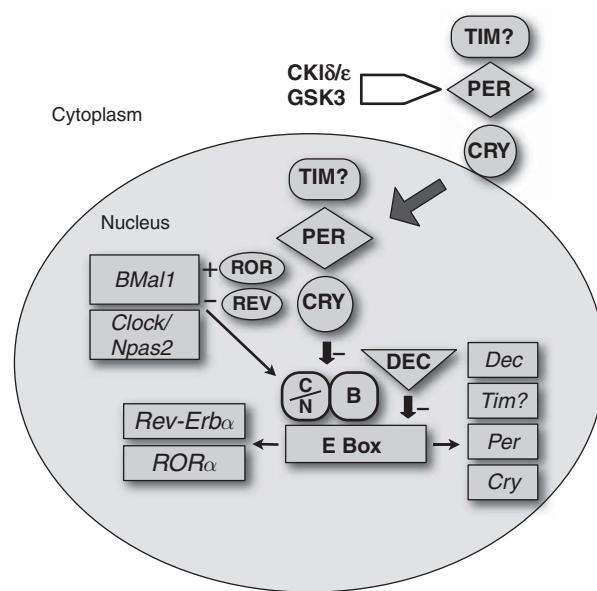


Figure 2 Molecular mechanisms of the mammalian circadian clock. Clock gene mRNAs are indicated by italics and proteins are in bold font. Fine arrows indicate transcription, block arrows indicate binding or interaction, the gray arrow indicates translocation, and the open arrow indicates posttranscriptional modification. Excitation and inhibition are indicated by plus (+) and minus (-) signs, respectively. C: CLOCK protein, N: NPAS2 protein, and B: BMAL1 protein.

Ultradian Rhythms

As the timescale of ultradian rhythms is so broad, from fractions of a second to hours, a large number of biological clocks fall into this category and a comprehensive discussion is not possible in this article. However, an example of a well-studied ultradian rhythm is the approximately 40-min cycle of cellular respiration observed under continuous aerobic culture conditions in the yeast *Saccharomyces cerevisiae*. Interestingly, the majority of the individuals

within a culture will become synchronized with one another, such that the O₂ concentration and intracellular pH of billions of cells become perfectly coordinated. This ultradian oscillator meets all of the requirements of a true clock. It is intrinsically rhythmic, temperature-compensated, and maintains a 40-min oscillation for months under constant culture conditions. The signaling mechanism responsible for the mass intercellular synchronization has not yet been established, but two diffusible chemicals, H₂S and acetaldehyde, are possible candidates, as they show rhythmic changes in concentration and can shift the phase of the ultradian metabolic clock. The molecular mechanisms of this clock are under investigation, and putative clock genes include *GTS1*.

One of the first ultradian rhythms to be studied in humans was the REM–NREM sleep cycle, which has a period of about 90 min and occurs 3–5 times in the average sleep episode. This rhythm is composed of the synchronous activity of a number of different processes including oculomotor activity, muscle tone, dominance of the autonomic nervous system, brain electrical activity, and energy utilization. Similar to many other biological processes, sleep shows rhythmicity at multiple timescales. In addition to a 90-min ultradian rhythm, a circadian rhythm is also evident.

Circadian Rhythms

With the exception of organisms living in extreme environments, such as the deep ocean floor, all others are exposed to the daily solar cycle and have an internal clock that cycles about once a day. For example, the unicellular marine algae *Gonyaulax polyedra* moves up to the higher levels of the ocean to absorb sunlight and CO₂ for photosynthesis during the day, but descends to the nutrient-rich lower layers during the night to absorb nitrogen and phosphorous.

In multicellular organisms, the molecular machinery for circadian rhythms is present in nearly every cell. These clocks perform functions specific to each individual tissue and are synchronized with the external environment by a master circadian pacemaker. In mammals, this master circadian clock is located deep in the brain in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN has all of the defining attributes of a biological oscillator: intrinsic rhythmicity, a free-running period under constant conditions, and temperature compensation. Substantial evidence indicates that the SCN is the master circadian clock as SCN lesions abolish circadian rhythmicity of locomotor activity, feeding, drinking, and hormone release. Furthermore, surgical transplantation of SCN cells can restore circadian rhythms of locomotor activity to recipient animals with SCN ablation. Hamsters with the tau mutation have an endogenous circadian rhythm with a period shorter than 24 h. Animals homozygous or heterozygous for the mutation have periods of 20 and 22 h, respectively, whereas wild-type animals have an endogenous period of about 24 h. SCN grafts taken from a homozygous tau mutant donor and placed in a wild-type recipient will restore locomotor activity rhythms with the same period as the donor animal, demonstrating that the circadian period is conferred by the donor SCN.

The SCN receives photic information directly from the eye via the retinohypothalamic tract. The synchronization of the master clock occurs by a process known as photic entrainment. The primary circadian photoreceptor melanopsin is found in the soma region of most retinal ganglion cells that innervate the SCN. Melanopsin-containing retinal ganglion cells are relatively insensitive to brief light exposure, but gradually become more activated in response to constant illumination of an intensity similar to that of the dawn sky.

Nonphotic cues have relatively little impact on the SCN master clock, but nevertheless, profoundly influence circadian behavior by altering individual peripheral clocks. The nonphotic stimuli known to affect circadian rhythms are numerous and include olfactory cues, food, pathogens, social interactions, emotional state, and conditioned stimuli. Perhaps the most important of these is feeding.

Feeding is a potent synchronizer of behavior. Nocturnal rodents will consume the majority of their daily caloric requirement during the dark portion of the light-dark cycle. If they are placed on a 24-h feeding schedule that limits food access to a short period during the daytime, nocturnal rodents will show a reliable increase in activity several hours prior to food availability. This food-anticipatory activity only occurs when the feeding schedule is in the circadian range, as rodents are unable to anticipate feeding schedules that are much shorter or longer than 24 h.

The semihierarchical organization of circadian oscillators in mammals allows peripheral oscillators to respond to relevant stimuli, but remain synchronized with each other and the external environment via the light-specific master clock. The SCN can synchronize peripheral oscillators either by direct neural projections or by a diffusible or blood-borne signal. Although entrainment is generally top down, there is also some evidence that peripheral clocks can influence the master circadian clock. For example, under conditions of constant bright light, the rodent SCN becomes arrhythmic, but can be entrained by scheduled meals delivered once every 24 h.

Molecular Mechanisms of Circadian Rhythms

Circadian rhythmicity at the molecular level is the result of both positive- and negative-feedback loops and includes mRNA transcription, protein translation, protein–protein interactions, and posttranscriptional protein regulation (see **Figure 2**). Three eukaryotic organisms have been fairly well characterized: *Neurospora*, *Drosophila*, and rodents. Only the latter will be described here; however, there is a high degree of similarity among all three. In mammals, genes *Clock* and *Bmal1* encode two basic helix-loop-helix (bHLH)-PER-ARNT-SIM (PAS) domain-containing transcription factors called CLOCK and BMAL1 (brain and muscle ARNT-like protein 1; also known as MOP3). CLOCK and BMAL1 dimerize and bind to E-box enhancers, activating transcription of genes *Period 1*, *2*, and *3* (*Per1*, *Per2*, *Per3*); *Cryptochrome 1* and *2* (*Cry1* and *Cry2*); *Rora*; and *Rev-Erbα*. ROR α activates *BMAL1* transcription, while REV-ERB α inhibits it, providing positive and negative feedback on the clock, respectively. The main negative-feedback loop is formed by clock proteins PER and CRY, which associate and are translocated back into the nucleus, where they inhibit their own transcription by interacting with the CLOCK/BMAL1 heterodimer. The rate of accumulation, association, and translocation of PER and CRY, critical for the period and phase of the molecular clock, is controlled by enzymes casein kinase I epsilon (CKI ϵ) and casein kinase I delta (CKI δ), the *Drosophila* shaggy homolog glycogen synthase kinase 3 (GSK3), and several other recently identified substances. Interestingly, it is a mutation of CKI that leads to the short-period tau mutation in hamsters described above. A number of other clock components have been studied. NPAS2, an alternate dimerization partner for BMAL1, seems to be important for clock function in cells outside the master circadian clock, but is virtually absent from the SCN. Other proteins, such as DEC1 and DEC2, can act as transcriptional repressors by interacting with E-box-binding sites. The mammalian homolog of timeless, a

core component of the *Drosophila* clock, also appears to have a role in the negative limb of the mammalian circadian clock. However, the roles of these and numerous other genes, proteins, and enzymes in clock function are still being delineated.

Relationship of Circadian Rhythms to Physical and Mental Health

The essential circadian clock genes are ultimately responsible for circadian rhythms of physiology and behavior, and disruptions of circadian rhythms may play a role in pathology. For example, shift workers, who habitually work during the late-evening and night hours, have a higher risk of developing long-term diseases, are more likely to be hospitalized, and consult more frequently with health care providers than individuals working a more typical day-oriented nine-to-five schedule. Shift work is associated with an increased risk of a number of serious health conditions, including cardiovascular disease, non-insulin-dependent diabetes, gastrointestinal illness, cancer, spontaneous abortion, premature birth, infertility, and psychological distress. Also at risk are transcontinental flight personnel, who experience a chronic asynchrony between their internal circadian rhythms and the external environment.

The link between circadian rhythms and cancer may be due to the direct role of the molecular clock in the regulation of the cell cycle. Experiments causing disruptions of the circadian system in animals, such as alterations of the light–dark cycle, SCN lesions, or clock gene mutations, result in an increased risk of cancer and faster growth of preexisting tumors. Mice lacking the *Per2* gene are more likely to develop tumors than wild-type mice, and do not show the typical induction of essential clock genes by gamma radiation, suggesting that in wild-type mice, circadian clocks respond to cellular damage in order to activate apoptosis (programmed cell death) and suppress tumors. A practical application of this research is the advent of chronotherapeutics, which aims to take advantage of the observed asynchrony in cell division and metabolic rhythms between healthy tissues and the tumor. Thus, treatment can be prescribed for a time of day where chemotherapy will be maximally toxic for the tumor, but have minimal effects on the patient.

The human molecular clock also has been implicated in certain sleep disorders. Mutations of the *PER2* and *CK1 δ* genes were found to be the cause of two familial cases of advance sleep phase disorder (bedtime = 6–9 p.m. and wake time = 1–3 a.m.), while the opposite condition, delayed sleep phase disorder (bedtime = 3–6 a.m. and wake time = 1–3 p.m.), was found to be associated with a polymorphism of human *PER3*.

Circadian clock genes have also been implicated in mental health. For example, the genes *CLOCK*, *PER3*, and *TIMELESS* have been associated with schizophrenia/schizoaffective disorder and bipolar disorder. Support for a role of *CLOCK* mutations in bipolar disorder has recently come from animal literature, with evidence that the *Clock* mutant mouse might constitute an animal model of mania. There is also evidence that the therapeutic action of the mood-stabilizing agent lithium may be related to direct effects on the circadian clock component GSK3. Even more interesting are findings that the inhibition of GSK3 may be common to other mood-stabilizing and antidepressant therapies, including drugs which target the serotonergic and dopaminergic systems as well as electroconvulsive therapy.

Relationship between Ultradian and Circadian Clocks

There is strong evidence that ultradian and circadian clocks are interrelated. In complex organisms, the master circadian clock synchronizes ultradian clocks with the external light–dark cycle and many processes have both ultradian and circadian rhythmic components. For example, the cell cycle has an ultradian rhythm that varies by the organism and type of cell, but cell populations tend to enter into the cell cycle at the same time each day. Another example is the secretion of numerous hormones that have both pulsatile and circadian secretion patterns.

Commonalities also are found at the molecular level. For example, lithium, commonly used as a mood-stabilizing agent, as described above, has been shown to lengthen the period of both ultradian and circadian clocks. Lithium lengthens the period of circadian rhythms in rodents and can lengthen the period of neuronal firing of cultured SCN neurons in a dose-dependent manner, via the inhibition of the core circadian clock component GSK3. The period of the ultradian metabolic clock of the yeast *Saccharomyces cerevisiae* is also lengthened by lithium. This suggests that there are common molecular mechanisms that underlie both of these clocks.

Some ultradian rhythms may actually share molecular components with the circadian clock. Like mammals, a core component of the *Drosophila* molecular clock is the gene *period*. A null mutation of *per* abolishes the circadian clock, while other mutations can lengthen or shorten its period. These mutations have the same effect on the ultradian mating song cycle. The short form of *per*, called *per^f*, not only shortens the period of the circadian clock, but also that of the ultradian mating song cycle. Similarly, *per^{L1}* lengthens both circadian rhythms and the song cycle, while the null mutation abolishes both rhythms completely. As the molecular mechanisms of other ultradian rhythms become known, other points of commonality between circadian and ultradian rhythms will likely emerge.

Conclusion

Rhythms are a ubiquitous property of all living organisms and not merely an anomalous phenomenon occurring in a few isolated systems. They are observable at all timescales from fractions of a second to years and are necessary for the coordination of the multitude of biological systems that characterize healthy cells, tissues, systems, and organisms.

See also: Depression; Feeding; Mouse Genetic Approaches to Psychiatric Disorders; Sleep Genetics; Sleeping, Waking, and Dreaming; Sleep: Medical Disorders.

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Neuropsychology of Sleep

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Glossary

Ascending reticular activating system (ARAS) – A diverse system of varying neuron types and neurochemical identities occupying the core of the brainstem and projecting to the diencephalon and cortex, where it produces activation reflected behaviorally by waking and dreaming and electrophysiologically by increased frequency and decreased amplitude of brain waves. The ARAS includes nuclei of the major neuromodulatory systems known to increase forebrain activity using acetylcholine, norepinephrine, and serotonin.

Coherence – The relative degree to which oscillatory activity, measured by electroencephalography (EEG) or magnetoencephalography (MEG), at a particular frequency tends to be in phase (synchronized) between different specified regions of brain.

Functional Magnetic Resonance Imaging (fMRI) – Functional neuroimaging of the brain measuring blood flow responses to regional changes in oxygen demand (hemodynamic response) that, in turn, change the blood-oxygen-level-dependent (BOLD) magnetic response to magnetic stimulation, which varies with the relative proportion of oxygenated and deoxygenated hemoglobin in the blood.

Magnetoencephalography – Regional activity of the brain measured at the scalp by detecting minute fluctuations in electromagnetic fields produced by underlying neuronal electrical activity.

Phasic activity – Brain activity occurring over a brief interval (milliseconds to seconds) measured by EEG or MEG such as the spindle and K-complex of nonrapid eye movement (NREM) sleep or the rapid eye movements of rapid eye movement (REM) sleep.

Positron emission tomography (PET) – Functional neuroimaging of the brain that measures regional brain activity in terms of regional blood flow or metabolism that is determined by measuring radiation emitted from injected isotopes.

Tonic activity – Brain activity that continues over sustained time periods of seconds to minutes such as the EEG- and MEG-rhythmic oscillations (brain waves).

The Neuropsychological Study of Sleep

The study of neuropsychological processes occurring during or dependent upon sleep presents unique challenges. Because persons asleep cannot respond to queries or enact volitional behavior, neuropsychological assessment of mental processes occurring during sleep must be done during waking, a limitation shared when studying the neuropsychology of dreaming. Investigators studying neurobehavioral processes and phenomena associated with sleep have employed five main techniques to overcome sleep's barriers to direct observation.

First, the amount of sleep can be varied and subsequent performance can be compared to that of the same individuals prior to this manipulation or to population norms. Such manipulations include acute deprivation of sleep for varying durations, chronic sleep restriction, sleep fragmentation by repeated awakenings, selective reduction or enhancement of specific sleep stages, and sleep extension. The many important discoveries resulting from such experimental techniques have included characterization of the attentional deficits resulting from total sleep loss as well as cumulative attentional impairment resulting from chronic sleep restriction ("sleep debt") – work pioneered by Robert Wilkinson in the 1950s and 1960s and recently by David Dinges and his colleagues. Indeed, in addition to attention, contemporary sleep researchers have demonstrated experimentally that most cognitive functions including several memory systems, executive functions, as well as social and emotional skills are negatively impacted to a greater or lesser extent by sleep loss. An important addition to performance studies of sleep deprivation has been the use of functional neuroimaging to examine brain physiological changes that accompany behavioral changes. Prominent among these studies have been those using functional magnetic resonance imaging (fMRI). From such data, Sean Drummond and colleagues have proposed a cerebral compensatory response to cognitive demands in the sleep-deprived state.

The second technique, a variant of the first, has been to utilize normal sleep and normal waking as contrasting 'interventions' to study their differential effects on memory consolidation. This approach, pioneered by Robert

Stickgold, Jan Born, Matthew Walker, and others has been used to demonstrate sleep facilitation of procedural learning in perceptual and motor domains as well as of declarative and emotional forms of memory. The specific sleep physiological correlates of different forms of memory consolidation have been examined by correlating performance changes with polysomnography (PSG) measures such as sleep stage percentages or by comparing performance change over the slow wave sleep (SWS)-rich first half of the night with the random eye movement (REM)-rich latter half. In addition to memory performance, these paradigms have shown sleep-related adaptive modifications of the relationships among different items stored in memory including increasing resistance to interference and integrative processes that facilitate categorical learning.

In a third technique, a sleeping individual is awakened from different sleep stages and performance is tested upon awakening. This procedure relies on a putative physiological carry over from sleep to the temporally proximal waking and has been used by Robert Stickgold and Matthew Walker to demonstrate differential effects of REM and NREM sleep on associative memory and cognitive flexibility.

The fourth and fifth techniques both probe neuropsychological functions occurring during sleep itself. Recent findings resulting from such research are the topic of this article. First, using the new technologies of functional neuroimaging, it has become possible to directly visualize activity in the sleeping brain. During the last decade, pioneering positron emission tomography (PET) studies have been done in the laboratories of Pierre Maquet, Allen Braun, and Eric Nofzinger. More recently, fMRI has been employed in the laboratories of Michael Czisch and others. From sleep-stage dependent regional activation data, cognitive processes taking place during as well as before and after sleep can be hypothesized. The relationship between regional brain activity and known performance and experiential phenomena during waking is, of course, the body of knowledge from which such inferences must begin.

The subjective correlate of neural processes occurring during sleep is dreaming and, hence, some of the neural processes underlying the phenomenology of dreaming can also be inferred from neuroimaging sleep. The last approach – observing changes in the phenomenology of dreaming resulting from brain injury – provides additional information to hypotheses derived from imaging the sleeping brain. Neuroimaging of waking performance can tell us which brain structures are recruited by particular functions or cognitive domains but do not establish the necessity of such active areas for enacting the behavior in question. Behavioral data following lesions resulting from stroke and other forms of brain injury can begin to address the actual requirement of a particular brain structure for a specific behavioral function and such

information forms the basis of traditional neuropsychology. The necessity of certain areas for particular dream elements or for dreaming itself can be similarly inferred by querying brain injured patients about their dreams. However, the same mediation of any such accounts by waking processes involved in recollection and recounting must be recognized.

Global Changes in Brain Metabolism and Blood Flow between Sleep/Wake Stages

With sleep onset and entry into NREM sleep, global cerebral energy metabolism and oxygen utilization decrease from waking levels. As sleep deepens through the stages 1 to 4 of NREM as characterized by slowing of the scalp electroencephalograph (EEG), further decrease in global energy metabolism and oxygen utilization in the range of 10% in light NREM to as much as 40% during slow wave sleep occurs. The cellular correlate of these changes is decreased firing rate of neurons in the wake-promoting regions of the ascending reticular activating system (ARAS). The behavioral correlate of these global physiological changes is the loss of consciousness at sleep onset with further decreases in arousability, alertness upon arousal and intensity of reported sleep mentation as NREM deepens. In contrast, during REM, global cerebral energy and oxygen metabolism return to levels equal to or exceeding those of waking. The EEG correlate of these changes is increased frequency and decreased amplitude, while the cellular correlate is an increased firing rate of specific components of the ARAS. The behavioral correlate of this REM reactivation is increased arousability, alertness upon arousal and intensity of reported sleep mentation.

Regional Changes in Brain Metabolism and Blood Flow

Deactivation of frontal cortices is one of the first physiological signs of human sleep. Early deactivation of frontal areas has been reported using the full spectrum of physiological recording techniques including EEG, magnetoencephalography (MEG), PET, and fMRI. Frontal deactivation early in sleep may be related to frontal regions' greater need for homeostatic recovery from waking activity. For example, Alexander Borbely and his colleagues have shown that a useful marker of homeostatic sleep pressure, slow wave activity (SWA), is greater in frontal than in parietal and occipital regions during the first NREM episode of the night. PET studies have then described further decreasing activation, as measured by regional cerebral blood flow (rCBF) or by glucose metabolism. PET studies in the laboratory of

Pierre Maquet have shown that decreased rCBF to the thalamus may be an early event following sleep onset that is then succeeded by deactivation of medial cortical, limbic, and striatal areas. These researchers have also shown that decreasing blood flow to the ventromedial prefrontal cortex (vmPFC) correlates with increasing SWA. The vmPFC is part of a network of primarily medial cortical areas that has been termed the brain's 'default network.' Correlated activity throughout this network occurs in waking when the brain is not engaged in any effortful task and such correlated activity may persist into the lighter stages of NREM.

Recent fMRI work by in the laboratory of Michael Czisch and colleagues provides greater detail on the widespread deactivations that occur from waking to NREM and as NREM deepens. Following sleep onset, blood flow decreases to frontal areas including inferior, middle, and superior frontal gyri, as well as medial frontal and primary motor cortices. As NREM sleep deepens, deactivation is also seen in midline subcortical (e.g., hypothalamus, caudate), thalamic (e.g., anterior nucleus), limbic (e.g., insula, hippocampal complex), parietal (e.g., inferior parietal), and occipital (e.g., precuneus, cuneus) structures. Such deactivations are greater in the right hemisphere.

Much of the lateral frontal and parietal cortices remain less active than in waking after the transition from NREM to REM. However, PET studies have shown that blood flow and glucose metabolism increase during REM in subcortical brain regions such as the pons, midbrain, thalamus, amygdala, hypothalamus, and basal ganglia as well as limbic-related cortices such as parahippocampal cortex, temporal pole, anterior insula, caudal orbitofrontal cortex, subcallosal anterior cingulate, and medial prefrontal cortex. Maquet and colleagues have shown that regions most consistently remaining hypoactive in REM sleep compared to waking are in lateral cortical association areas, specifically those in middle and inferior frontal gyri, inferior parietal and temporo-parietal cortices.

In contrast, there are areas where the increase in regional glucose metabolism during REM exceeds even that of waking. Eric Nofzinger and colleagues have termed these areas the 'anterior paralimbic REM activation area,' a midline region comprised of structures including hypothalamus, ventral striatum and pallidum, hippocampus and uncus, as well as supplementary motor, pre- and subgenual anterior cingulate and insular cortices.

Thomas Balkin and colleagues have shown that frontal reactivation lags behind that of the rest of the brain following awakening. Such a lag in frontal reactivation may be the basis of reduced alertness and performance measured immediately following awakening, especially after sleep that follows sleep loss, a phenomenon termed 'sleep inertia.'

The Effects of Brain Lesions on Dreaming

Traditional neuropsychology infers regional brain function from the effects of stroke and other brain injury on behavior. Mark Solms has applied this approach to the study of dreaming by surveying hundreds of his patients and reviewing older neurological and neuropsychological reports asking the question what happens to dreaming when different parts of the brain are damaged. Solms has identified brain-injury-related syndromes of dreaming. In global anonera, total cessation of dreaming results either from bilateral or unilateral inferior parietal lesions or deep bilateral lesions of the ventromedial prefrontal white matter as was produced by lobotomy. In visual anonera, complete or partial loss of dream visual imagery results from bilateral lesions of visual association cortices in medial occipito-temporal areas. Solms found that patients with visual anonera were also unable to produce mental imagery in waking, a syndrome termed visual irreminiscence. Visual anonera need not be total, and there exist partial syndromes in which the dreaming counterpart to the waking function of an extrastriate area is lost when that area is damaged (e.g., 'kinematic anonera' or 'facial anonera'). Solms also identified disorders in which seizure activity increased the frequency and intensity of dreaming or anterior subcortical damage appeared to disinhibit dreams.

Damage to areas that remain relatively deactivated during REM sleep, the primary visual and dorsolateral prefrontal cortices, has little effect on dreaming. In contrast, several of the areas where lesions do affect dreaming are notable in that they do show increased blood flow during REM. Such areas include visual association cortices of the inferior temporal lobe, and particularly those associated with the ventral stream of visual processing – the portion of the visual system that identifies objects, faces, and places. Another such area is the anterior limbic cortex and subcortex corresponding to Nofzinger's "anterior paralimbic REM activation area."

Both Solms and earlier investigators found that isolated damage to the inferior parietal cortex, especially on the right side, can eliminate dreaming. The right inferior parietal lobe (rIPL) is an area essential to spatial attention and its damage, or even temporary inactivation using transcranial magnetic stimulation (TMS), can result in a neglect of left hemispace. The rIPL has also been linked to a number of high-level cognitive skills, including visual working memory, preparation of serial actions, allowing an egocentric frame of reference, and visual self-other discrimination. However, paradoxically, Maquet and colleagues have shown that, in most PET studies, the inferior parietal lobes have remained deactivated in REM. Notably, however, in an early PET study, the Maquet group found that an anterior portion of the right

supramarginal gyrus was activated in REM. Explanation why this multifunctional high-level association cortex should be essential to dreaming remains an intriguing question.

The Electroencephalographic Correlates of Dreaming

After REM sleep was discovered by Aserinsky and Kleitman in 1953, investigators noted a high probability of obtaining a dream report when a sleep laboratory subject was awakened from REM. Such observations led early dream researchers to speculate that REM sleep was the exclusive time when dreaming occurred. However, soon thereafter, additional sleep laboratory studies also demonstrated substantial recall of dreams following NREM awakenings. Nonetheless, subsequent laboratory studies have clearly shown that a larger percentage of REM than NREM awakenings are associated with dream recall. Moreover, it has been consistently shown that, compared to NREM dreams, REM dreams are longer as well as more bizarre, motorically active and emotional. In a review of 29 studies, Tore Nielsen has estimated a NREM recall rate of 42.5% contrasted with a REM recall rate of 81.8 %. Nielsen hypothesizes that NREM dreams may result from REM-like physiological conditions that fail to generate the particular suite of biosignals used to score REM by standard criteria – a phenomenon he terms “covert REM.”

Fast neuronal oscillations at gamma frequencies (30–80 Hz) appear in waking in association with attention to stimuli, working memory, and other forms of effortful, directed cognition. Oscillations at this frequency are greatly diminished during NREM; however, EEG and MEG studies have shown that these fast oscillations return in REM sleep. It has been hypothesized that, in waking, the functional correlates of these gamma oscillations are long-range binding of the multimodal components of complex percepts. David Kahn and colleagues have suggested that the gamma frequency oscillations of REM sleep may play a similar role with regard to the construction and cohesion of dream elements.

Contrasting with REM, in human NREM sleep and especially stage 2 and SWS, gamma frequency activity is greatly attenuated and, instead, the slow oscillatory rhythms generated by thalamocortical networks, including spindles, delta waves the cortical slow (<1 Hz) oscillation predominate. The lesser frequency and intensity of dreaming in NREM may, therefore, be due to interference with ongoing mental activity by such intrinsic oscillations.

Spectral analysis uses fast Fourier transformation of EEG data to compute the percentages of total EEG power

that occur in the different sleep-relevant frequency bands of the EEG spectrum. This technique has been used to investigate whether greater power in particular frequency bands before awakening is associated with dream recall. Greater percentages of EEG power in faster frequencies (e.g., beta) or lower percentages in slower frequencies (e.g., delta) have been associated with greater frequency, length, or dream-like character of reports in some but not all studies. Since faster EEG frequencies are associated with greater cortical arousal, their association with sleep mentation is predicted and the weight of evidence across majority of studies generally supports this prediction. However, there have been a number of studies that found no such dream correlations, dream correlations that were only seen at certain scalp locations (EEG derivations), were opposite to the predicted direction, or occurred with post- versus pre-awakening EEG spectral composition. In addition, correlations of spectral power distribution with dream recall were not identical between studies of REM and NREM awakenings. Moreover, most studies did not associate dream variables with spectral power in the fastest (gamma) or slowest (<1 Hz cortical slow oscillation) EEG frequencies.

A great deal of attention has been paid to cognitive correlates of phasic (brief, intermittent) events during sleep in relation to dreaming. For example, in stage 2 NREM, Russell Conduit and colleagues have shown that auditory stimuli below the threshold for awakening increase the frequency of reports of visual imagery in subsequent awakenings. These investigators also showed that NREM awakenings preceded by eyelid movements (ELMs) yielded a higher frequency of visual imagery reports than awakenings not preceded by ELMs. They attribute their observations to imagery being stimulated by transiently increased brain arousal that either follows sensory stimulation or occurs spontaneously producing both the ELM and associated imagery.

Most attention, however, has been paid to dream correlates of the phasic events occurring during REM, especially the REMs themselves. Investigators have traditionally subdivided REM sleep into phasic periods during which clusters of REMs occur and tonic periods lacking or with only occasional, isolated REMs. Intense interest in phasic REM has resulted from the close temporal association of REMs with ponto-geniculo-occipital (PGO) waves discovered in the cat. These ascending potentials, originating in the brainstem, suggested to Allan Hobson and Robert McCarley, in their activation-synthesis hypothesis, a means by which, during REM, a phasic signal, arriving via the visual pathway, might be interpreted as sensory input by the visual cortex thereby leading to dream hallucination.

Since the publication of Hobson and McCarley's hypothesis in 1977, tantalizing suggestions of human PGO waves have been reported from EEG and

neuroimaging studies. Three very recent findings, however, have provided the most powerful evidence of the human PGO and of cortical correlates of phasic REM respectively. First, Lim and colleagues described phasic signals with wave form and temporal characteristics very similar to the feline PGO, originating, during REM, from the pons of a Parkinson's Disease patient with depth electrodes placed in the pedunculopontine nucleus (PPT). Second, using MEG tomography, Ioannides and colleagues have shown that preceding and correlated with rapid eye movements (REMs), there is phasic brain activity in the pons that is accompanied by simultaneous activity in frontal eye fields (FEF). Notably, just prior to REMs, activation was also seen in the amygdala and limbic cortices. Third, using fMRI, Renate Wherle, in Michael Czisch's laboratory, has shown that characteristic changes in forebrain activity accompany phasic REM episodes arising from a tonic REM background. Changes included disappearance of the residual responses of the auditory cortex to sound stimulation seen in tonic REM as well as enhanced synchronization with thalamic activity of a broad cortical-limbic-striatal network. These investigators posit that this represents activation, during phasic REM, of networks important to memory and emotional processing that are simultaneously shielded from interference by an increased cortical threshold to external sensory input.

Cortical Connectivity in Sleep

Profound changes in addition to those used to stage sleep can be seen in the EEG of the sleeper when techniques of quantitative EEG are applied. Such techniques suggest both increased synchrony of slow oscillations and loss of coherence among fast EEG rhythms in NREM and REM sleep, respectively.

The slowing and synchronization of the NREM EEG is accompanied by the emergence of waveforms that reflect underlying thalamocortical oscillations such as spindle- and delta-frequency rhythms and the slow cortical oscillation discovered by the late Mircea Steriade. A widely replicated finding has been that slow wave activity is greater in frontal cortex following sleep onset and then spreads posteriorly as NREM deepens. For example, using mathematical estimates of functional coupling and direction of information flow, Luigi DeGennaro and colleagues have demonstrated an anterior-to-posterior directionality in the progressive EEG synchronization following sleep onset.

While such slow oscillations suppress cognitive activity in NREM, loss of long-range coherence in rapid gamma frequency oscillations may contribute to disorganization of cognition in REM dreaming. Unlike the wide-range coherence of gamma frequency oscillations seen in

waking, in REM, such oscillations become desynchronized and lose coherence between frontal and posterior cortex or between cortex and hippocampus. Researchers, such as Maria Corsi-Cabrera, suggest that such gamma desynchrony may reflect functional disconnections between different brain regions that could contribute to the hypofrontal features and weirdness of REM sleep dreaming. In the last several years, transcranial magnetic stimulation (TMS) studies have been employed to experimentally demonstrate such functional disconnections between different brain regions during sleep.

A Descriptive Neurobiological Model of Dreaming

Empirical demonstration of regional brain activity preceding specific dream reports awaits experiments combining EEG and neuroimaging techniques. Nonetheless, neuroimaging studies of sleep, phenomenological and brain-lesion studies of dreaming, and, especially, the ever-increasing understanding of the brain basis of waking skills and subjective experience allow us to construct a speculative, descriptive neurobiological model of the brain basis of dreaming. This model is summarized here and is detailed in two book chapters by this author and a review by Hobson *et al.* cited below.

First, since dreaming is a form of consciousness, albeit one lacking insight into one's mental state, portions of the forebrain must be activated to a level that will support such fictive experience. As described in preceding sections, during REM sleep, the ascending reticular activating system (ARAS) activates the thalamus and basal forebrain that, in turn, activate subcortical limbic structures as well as specific parts of the cortex described above. Importantly, however, during REM, this ascending activation is driven by the cholinergic nuclei of the mesopontine brainstem rather than by the full complement of wake-promoting aminergic (noradrenergic, serotonergic, and histaminergic) systems that accompany cholinergic activation in waking.

Relative to forebrain activation in waking, such aminergically deficient and cholinergically biased forebrain activation in REM undoubtedly contributes to the unique quality of dream awareness. For example, differences in waking and sleep neuromodulation of the forebrain might underlie the differing regional pattern of forebrain activation seen in these two states of consciousness. Alternatively, or additionally, since aminergic systems have been abundantly demonstrated to facilitate higher cognitive functions such as attention and working memory, the nadir of such systems' influence on the forebrain during REM may subtract certain key capacities of waking consciousness (e.g., insight) from that of dreaming.

The regional pattern of brain activity in REM includes Nofzinger's "anterior paralimbic REM activation area" as well as brainstem activity in the pons and midbrain. Included in this midline activation are the hypothalamus, basal forebrain, amygdala, ventral striatum and pallidum, as well as anterior cingulate (ACC) and other limbic cortices. Activity in these regions may generate the emotionally salient features of dreaming. For example, anger, sexuality, or panic states in dreaming may result from activity in specific regions of the hypothalamus that trigger such instinctive programs. Similarly, appetitive behaviors, Freud's dream 'wish fulfillment' that is emphasized in Mark Solms' dream theory, may result from activity in the brain's reward system that includes midbrain dopaminergic cells of the ventral tegmental area (VTA) as well as the ventral striatum and pallidum and the medial prefrontal cortex. Similarly, fear and anxiety, ubiquitous if not predominant dream emotions, may result from activity in the amygdalar, hippocampal, basal forebrain (e.g., bed nucleus of the stria terminalis), and medial prefrontal (e.g., subgenual ACC) areas that support the encoding of fear and extinction memories. Ross Levin and Tore Nielsen's recent theory on the emotion regulatory function of normal dreams as well as dysfunction of this system in nightmare disorders focuses on REM-related activity in these fear circuits of the limbic forebrain.

In addition to a role in reward processing and fear regulation, regions of the medial prefrontal cortex are essential to social cognition and social judgment, prefrontal functions emphasized in Antonio Damasio's somatic marker hypothesis. Notably, dreaming is a highly social experience in which the dreamer interacts with a panoply of fictive characters of a variety sometimes wider than in waking, such as when characters from the distant past reappear. Moreover, many of the sophisticated social skills deployed in waking such as the ability to 'mind-read' – i.e., infer and proactively or reactively respond to what a dream character may be thinking or feeling – are preserved in dreams. Additionally, high-level social emotions such as guilt and empathy are not uncommon in dreams.

While episodic memory in the form of reenactment of events experienced in waking is notably deficient in dreams as reported by Roar Fosse and colleagues, recognition memory is intact and sometimes bizarrely enhanced. For example, in a study by David Kahn and colleagues, almost half of dream characters were identified on the basis of 'just knowing' who they were. Moreover, it is also not uncommon for a dream character to substantially differ (e.g., in appearance, age, sex, or mortal status) from its waking counterpart but nonetheless be confidently identified as that individual.

Altered distribution of activity in among different components of the hippocampal complex in REM

compared to waking may underlie this altered distribution of mnemonic activity and accuracy in dreaming. Neuroimaging studies of waking are increasingly assigning roles to frontal cortices in memory function. Lateral and polar frontal areas are involved in effortful, strategic encoding and retrieval, whereas posterior medial areas are involved in feeling-based confirmatory aspects of memory. In this regard, poor working memory, loss of orientation and the illogical, non-volitional appearance of dream persons, places, and things may reflect the above-noted hypo-activity of the lateral prefrontal cortex. Conversely, the confabulatory nature of dream narratives may reflect the REM-related hyperactivity of posterior-medial frontal areas and adjacent basal forebrain – the very areas whose disruption in waking leads to amnesic confabulatory syndromes.

Fictive motor activity is being initiated, controlled and inhibited during dreaming just as its actual counterparts are enacted in waking. In REM, however, actual contraction of skeletal muscle for the enactment of motor commands is inhibited by the active atonia described in previous sections. Prominent activity in those areas involved in the initiation and control of motion, specifically the basal ganglia and cerebellum, have been noted in neuroimaging REM, such as in the PET studies of Allen Braun. Cerebellar activity may specifically result in the vestibular anomalies of dreaming such as sensations of flying, falling, or moving in a vehicle. Interestingly, primary motor cortex and lateral pre-motor areas are not reported to be re-activated in REM. It is possible, however, that motor functions of medial areas, such as the dorsal anterior cingulate or pre-supplementary motor area (pre-SMA), produce motor commands in dreaming. As in the case of most aspects of the functional neuro-anatomy of dreaming, such hypotheses suggest important studies yet to be performed.

Perhaps the most striking aspect of dreams is their remarkably life-like visual character. ARAS activation of the visual forebrain as well as phasic signals from brainstem to visual association cortices may account for dreams' visual content. For example, complex hallucinosis of animate and inanimate objects may be subserved by activity in visual association cortices of the ventral stream of visual processing. Braun's PET studies have identified REM-related activity in extrastriate areas of the ventral stream and, as noted above, Solms' studies have associated damage to these areas with nonvisual dreaming. This may be a process analogous to the visual hallucinosis of waking as is suggested both by neuroimaging of complex hallucinosis in waking (e.g., in Charles Bonnet syndrome), and by the co-occurrence of nonvisual dreaming and inability to visualize during waking reported by Solms. Notably, specific areas identified with waking perception of faces and scenes, the fusiform gyrus of the inferior temporal

cortex and the parahippocampal gyrus, respectively, have also been shown to increase their activity during REM.

Although dream hallucinosis is thus neurobiologically explicable, the integration of these hallucinations into identifiable scenes as well as into elaborate dream plots is a remarkable phenomenon that suggests a predisposition of the brain to generate narratives even absent the awareness of doing so. This is all the more remarkable given the deficits in executive and higher-order functions during dreams, deficits that can be explained by the relative inactivity of lateral frontal and parietal cortices during REM as compared with waking. Such executive deficits include the attenuated attention, working memory, logic, volition, and orientation that Allan Hobson and colleagues have described. The persistence of narrative capacity in the face of such deficits may involve activity of medial prefrontal structures shown by Braun and colleagues to be activated by narrative generation whether it is in spoken language or in sign language.

A second mystery, as discussed above, is the role assigned to the inferior parietal cortex, especially in the right hemisphere, that, although typically remaining deactivated in REM sleep, has been shown by lesion studies to be essential to dreams. It has been suggested that its role is to generate the fictive space in which dreaming occurs. However, there may be other functions dependent upon this high-level multimodal association cortex, such as the establishment of an egocentric spatial perspective, without which dreaming cannot occur.

Conclusion

Laboratory awakening studies on the polysomnographic correlates of dream recall frequency or intensity have generated important hypotheses and provided valuable, replicated findings. Similarly, neuroimaging of the sleeping brain has provided replicated observations of regional cerebral activity corresponding to polysomnographically defined sleep stages, albeit without accompanying subjective reports. Inevitably, awakenings from sleep and their dream reports will be accompanied by both spatially precise functional images of the dreaming brain and temporally precise EEG measures. When such techniques become widely employed, activity of the sleeping brain as it generates a dream may at last be directly observed. As has been the case throughout the history of dream research, however, such technical advances will undoubtedly generate yet more new and intriguing questions.

See also: Animal Models of Learning and Memory; Bioenergetics of Sleep; Brain Imaging; Circadian and Ultradian Clocks/Rhythms; Cognitive Control in the Service of Self-Regulation; Conscious and the

Unconscious; Disorders of Face Processing; Emotion–Cognition Interactions; Episodic and Autobiographical Memory: Psychological and Neural Aspects; Hallucinations in Neuropsychiatry and Drug Abuse: From Phenomenology to Pathophysiology; Neural Basis of Working Memory; Neural Systems of Motivation; Physical Cognition and Reasoning; Short-Term Memory: Psychological and Neural Aspects; Sleep Genetics; Sleep: Learning and Memory; Sleep: Medical Disorders; Sleeping, Waking, and Dreaming; Social Cognition: From Behavior-Reading to Mind-Reading; Subjective Experience and the Expression of Emotion in Man; Temporal Lobe and Object Recognition; Vision.

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Sleeping, Waking, and Dreaming

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Introduction

To make a faithful copy of the world is one good act a brain can do. However, to correctly predict the world's features is even better. It is the general theme of this article as well as the articles related to sleep that the inherent capacity of the brain to change its state, as it does in sleeping, waking, and dreaming confers significant adaptive advantage upon those organisms that can do so regularly. One clear implication of the state changes from waking to rapid eye movement (REM) is to reveal the brain's built-in capacity to predict.

Timing by Neuronal Clocks

It is already clear that all cells, be they animal or vegetable, anticipate the light-dark cycle of the world by means of intrinsic rhythms of about 24-h circadian length. Even individual molecules of DNA are clock-like mechanisms that can self-regulate so as to operate in the most favorable light and temperature phases of each day.

On the other hand, higher animals with elaborate brains can do more than this. They can take advantage of their intrinsic circadian rhythms to program sleep during the rest phase of their circadian rhythm and within that sleep they can program an oscillation of sleep phases in one of which we humans dream. This article, attempts to show that animals take advantage of these extrinsic and intrinsic rhythms to conserve energy (and manage its expenditures) and to organize information (and apply its instructions) in an admirably supple way that varies markedly according the ecological realities to which each species must conform.

Why do we dream? The theory that we develop here is that we dream because our brains are performing genetically programmed virtual behaviors of use to us in the sensorimotor development of our learning about the world.

The Concept of State

Most simple organisms have two states, activity (on) and rest (off), which depend upon ambient temperature. Thus, at any given warm temperature, they have

only one state, waking. They may be more or less on as a function of temperature-mediated activation. At all other cool states, they become torpid. Notable exceptions to this rule have raised hopes that a genetically detailed but simple neuronal model could be developed for basic sleep research. The fruit fly (*Drosophila*) and the round worm (*Caenorhabditis*) have both been shown to reduce activity and responsiveness with changes in temperature.

Mammals and birds are the only creatures that maintain a high (+37 °C) body temperature over a wide range of ambient temperatures. We say that they are homeothermic (rather than poikilothermic like their less-sophisticated forebears). Some mammals adopt an intermediate strategy: hibernation. Hibernation is an active state in which core body temperature is reset at 0.5 °C in the late fall and held at that level until ambient temperature rises in the spring.

Homeothermy conveys an adaptative advantage. Behavior can be maintained despite relatively unfavorable environmental conditions. The behavior that is maintained by homeothermy is also more versatile than that of poikilothermic animals. This versatility not only extends to such motor skills as walking, swimming, or flying, but also to the succession of global states such as sleeping and waking. Most spectacular of all, and crying out for deeper understanding, is the brain-activated state, called REM, which regularly punctuates sleep and is associated in humans with vivid hallucinoid dreaming.

These sets of conditions are called 'states' because they are so global, so enduring, and so clearly distinguished from each other as to imply distinct regulatory mechanisms and differential functions. It is the burden of this article as well as related ones to communicate the insights that have been gained regarding these states over the past half century.

To anticipate a bit, we will show that mammals have modified these interrelated states in order to achieve an even more impressive set of capabilities that allow their brains (1) to anticipate the future and prepare for it through development; (2) to incorporate the past, and learn from it; and (3) to maintain homeostasis so as to guarantee optimal achievements in the planning, coping, and creative behaviors of the future.

Spontaneity and the Reflex Model

For most of the twentieth century, neurobiology and psychology were both dominated by Ivan Pavlov and BF Skinner who applied the reflex paradigm to psychology. This paradigm was also very fruitful in the hands of the father of neurophysiology, Charles Sherrington, and it came to define the first century of experimental neuroscience from 1900 to 2000 and beyond. In the reflex paradigm, a set of resting conditions is established. Then a stimulus (S) is applied and a response (R) is measured. The characteristics of the S–R complex are then analyzed and intervening brain processes are inferred. New hypotheses can be derived and tested by modifying the stimulation parameters.

Essentially, the same format was used in psychology throughout the twentieth century. Pavlov combined stimulus pairs to evoke conditioned responses from unconditional stimuli and Skinner altered response pattern to spontaneous stimuli arising from the behavior of animals, the operant which was to be conditioned. In Skinner's model, the response became a stimulus which structured an environmental reward placing the reflex loop outside the brain.

The brain was thus seen as either a black box (a la B.F. Skinner) or a black board (a la John Locke) upon which the outside world wrote its messages and dictated our behavior. Prior to 1950, there was little room in physiology or in psychology for much of value to arise from within the brain itself. By postulating the id, Freud began to help build the case for spontaneity, for oscillation, and for built-in order and purposeful design. However, with the discovery of the electroencephalogram (EEG) in 1928, the recognition of the sleep cycle in 1936, the conceptualization of the reticular activating system in 1949, and, especially, the discovery of REM sleep in 1953, it was possible to realize how much useful information came from within the brain itself.

Activation (A)

Until Hans Berger discovered the EEG in Jena in 1928, there was no objective instrumental means for studying sleep. Charles Sherrington, the father of neurobiology, opined in *Man on His Nature*, that the brain turned off in sleep, while the great experimental psychologist, Ivan Pavlov, ascribed sleep to cortical inhibition. Both men shared the hypothesis, promulgated by the Belgian physiologist, Frederic Bremer, that sleep was caused by the decline in sensory stimulation that occurred at nightfall and was triggered by turning out the light in our bedroom. Not until morning was external stimulus strength sustained enough to support waking.

The Overthrow of the Deafferentiation Hypothesis

By 1930, when Hans Berger had put the EEG into the hands of scientists like the Belgian physiologist, Frederic Bremer, the sensory deafferentation hypothesis was so deeply engrained that it took almost 20 years to dislodge it. Yet nothing in Hans Berger's work suggested that brain activity ceased at sleep onset. In fact, the characteristic spindles and slow waves of sleep helped to convince early skeptics such as Edgar Adrian in Cambridge that the EEG signals were really of brain origin and not muscle artifacts.

The brain of Berger's EEG subjects had not turned off at sleep onset; instead, it had changed state and we now know that the activation level (A) declines by 20–40% overall in sleep. This signifies that the brain, without external stimulation, is capable of maintaining a remarkably high level of activation, but one that is not high enough to support consciousness and to be fully compatible with significant information processing. Bremer tried to prove his deafferentation hypothesis by cutting across the midbrain creating the *cerveau isolé* whose EEG rostral to the cut was permanently that of sleep. Bremer assumed that this effect was caused by the loss of information arising from the lower body. However, when he found that the EEG of a cat with a C-1 spinal cord transection was constantly activated, he was obliged to posit that the activating force was entirely due to sensory stimulation arising in the trigeminal nerve which entered the brainstem between the levels of the two cuts!

Never once did Bremer, or any other scientist, take the hypothesis seriously that activation arises within the brain itself. This failure to consider an internal activating system is more surprising, given the impact of the somnolent effects of the 1918 flu epidemic which Von Economo tentatively but presciently ascribed to damage in the midbrain and hypothalamus.

In part because of this conceptual block and in part because of the complexity of the new technology, progress was slow through the 1930s and 1940s and stopped entirely with the outbreak of World War II. In 1936, Loomis and Harvey did describe periodic activation and deactivation of the EEG in human sleep with a period length of about 90–100 min; however, they failed to observe REM and hence to conclude that the cyclic activation of the brain in sleep was entirely intrinsic. This discovery, when it was finally made, was entirely accidental!

The Reticular Activating System (1949)

Giuseppe Moruzzi was a friend and colleague of Frederic Bremer. After World War II, Moruzzi went from Pisa, Italy, to the Northwestern University in Chicago to study

the motor system with the American neurophysiologist, Horace Magoun. They used sharpened insect pins to record the action potentials from pyramidal tract cells of the cat motor cortex. Subsequently, they stimulated pyramidal tract fibers in the medullary brainstem hoping to identify pyramidal motoneurons via antidromic invasion. To monitor level of anesthesia in the animals, Moruzzi and Magoun recorded the cortical EEG as well.

To their great surprise, Moruzzi and Magoun observed that whenever they stimulated the brainstem the cortical EEG generated low-voltage fast waves indicative of activation. Having excluded the possibility that the effects were due to the afferent sensory inputs emphasized by Bremer, they suggested, for the first time, that brain activation could be intrinsically mediated by the brainstem itself.

Although the brain-activating effects observed by Moruzzi and Magoun could be obtained at many points from the hypothalamus to the medullary reticular formation of the brainstem, the anterior midbrain was the most potent activating site. We now suspect that this is because of the concentration of both aminergic and cholinergic modulatory neurons at the site, which are known to participate differentially in the activation process of both waking and REM sleep. Because of its anatomical complexity, the brainstem reticular formation had been dubbed ‘a wearisome labyrinth’ by Santiago Ramon y Cajal. It is important to emphasize that even today we are only beginning to understand this small but critically important part of the brain.

The Discovery of REM Sleep (1953)

Similar to the 1949 discovery of the reticular activating system, which was an accidental by-product of a study of the motor system in cats, the discovery of REM sleep was an accidental by-product of the study of attention in children. Nathaniel Kleitman’s graduate student, Eugene Aserinsky, placed electrodes beside the eyes of his young subjects in order to monitor their alertness during the performance of an attentional task. When they became bored, these subjects dozed off. As they were young, they often had sleep-onset REM periods and, when awakened, reported vivid dreams. (One of the subjects was Aserinsky’s 9-year-old son, Armand.)

Following Aserinsky’s report on his findings in children to Kleitman, it was decided to record the nocturnal sleep of adults. When they observed the periodic EEG activation of sleep, together with the eye movements, Aserinsky and Kleitman performed instrumental awakenings in an attempt to elicit reports of mental activity. Awakenings from REM sleep yielded detailed reports of vivid, bizarre, and sustained dreams, a finding which has been confirmed and elaborated upon in the subsequent half century. Aserinsky and Kleitman also described the

heart and respiratory rate increases and irregularities during REM sleep. Their study ended forever the myth that dreams were mysterious imaginations coming from outside of the body or even from some obscure part of the mind. The naturalization of the human psyche can be said to have commenced, in earnest, in 1953!

By 1957, William Dement had established the existence of REM sleep in the cat. Dement’s discovery was confirmed by Michel Jouvet and Francois Michel in Lyon, France, in 1959. Wishing to monitor alertness in their cats, which were subjected to Pavlovian conditioning in order to study learning, the neck muscle electrodes that were implanted showed a complete cessation of electromyography (EMG) activity as the cats postural muscle tone was inhibited at REM sleep onset. This observation set the stage for the many fascinating findings that are described as the input–output gating functions of REM sleep. Before turning our attention to this topic, let us emphasize that up to this point, brain activation was thought to be a global and uniform process because its regional and chemical differentiation were still unknown.

The Non-REM–REM Sleep Cycle

The easiest way to think of variations in activation over the sleep cycle is by picturing a damped oscillator with its greatest amplitude and longest stays in non-REM (NREM) sleep early in the night and shallower depth with longer stays in REM later in the night. Period length is relatively constant, for adult humans, at 90–100 min.

The damping of the NREM–REM sleep oscillator is a function of the interaction of the circadian tendency to alternate rest and activity with the homeostatic tendency for NREM sleep drive to increase with increasing time awake. Alex Borbely has incorporated these data in his elegant two-factor model of sleep propensity over time.

Sleep is a highly conserved behavior indicating its priority among survival strategies. Most adult humans sleep 4–10 h a day and suffer with less than their baseline quota. Lost sleep is recovered scrupulously: about 50% is recovered as time asleep and more as the intensity of rebound sleep. Short sleepers tend to be energetic and productive; long sleepers are more likely to be lethargic and reflective. Deprivation of sleep leads to fatigue, sleepiness, irritability, difficulty in concentrating, feeling cold and, in extreme cases, to psychosis. There is no difference between the effects of REM and total sleep deprivation but the universally fatal effects of both suggest that REM may be a more efficient recovery process. Rats which are sleep deprived for 4–6 weeks lose weight in the presence of abundant food, fail to regulate their body temperature, and become susceptible to infections they normally resist (see the section titled ‘Functional implications’).

The Concept of the Brain–Mind

So closely do the psychological features of cognition and emotion parallel the physiological features of sleep and waking that we can now hypothesize that mental activity is a brain function and the brain–mind is an integrated system. It is obvious to most people, and certainly to most scientists, that mind and brain are inseparable even though we do not yet understand how they could be as unified as they appear to be. This is what philosopher David Chalmers calls ‘the hard problem.’ One possibility is that brain–mind is only one entity but an entity which possesses subjective as well as objective features. This formulation, which seems philosophically and scientifically inescapable, does not yet resolve the mind–brain problem (or even make it easy) but it does seem to make a unified theory inevitable.

A working hypothesis of those scientists who study brain–mind states is that a deeper understanding of how brain activity becomes mental can be gained by the strategic study of sleeping, waking, and dreaming. Such studies have already proved illuminating with respect to sensation, perception, orientation, emotion, and memory. These modular functions contribute to the kind of consciousness that we experience in the several states. For example, it is dramatically clear that while waking is dominated by external perceptions and by abundant thought, dreaming is dominated by intense perceptions of internal origin and by greatly impoverished thought.

Thus, the kind of consciousness that we experience is clearly a brain function and we can now specify how and why this is the case. We can also model both the internal percepts of dreaming and the deficient cognitive capacities of that state and suggest how they might derive from changes in perfectly normal neurophysiology. It remains to be deduced what advantage may arise from these modular differences between dreaming and waking. Our suggestion is that the capacity to form visual images in the absence of external inputs is an important skill for both normal exteroception and for imagination. The creature with a well-developed capacity for these skills will be at a competitive advantage.

The EEG in NREM Sleep

The alert brain–mind is characterized by a low-voltage fast EEG; a gamma rhythm of $40\text{--}60\text{ c s}^{-1}$ can be seen. This rhythm is generated by the activated and synchronized neuronal firing in widespread cortical circuits. Drowsiness is characterized by EEG slowing in the $4\text{--}8\text{ c s}^{-1}$ theta range. When thalamocortical circuits escape from brainstem control, stereotyped clusters of $12\text{--}15\text{ c s}^{-1}$ spindles dominate the cortical EEG and sleep proper (stage II) commences. As sleep lengthens and deepens, $1\text{--}3\text{ c s}^{-1}$ slow waves come to be associated with spindles

in stage III and then to dominate the record of stage IV NREM sleep (**Figure 1**). This progressive process reverses itself when REM sleep supervenes. All spindles and slow waves are suppressed as their neuronal oscillators are blocked and the EEG reverts to wake and drowsy-like patterns of low voltage, fast activity, including alpha, theta, and gamma rhythms (**Figure 2**).

REM sleep is at its peak prominence at 30 weeks *in utero* when the brain is rapidly developing. It falls steadily during the first year of postnatal life during which time it may decline as much as 400% while waking greatly increases. The correlation of REM sleep with brain immaturity suggests a developmental function consistent with the idea that the sleeping brain is capable of activating circuits that are crucial for waking behavior and for consciousness. Contrastingly, the capacity to generate robust NREM sleep increases with age and with brain development from early infancy to age 30 when it sharply declines only to disappear at age 40. With the disappearance of NREM sleep goes brain secretion of such anabolic chemicals as growth hormone (GH) and luteinizing hormone (LH).

Both NREM and REM sleep diminish in strength over the balance of a lifetime when cognitive and other functions show parallel declines.

Many other functions parallel the NREM–REM change with a night of sleep. Heart rate, respiratory rate, and blood pressure decline in NREM only to rise again to waking levels or beyond in REM. Erection (in males) and clitoral engorgement (in females) are common in REM whether or not the associated dreams are erotic. These and other changes in bodily function are often pathologically exaggerated. These clinical findings destroy forever the myth of sleep as inwardly peaceful. Under the cover of relative unconsciousness and immobility lurks a wide variety of physiological activities, not all of which are beneficial to health.

In animals, a highly rhythmic theta EEG rhythm can be recorded from the hippocampus. Such activity is also seen during intense exploratory activity in waking. In the cat, REM is associated not only with strong hippocampal theta but also with large biphasic sharp waves often occurring in clusters together with the eye movements. These waves are created in the pons (P), lateral geniculate body (G), and the occipital cortex (O). They are therefore called ‘PGO waves,’ named by their discoverer, Michel Jouvet of Lyon, France.

The many provocative similarities between waking and REM stimulate hypotheses about a functionally significant homology between the two brain states in which one (REM) is seen as the benefactor of the other (wake) and vice versa. This idea is at the heart of the unified theory developed here: REM sleep is an activation state of the brain–mind, which both anticipates and plans for waking. The brain–mind then uses data from waking state to shape its expectations of the future.

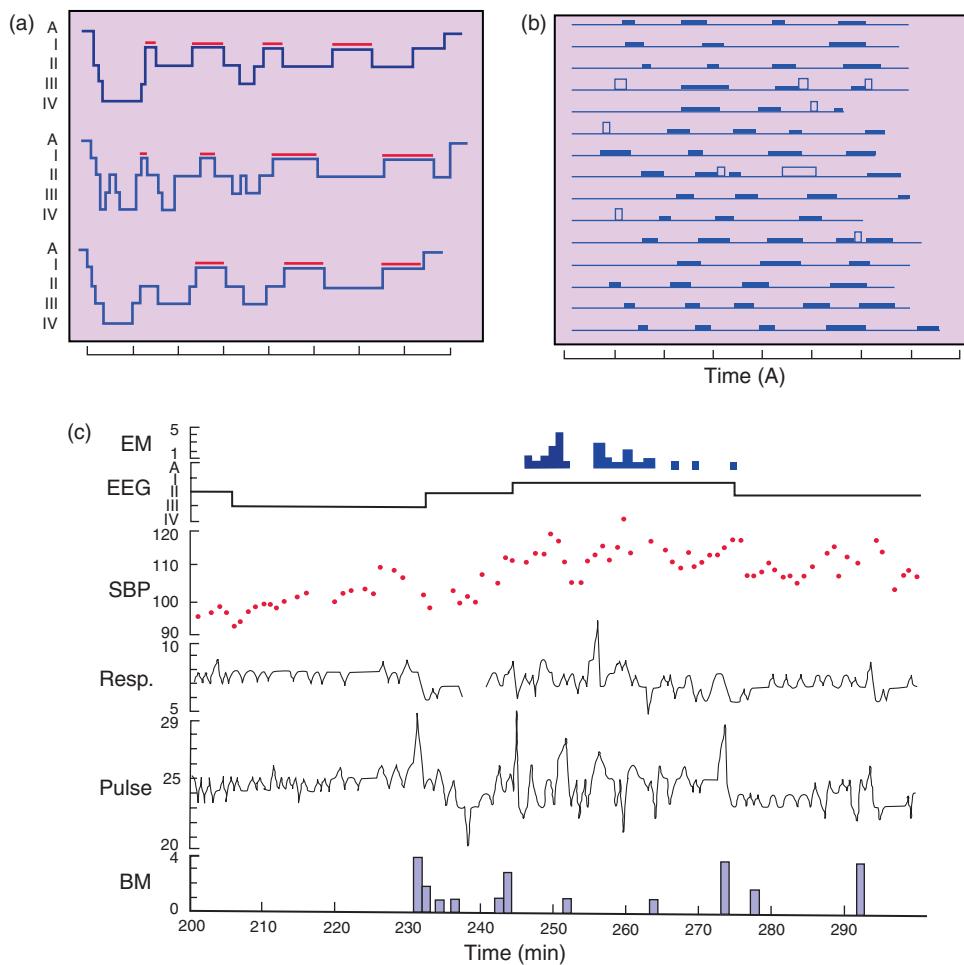


Figure 1 Stages of sleep: (a) standard polygraphic representation of three nights of human sleep; (b) ten nights of human sleep showing recurrent periods of brain activation; and (c) a single cycle of human sleep showing that clusters of eye movement in REM are associated with increases in systolic blood pressure and cardiorespiratory irregularity.

The dynamic interaction of sleep and wake states plays itself out at many levels of brain organization and benefits temperature control, cerebral homeostasis, and learning and memory. It is the unique task of the brain–mind to process information. This is the main assumption of the reflex paradigm in its focus upon external information. But what about internal information? Much internal information is in the service of the reflexive processors of the sensorimotor systems. This is particularly true of many autonomic pathways. However, just as the heart contracts rhythmically on its own, so does the brain manufacture its own data without any help from the outside world. An infant does not need to learn how to cry; it is born with that innate, powerful, and useful motor skill. In addition, there are many other such survival-enhancing skills, like sleep itself, which are innate and instinctual.

Information Source and Fate (I)

In this section, we summarize what sleep research has learned about how information processing is altered by sleep so as to help us understand both external (reflexive)

and internal (innate) information processing. Throughout this discussion, we will have reference to two aspects of this process: (1) those which regulate information flow to and from the world (via input–output gates) and (2) those which arise with the brain itself.

Sensory Input Gating

In order to preserve sleep in the face of strong central activation, it is necessary not only to suppress motor output but also to block sensory input. In the course of his work on the motor inhibition of REM, the Italian neurophysiologist, Ottavio Pompeiano, had shown that the sensory threshold was raised through the mechanism of presynaptic inhibition during REM sleep, especially when the eye movements were present. It is at such times that dreaming is most intense.

A second, important mechanism is the raised threshold to external sensory signals that occurs at secondary relay stations, especially in the thalamus, the sensory gateway to the cortex. As an intrinsic mechanism of sleep onset,

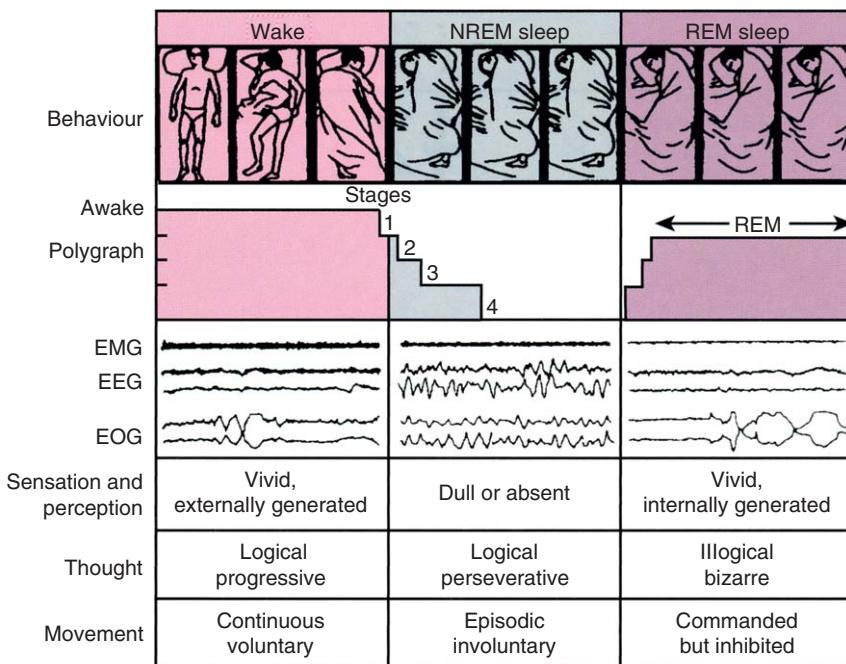


Figure 2 Behavioral states in humans. Posture shifts and changes in mental activity are associated with shifts in brain physiology.

the spindles of stage II NREM constitute spontaneous rhythmic discharge in thalamocortical and corticothalamic neurons. These paroxysmal discharges do not give rise to sensation; rather, they obliterate awareness and thus constitute usurpation of the sensory pathways by which an external stimulus might reach the cortex. This is deafferentation with a vengeance but it is not the lack of sensory input that Bremer imagined it to be. It is the result of an active blockade of neurotransmission called ‘occlusion.’

The neuronal dynamics of spindle and slow-wave production was first described by Dominic Purpura and his colleagues at the Columbia University in New York. They were later extensively elaborated upon by the late Mircea Steriade working at the Laval University in Quebec. One of the great triumphs of behavioral neuroscience indicates that spindles and slow waves exist not only to guarantee deafferentation by putting the forebrain offline but also to allow the actively deafferentiated brain to process data of relevance to learning and memory. Frederic Bremer and Giuseppe Moruzzi owe Mircea Steriade the honor for resuscitating the deafferentation concept in the service of neuronal plasticity.

The mechanisms by which plasticity may be enhanced by sleep involve pontomesencephalic neurons which are intrinsic to Moruzzi and Magoun’s reticular activating system. As long as tonic input from the brainstem cells of the reticular formation instructs the cells of the reticular shell of the thalamus to fire, they do so, suppressing the spontaneous rhythmic activity of the thalamocortical circuits. In this open-gate condition, the brain is awake and

the thalamus can relay external data to the cortex. At sleep onset, when reticular nucleus activation subsides, the thalamocortical circuits become co-opted by their own intrinsic rhythmicity and are no longer available for their wake-state external monitoring tasks.

This is a prime example of input–output gating by which the brain shuts its eyes, ears, and other sensors to the outside world so that it can attend more effectively to the many internal bookkeeping tasks of sleep. So far, we have emphasized the benefits of development and learning and memory. We will add benefits to temperature control in the next section.

Gyorgi Buzsaki has discovered that the flow of information within the brain is also altered in sleep. Not only are the external gates closed but also the flow of data out of the subcortical hippocampus (toward cortical storage sites presumed to be the home of long-term memory) is favored over flow into the hippocampus (where information is presumably captured and temporarily stored as short-term memory during waking). This observation serves to emphasize the general point that sleep is not so much a question of more or less information processed as about what kind of information processing is favored: Is it capture of information in short-term storage (waking) or is it consolidation and elaboration of long-term memory stores (sleep)?

As indicated above, occlusion is the term which is used to describe the internal occupation of sensory circuits by pseudo-sensory stimuli. Of course, the term ‘pseudo’ is only relative to the provenance, not the significance of such stimuli. Stimuli may be equally as meaningful when

they come from within the brain as when they come from without. Dream consciousness commonly and wrongly ascribes internal stimuli to external provenance. Hence, one of the cardinal features of dream psychology is the conviction that we are awake. This delusional property is just as strong a testimony to how well the brain activation of sleep mimics that of waking as it is a comment on how fully impaired is our insight and judgment during dreaming.

Motor Output Gating

The same Horace Magoun who, in 1949, co-discovered the reticular activating system of the brainstem worked with Ruth Rhines in 1947 and showed that electrical stimulation of the medullary brainstem could induce powerful suppression of spinal reflex activity. Postural muscle tone was observed to suddenly decrease in the decerebrate cat by Philip Bard and David Rioch at the Johns Hopkins Medical School who reported their observation to Michel Jouvet. With Francois Michel, Jouvet had reported periodic obliteration of muscle tone measured as the EMG during the REM sleep of cats.

In 1959, Jouvet reported the effects of preoptine transection of the brainstem at the same level that Frederic Bremer had interrupted to produce the perpetually sleeping *cerveau isolé*. Jouvet was interested in testing the hypothesis that a descending signal from the brainstem to the spinal cord mediated the muscle inhibition of REM sleep. He hypothesized that it was an ascending signal from the brainstem which mediated the EEG activation of REM.

Thus, Jouvet's results killed two birds with one stone. It was from the pontomesencephalic midbrain reticular formation that emanated both commands, an ascending pathway activated the forebrain and a descending pathway inhibited postural muscle tone! Jouvet's 1962 paper proposed, for the first time, that the pontine brainstem was a control region for REM sleep. In other words, the pontine brainstem had a way of waking up the forebrain while simultaneously preventing movement. This combination of changes caused Jouvet to coin the term, 'paradoxical' sleep. "The animal is asleep but the EEG shows it to be awake," he wrote in his laboratory notebooks of that era (**Figure 3**).

The later work by Ottaviano Pompeiano in Moruzzi's lab in Pisa, Italy, and by Michael Chase in the Brain Research Institute at the University of California, Los Angeles (UCLA) showed that the inhibition of muscle tone in REM sleep was mediated by glycine released by fibers impinging on final common path motoneurons at all levels of the brainstem and spinal cord during REM sleep in the cat. Nature had thus tinkered up a mechanism for turning on the upper brain and simultaneously turning off motor output! Of course, the upper brain did not really

wake up – and we will find out why anon – but it did activate; and it did generate eye movements, and we do dream in REM sleep.

The fact that almost all REM dreams are animated indicates that REM sleep is a virtual – or simulated – motor activation pattern for the brain–mind. We move, however fictively, in our REM sleep dreams. The activation pattern is not random or even chaotic as some aspects of dreams might have us believe. Rather it is reliably stereotyped allowing us to stand, walk, and even run – offline as it were – while floating in amniotic fluid in our mothers' bellies or lying safely in our beds. This is what is meant by 'fictive' movement. As far as the brain is concerned, the movement is real in that it is patterned. That neurophysiologically real pattern could be used to create the appropriate connections with other circuits necessary to support and be supported by motor activation. Such a system would be particularly not only useful during development but also in supporting the learning of new motor skills throughout life.

Pontogeniculo-Occipital Waves

Just as spindles have become the EEG signature of NREM sleep, so the PGO wave has become the EEG signature of REM sleep. EEG spindles are primarily corticothalamic. As PGO waves can be recorded in the pons, the lateral geniculate body of the thalamus, and the posterolateral cortex, they are thus also corticothalamic. PGO waves not only are most easily recorded in those visual brain structures in cats but are also widespread in the brainstem and posterolateral forebrain of both cats and humans. From a functional point of view, it is clear the PGO waves convey information about upcoming eye movements from their motoric sources in the brainstem to visual information processing centers of the upper brain. Thus, they are of interest not only to dream image theory but also to broader state-independent aspects of sensorimotor integration. They predict impending changes in visuomotor circuits.

PGO waves are seen as the first clear sign of an impending REM sleep period in the cat. They are 250-mV biphasic EEG deflections of about 25 ms in duration. They occur at first as singlets then doublets and finally in clusters of up to 25 waves as the REMs appear once the EMG and EEG changes are complete. Under favorable conditions, each PGO wave can be seen to be of greater amplitude in the lateral geniculate body (LGB) on the side of the brain to which the eyes are moving.

The firing of an ipsilateral PGO burst cell of the peribrachial pons is the initial event, followed in 15 ms by the PGO wave and in 25 ms by the eye movement. An inescapable conclusion about this sequence is that the burst cell is first apprised of the oculomotor system's intention to produce a saccade to one side or the other.

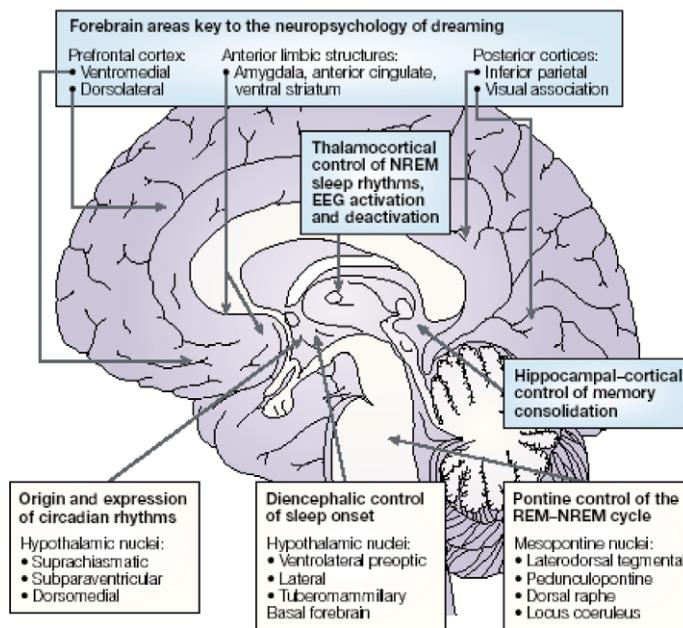


Figure 3 Regions of interest to sleep and dream research are shown on a sagittal schema of the human brain.

The geniculate (and cortex) are then notified presumably so that appropriate adjustments can be made before the eye movement is actually executed. Here is another example of a predictive brain function.

As eye movements are easily visible in REM sleep, it is surprising that it has taken millennia to begin to appreciate their significance for behavioral neuroscience. Of course, they excite our interest as candidate dream image stimuli (or more cautiously, as dream image shapers). However, as important as this hypothesis may be, we are faced with incontrovertible evidence of entirely spontaneous and highly organized sensorimotor integration in sleep. This evidence strongly supports theories of brain development and maintenance by showing that, in sleep, the neural substrate of motor commands and their sensory accompaniments can be activated and run in sleep. Such a capability has significance for the future life of the animal by allowing the offline construction, revision, and tuning of crucial neural circuitry.

Modulation (M)

In 1960, when Michel Jouvet was struggling to explain how the brainstem might mediate waking, sleeping, and dreaming, the Swedish neuroanatomist Kjell Fuxe and his team were describing brainstem neurons, which manufactured and used as their neurotransmitters, the biogenic amines, norepinephrine, and serotonin. Tying these together with dopamine (DA; the already-recognized modulator of the substantia nigra and other diencephalic nuclei), Jouvet imaginatively speculated a tripartite role for these three

biogenic amines: dopamine-mediated waking, serotonin-mediated NREM sleep, and norepinephrine (NE)-mediated REM sleep.

Jouvet's neuropharmacological and lesion experiments in the 1970s provided some evidence for the dopamine (waking) hypothesis and for the serotonin NREM sleep hypothesis. However, subsequent microelectrode studies have clearly shown that all three biogenic amines are released predominantly in waking. The noradrenergic and serotonergic systems are all at their peak during waking, while their output in sleep decreases. DA, 5-hydroxytryptamine (5-HT), and NE must therefore be wake-state mediators. The aminergic neuromodulators, NE and 5-HT, must decrease their influence to allow sleep and especially REM sleep to occur (**Figure 5**).

These discoveries were made in the late 1960s and early 1970s by Allan Hobson and Robert McCarley using chronic microelectrode recording techniques. These techniques were developed by David Hubel and modified by Edward Evarts for early work on sleep, but were then applied to the very successful studies of the visual and motor systems, respectively. One reason that Hubel and Evarts abandoned their pioneering work on sleep in favor of sensorimotor neurophysiology was that their neuronal data of sleep were so counterintuitive. Both NREM and REM sleep evinced less overall decrease in neuronal activity than was expected and it was not at all clear how the massive reorganization of spontaneous neuronal activity could be measured or interpreted.

Based on Jouvet's observations, it seemed imperative to record neurons not just in the cortex (as Hubel and Evarts had done) but also to develop the moveable microelectrode

method for use in the brainstem to follow up on Jouvet's important transection studies, which suggested a control region for REM in the anterior pons. However, the brainstem was not an attractive target; it was in the physical depths of the brain and was characterized by complex neuroanatomy. Besides, microelectrode recording there was problematical because of adventitious movement. Many neuroscientists doubted that the relatively small cells of greatest interest could be resolved or identified and then held for sufficiently long times to be informative.

REM-On and REM-Off Cells

As it turned out, Hobson and McCarley did encounter many difficulties. However, there were as many pleasant surprises, such as:

1. The pontine reticular formation contains numerous huge (75 M) neurons which send their axons into the spinal cord from which they can be unequivocally identified as reticulospinal. When the animal's head is immobilized, such neurons are easily isolated and held through 10 successive NREM–REM sleep cycles, during each of which they undergo robust and reliable increases in excitability in REM sleep. They share this REM sleep excitability increase with the cholinergic neurons they were initially considered to be. Hobson and McCarley worked out their quantitative measures of REM sleep generation for this population of REM-on cells.
2. The spontaneous firing of REM-on cells was typically suppressed (in waking) and only gradually increased firing in NREM sleep. With REM sleep onset, their firing rates increased exponentially. Intense bursting was associated with the REMs and often preceded them. The exponential increase in spontaneous activity of these REM-on cells at the onset of each REM period peaked in the first half of the REM period, declined slowly thereafter, and dropped precipitously at REM offset as the NREM–REM cycle began again.
3. After their brainstem neuron recording sessions became standardized, Hobson and McCarley discovered three loci-containing cells of an exactly opposite type which they therefore called 'REM-off cells.' To their surprise, these smaller and regularly firing REM-off cells were localized in the locus ceruleus, the dorsal raphé nucleus, and the peribrachial region, all brainstem areas known to contain aminergic neurons. The metronome-like firing pattern of REM-off cells during waking was stereotyped and regular at rates of $2\text{--}4 \text{ c s}^{-1}$. These rates declined in NREM and the cells often stopped firing completely in REM, only to resume firing again in post-REM waking. Using microwires, it was possible for Ralph Lydic to record

from these small REM-off cells for 7 consecutive days. In each and every REM period, Lydic observed cessation of firing by raphé neurons.

The Reciprocal Interaction Model

Based on the out-of-phase discharge of the putatively cholinergic REM-on cells and aminergic REM-off cells, Hobson and McCarley proposed in 1975 that the two cell groups were interconnected so as to constitute the oscillator shown in **Figures 4 and 5**. The model shared the formal characteristics of a mathematical model first introduced to explain reciprocally fluctuating levels of prey and predator populations in field biology. Most important, the model was testable using neuropharmacological techniques.

AIM: A Brain–Mind State Space Model Based on Activation (A), Information source (I), and Modulation (M)

In the past 35 years, our scientific knowledge of sleep and dreaming has advanced much more than in all of previously recorded human history. Many egregious errors have been reversed so that, today, we stand on the threshold of a new vision of the human brain–mind and its surprising capabilities. To assist the reader to comprehend the import of these discoveries, we narrate the story again through the emergent AIM model. We do not insist that this model is necessarily the best one that we could have devised. It is certainly incomplete in ways that we will point out. However, we will show that a long list of otherwise disconnected facts each may be assimilated and understood in terms of the model.

Three factors have clearly distinguished themselves from this list of facts. The state of the brain–mind is a combined function of:

1. activation: the degree to which the brain is turned on;
2. information source and fate: the degree to which input–output gates are open (while internal stimuli are excited or inhibited); and
3. modulation: the chemical microclimate of the brain which is determined by specialized neurons in the brainstem.

Before defining and discussing each of these three factors note, let us note that factors A, I, and M can be quantified and arrayed to form the three dimensions of a virtual state space as shown in **Figure 6**.

Depending upon the value of A, I, and M, the state of the system at any given time, t , is denoted as a single point in that space. As A, I, and M change in time, the point migrates to new positions in the state space. Thus AIM is, in effect, a four-dimensional model.

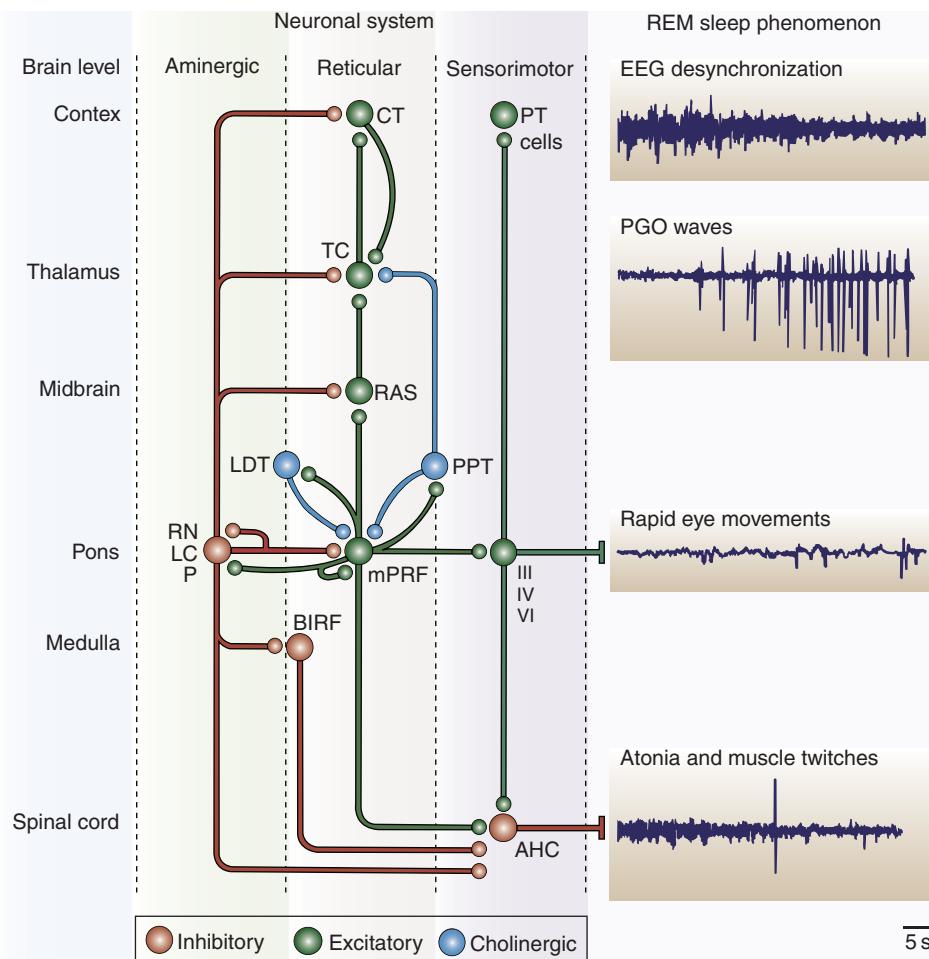


Figure 4 Cellular mediation of REM sleep phenomena. The polygraphic manifestations of REM sleep in the cat are displayed on the right side. The neurons and circuits thought to mediate those changes are shown on the left side.

- Factor A: activation is taken as the x -axis of the model. It represents the energy level of the brain–mind system. The high values of A fall in the right side of the state space and are essential for all normal values of A. Both waking and dreaming states have high values of A.
- Factor I: information is either external or internal in source and results in either real or fictive behavior, depending upon the condition of input–output gates and the excitability of intrinsic sensory and motor gates and circuits. This factor, which is measured on the z -axis of the model, determines one of the important differences between waking and dreaming. Waking is both interoceptive and exteroceptive, whereas dreaming is almost entirely interoceptive. In other words, waking is characterized by high values of I, whereas in dreaming I is low. In addition to this shift in the provenance of sensory data, motor output is blocked so that the internally generated sensory stimulation does not result in motoric actions.
- Factor M: modulation (shown on the y -axis of the model) determines the way in which the brain–mind

process information and is a function of the modulation ratio of aminergic to cholinergic. When the system is highly aminergic, waking is the result. When aminergic activity is at its nadir, the brain–mind is in the REM sleep dreaming state. The system has gained its sensorimotor freedom from the outside world at the expense of impaired cognitive functions.

Waking (W) and REM (R) are both high A states but they are differentiated from each other by both I and M. Low values of I and M are shared by dreaming, whereas waking has high values of both. As already noted, NREM sleep has intermediate values of all three variables.

As the values of A, I, and M are constantly changing, the point solutions of A, I, and M are seen as a sequence of dots. Overnight, this sequence forms a series of four to five elliptical trajectories through the state space. Each ellipse corresponds to an NREM–REM cycle. Notice that successive cycles go less deeply into the S (or NREM sleep domain) and go more deeply and remain longer into the REM domain.

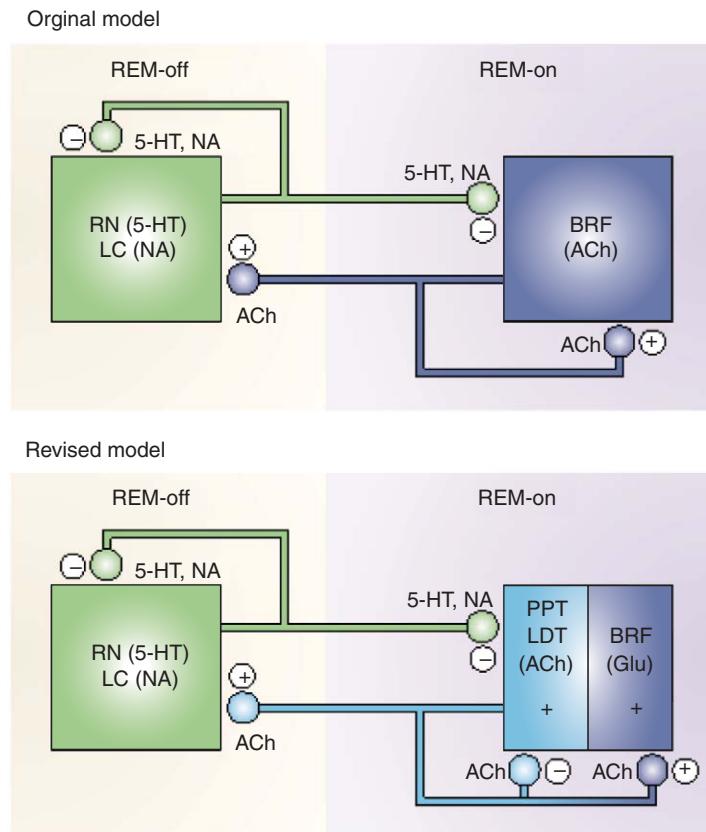


Figure 5 The reciprocal interaction model postulates cyclical changes in balance between REM-on and REM-off neurons of the cat pontine tegmentum in the original and revised versions.

The new four-dimensional AIM model has many obvious advantages over the standard two-dimensional model of sleep science (see **Figure 1**). The most obvious advantage is that because it takes account of basic research data it accounts for important differences between REM and wake. However, the model also provides for changes in trajectory and for important excursions from canonical trajectories that may be seen in sleep disorders. The AIM model also helps us to understand experimental and clinical drug effects. We know that the AIM model is overly simplistic in failing to take account of regional changes in A, activation level, in the brain.

Cholinergic REM Sleep Induction

Microinjecting cholinomimetic drugs enhances cholinergic synaptic efficacy at various sites in the brainstem. Helen Baghdoyan was responsible for systematizing this highly successful experimental paradigm. The short-term REM enhancement that she studied was dose dependent and could be obtained by blocking the enzyme, acetyl cholinesterase, as well as by direct cholinergic agonist

administration. The effects were localized to the pons and could be blocked by atropine. Compare **Figures 1 and 6**.

By 1980, we knew from Marcel Mesulam's anatomical work that the cholinergic neurons of the brainstem were not our paramedian pontine reticular REM on cells. Those paramedian giant cells were, however, cholinceptive, but they probably excited each other and their extended synaptic domains via glutamine. Thus, even though they did not manufacture acetylcholine, they could be excited by it. When first Ennio Vivaldi, and later Subimal Datta and José Calvo, explored the peribrachial PGO burst cell zone in the far-lateral pons, they were able to evoke immediate ipsilateral PGO activity and 24 h following microinjection of carbachol, prolonged (6–7 days) REM enhancement.

Since the development of the reciprocal interaction model, there have been numerous replications of its basic findings, extensive refinements and revisions of a few of its postulates. The model has been incorporated in the circadian-homeostatic schemata of Alex Borbely and in the new flip-flop model of Clifford Saper. It can thus be considered to be widely accepted, which is important since it constitutes the physiological basis of a new

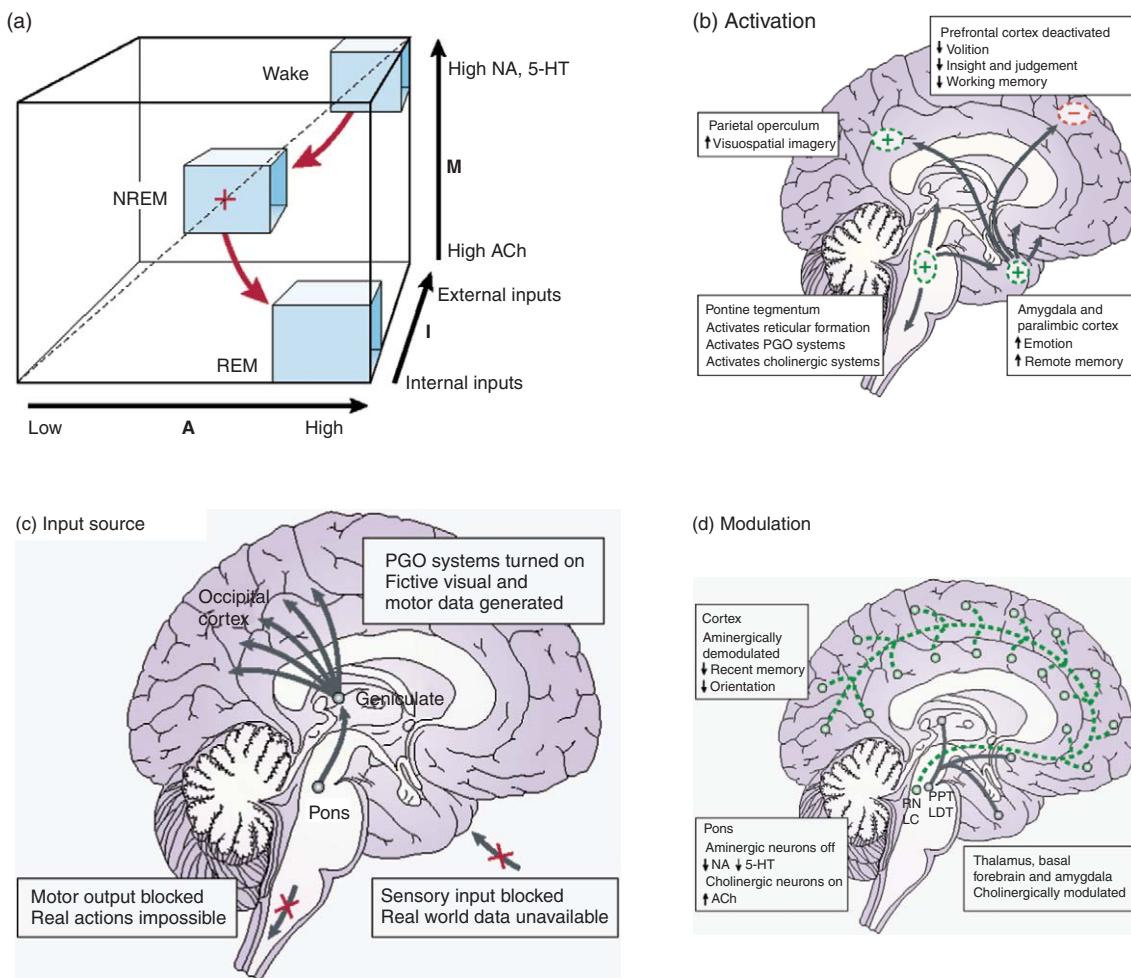


Figure 6 AIM model of brain–mind state control. A three-dimensional state space is constructed based on electrophysiological measures in the cat. (a) AIM state space showing the zones occupied by values from the cardinal states of waking, NREM, and REM sleep. (b–d) Schematized are the variable dimensions of the model: activation, A, input source I, and modulation, M.

approach to the study of dreams. It allows us to integrate the approaches to neuropsychology, learning and memory, and sleep disorders that are taken in subsequent articles.

Dreaming

Dreaming is normally difficult to recall unless awakening from REM sleep occurs spontaneously or by experimental design. REM sleep dreams are normally bizarre, animated, emotional, and nonlogical, suggesting that these anomalies may be cognitive epiphenomena rather than the profound mysteries that many psychologists have supposed them to be. The study of dreaming and REM sleep represents a unique opportunity to understand consciousness and its basis in brain activity.

Until 1975, science had no recourse to a brain-based dream theory. From time immemorial, dreaming had been regarded as otherworldly and spiritual, tied to bodily functions only in symbolic, mystical, and evanescent ways. Even in the works of would-be dream scientists

such as Sigmund Freud, dreaming was regarded as a mental process with no certain connection to any specifiable brain or other bodily process. Aserinsky and Kleitman's 1953 discovery of REM sleep dreaming opened the door to the new science of dreams.

Content and Formal Analytic Approaches

In most subsequent studies, dream researchers have clung to the conviction that a science of dreams could regard content as narrative. The narrative approach to dream content was used to detect the otherwise obscure features of the human personality. Furthermore, content analysis was both scientifically valid and reliable.

Formal Analysis

With the publication of the reciprocal interaction model, Hobson and McCarley articulated the activation synthesis hypothesis, which based its formal approach to dream

data on neurophysiology and sought isomorphisms in correspondence between the physiological and psychological domains.

For example, it was reasoned that if dreaming was predominantly visual then the visual brain should be activated in REM sleep. If REM sleep was a brain state and dreaming was a mind state, then REM sleep-dreaming might be a unified brain-mind state. More surprising was the discovery that dream vision was always associated with hallucinated body movement even though the body was actively immobilized in REM sleep. Since animal work had indicated brainstem motor pattern activation in REM, it was obvious to propose that dream movement was as fictive as was dream vision. The visual and motor systems were thus unified in the creation of visually rich, motorically active dream scenarios. It was intrinsically difficult to reduce these scenarios to stories and to narratives without losing important details such as movement. An important implication of these insights was that REM sleep dreaming was our conscious experience of an internal model of the world.

The initial success of formal analysis then led to a series of studies of dream form which had the goal of making activation synthesis a scientifically sound, brain-based dream theory. Hobson never denounced the psychodynamic emphasis on dreaming as an emotionally meaningful experience worth sharing with a family member, a friend, or even a therapist. Activation synthesis even stressed that insights about the otherwise overlooked aspects of the self might result from such discussion. But activation synthesis denied, vigorously, that such meaning as could be found in dreams was disguised and obtainable only through psychoanalytic decoding. In this section, we restrict our attention to those studies of dreaming which have neurobiological relevance.

Dream weirdness

It was the ill-defined strangeness of many dreams that tempted Freud to develop his disguise-censorship hypothesis in the first place. When dream weirdness was descriptively and dispassionately analyzed, it was found to be both robust and reliably identifiable in REM sleep reports. Most surprisingly, dream weirdness reduced to the discontinuity and incongruity of dream times, places, persons, and actions. This common dream feature was thus a case of microscopic disorientation. The Aristotelian unities are fractured in dreams just as they are in organic mental syndromes of which dreaming suggested itself as an example!

Dream amnesia

Dream recall is notoriously evanescent. Subjects rarely remember detailed dream plots despite laboratory evidence that there might be as much as 2 h of dreaming per night. Subjects are often aware that they have been

dreaming only to find that their recall evaporated rapidly after awakening.

Despite all the evidence for memory's fallibility at the edges of sleep, it was generally assumed that dreaming was fashioned out of stored memories. Yet, when subjects were asked by Magdalena Fosse and her co-workers to identify the memory sources of dream plot items, they were unable to trace more than 20% of their dream details back to previous wake-state experience. Whole dream plots, not just details, appeared to be synthesized *de novo*. This experimental finding indicated that the activated, input-output gated, and demodulated brain was capable of a high degree of creativity. Far from deforming and degrading a previous experience in the interest of protective disguise, the dreaming brain-mind was every bit as creative as a surrealist artist.

In other words, the formal approach to dreaming had suggested that, in REM, the brain-mind was both amnesic and confabulatory, as well as visually hallucinated and disoriented. These four qualities define the organic mental syndrome. It was thus possible to suggest that dreaming was not like delirium, it was delirium by definition.

Dream emotion

Dreams may be intensely pleasant (as when we behold remarkable scenes, have deeply gratifying social relationships, or even defy gravity and fly). They may also be intensely unpleasant (as when we are anxious owing to such incomplete arrangements as nudity or improper dress, turning up unprepared and in the wrong place for an exam or a lecture, or arriving at a transport terminal without the proper documents). Worse yet, we may perceive ourselves to be pursued by hostile characters whom we must fight. Such dreams may be accompanied by elation or by fear and anger. Social emotions such as shame, sadness, or guilt are surprisingly rare in dreams.

This finding suggests the hypothesis that dream emotion is not what we would expect if dreaming were autobiographically inspired. Instead, dream emotion may reflect the automatic enactment of highly adaptive survival-related behaviors. This hypothesis substitutes Darwin for Freud and behavioral offense (fight) and defense (flight) for the disguise of forbidden wishes as critical to understanding REM sleep dreams. The neurobiological and psychodynamic dream theories come close to each other here in their acknowledgment of the importance of instinctual drives, but the functional emphasis is entirely different.

The characterization of dream emotion in Darwinian terms is not only relevant to models of cerebrogenesis but also contributes to the functional theory that adaptive behaviors are being prepared and rehearsed every night of our lives. We stand ready to be afraid, to fight, or to flee whether or not we ever really do so in waking reality. We

have rehearsed these life-saving scenarios during our sleep. This is the essence of Michel Jouvet's survival theory, recently taken up by Antti Revonsuo. Whether or not one remembers the dreams does not matter. The brain keeps its own record of the fixed action pattern it has rehearsed and is ready to enact it, when called for, in waking.

Dream movement

Having discovered that dreamers were constantly moving through a dream space, Helene Porte set out to use fictive dream movement as a test of three dream theories. She reasoned that the Freudian model would not preferentially predict one kind of movement over another, nor would the activation-only theories of David Foulkes or John Antrobus predict a ubiquity of somatic movement in dream scenarios. Only a brain-based theory which gives pride of place to major movement pattern generation makes such a prediction.

Confirming McCarley and Hoffmann, Porte found that almost every sentence of every REM sleep dream report contained an action verb and that a great majority of those verbs connoted lower extremity movement. That is to say, postural displacement was a rule of dream movement. This is autobiographically unpredictable since many of us engage in fine motor activity such as reading, writing, typing, computing, or talking on the telephone during the daytime and only rarely in waking do we walk, run, ski, or fly. Our dreams are quite different from our waking habits in that vigorous athletic scenarios are common but sedentary activity is rare. Could that be because major movements of the body have higher survival value?

Dream thinking

Content analysts, such as William Domhoff, often deny that dreaming is either bizarre or illogical. This not only

flies in the face of commonplace experience but also runs against the robust findings of other scientists. While it is true that there is some dream content that is not characterized by discontinuity and incongruity and there is some dream thought which is logical, there is far less of both kinds of thinking than in waking and waking consciousness is almost never as grossly bizarre and nonlogical as dreaming.

To investigate the question of the frequency and quality of dream thought. Fosse *et al.*, first counted the references to thinking in 3000 reports elicited from normal students. As they led their normal waking and sleeping lives, these students were automatically beeped and asked to describe their mental content in the minutes before the signal. Their sleep was objectified through a portable home-based system (See **Figure 7**).

Thinking and hallucination are reciprocally state-dependent mind states. Dreaming is strongly hallucinatory and weakly cognitive while waking is weakly hallucinatory but strongly cognitive. Hence, the brain-mind is programmed to do one thing or the other. It either imagines or it thinks critically, but it does not do both at the same time. This reciprocity is so robust as to suggest a general law of psychology. The law, which states that a person is either rational and nonhallucinatory or irrational and hallucinatory, is true of both normal and abnormal mental states. Dreaming is not only delirium, by definition, but it is also formally akin to other forms of mental illnesses.

Dream logic

Roar Fosse's finding that references to thinking were rare in REM sleep reports might indicate an important deficiency in reasoning capacity that could tip the normative balance away from the examples of logical reasoning that are sometimes seen in dreams.

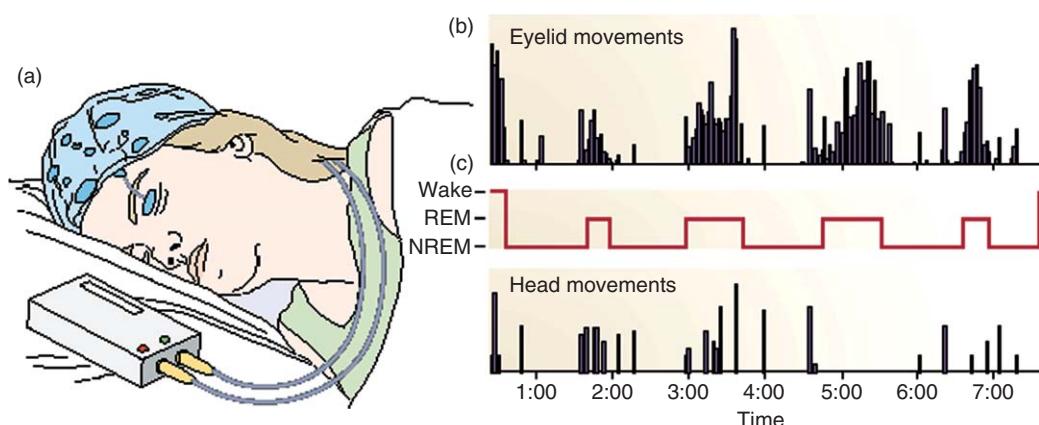


Figure 7 Human subjects, sleeping at home, give reports of mental activity which can be compared across the states of waking, NREM and REM sleep. See **Table 1** for a summary of results.

Table 1 Some formal properties of dream consciousness

<i>Phase I 1980–1994 Laboratory and unmonitored home reports</i>	
Perception. Vision and sense of movement predominate	Hoffmann and McCarley (1981)
Bizarre cognition. Reduces to discontinuity and incongruity	Hobson <i>et al.</i> (1987)
Fantasy. Chimeric characters common in dreaming absent in fantasy	Williams <i>et al.</i> (1992)
Children. Adult-type dreaming begins at age 5	Resnick <i>et al.</i> (1994)
Emotion. Anxiety (fear), elation, and anger present	Merritt <i>et al.</i> (1994)
Plot Sequence. Gradual drift of subject within scenes	Sutton <i>et al.</i> (1994)
Radical shift of subject between scenes	
Splicing. Judges cannot recognize continuity across scenes	Stickgold <i>et al.</i> (1994)
<i>Phase II. 1995–2004: Home dreams with physiological monitoring</i>	
Nightcap. REM report word length $7 \times$ NREM	Stickgold <i>et al.</i> (1994)
Movement Perception more common in REM	Porte and Hobson (1996)
Character recognition is arbitrary. ‘Just so’	Kahn <i>et al.</i> (2000)
Thinking and hallucinating. Reciprocal across states	R. Fosse <i>et al.</i> (2001)
Contrasting with waking and REM	
Memory source in waking identified in only 20% of dream incidents	M. Fosse <i>et al.</i> (2003)
Gender of dreamer assessed accurately slightly above chance	Kahn and Hobson (2007)
<i>Phase III. 1995–present: Home dreams with physiological monitoring – focus on secondary consciousness (metacognition)</i>	
Theory of mind. Dreamer recognizes mind of other dream characters	Kahn <i>et al.</i> (2002)
Logic - some thinking rational, most not rational	Hobson and Kahn (2005)
Authorship. Reports of subjects incorrectly grouped by judges	Hobson and Kahn (2007)
Schizophrenia. Patients and normals have equally bizarre dreams	Scarone <i>et al.</i> (2008)
Patients, but not normals, also have bizarre TATs	
Logic. Illogicality of most dream events is unnoticed	Hobson <i>et al.</i> submitted for publication

To try to capture this possibly diminished cognitive capacity. David Kahn and Allan Hobson constructed a measure of logical inference that could be used by judges who scored reports from REM dream and waking in normal subjects.

Many dream features were found to be associated with logical oversights that were unnoticed by the dreamers. For example, riding a bicycle in London traffic with a long-handled tree trimmer was not noticed as either bizarre or nonlogical by the dreamer but, in the light of waking, was found to be so by the judges. The implication is that dream thinking is not only impoverished by virtue of its rarity but the thought impoverishment is a manifestation of a state-dependent impairment of the capacity to generate logical inference. Without the capacity for logical inference, it is impossible to notice the presence of dream bizarreness. Thus, critical thought declines in quantity and quality together with the tendency toward the cognitive discontinuity and incongruity detected in bizarreness scoring.

Dreaming and Mental Illness

The formal similarities between dreaming and mental illness include the internally generated percepts (hallucinations) and inferentially deficient thought (delusions). Both features are distinctly disadvantageous if they occur in waking. When they do, we still call the new state a ‘mental’ illness even though it is clearly a disorder

of the unified brain-mind. This is because we cannot yet see how a physical object, the brain, could possess self-awareness. We thus remain language dualists even if we are philosophically committed to monism.

For the past century, psychiatry has properly regarded dreaming as potentially informative. Now, we see just how prophetic this hypothesis may be. Instead of being simply a mental illness, major psychosis may be thought of as a functional disorder of the brain-mind. As such, it may well share features with normal dreaming, another functional alteration of the brain-mind. In my writings, I have attempted to show how this scenario might play out in order to establish a firm link between apparently abstract and irrelevant details of basic research and clinical problems of the greatest complexity and practical importance for our theory of both sanity and insanity.

Chief among the encouraging signs for this concept are the potent effects on psychotic symptoms of the many psychoactive drugs which interact with the brain’s own state-control chemicals, the neuromodulators. The neuromodulators we attempt to influence with prescription drugs are the same substances which sleep research has shown to be fundamental in orchestrating the state-dependent characteristics of the normal brain-mind. The control of brain-mind state as studied over the normal sleep-wake-dream cycle is clearly of crucial psychobiological significance in understanding both the normal vicissitudes of consciousness that we all experience, and also those unwanted distortions that beset those

humans with hallucinations, delusions, emotional exaggeration, and memory problems.

Schizophrenia and dreaming

In the early days of the sleep lab era (1953–75), dream content analysts were quickly attracted to laboratory studies of sleep and dreaming in patients in order to test psychoanalytic dream theory. They were disappointed. Whether they predicted more or less REM as the basis of one or another reading of Freud's theory, they found neither.

Recently, Silvio Scarone and his colleagues in Milano asked their schizophrenic patients to keep a dream journal. With those patients who were compliant he also used the projective Thematic Apperception Test (TAT) test to elicit wake-state control reports. In a small but promising sample, Scarone showed that bizarreness scoring of schizophrenic and normal dreams revealed no difference between the two groups. However, schizophrenics reacted bizarrely to the same TAT cards that normals read without introducing bizarre mentation. This preliminary result indicates that the brain–mind of waking schizophrenia patients operates as if it were asleep. The schizophrenic brain–mind lacks the sharp differentiation between the states shown by normal subjects.

The results do not imply an identity between schizophrenia and dreaming. They only suggest a formal similarity. That formal similarity may also be found at the level of brain function. Normal and abnormal states may thus show changes in underlying neuromodulatory mechanisms. Reductionism is successful when it explains a wide range of phenomena with a few basic assumptions. The brain–mind of a schizophrenic patient awake is formally similar to that of a dreamer, whether the dreamer is schizophrenic or normal.

The Artistic and Literary Aspects of Dreams – Dream Content and Dream Form Revisited

While a dream report may contain a story line or plot, dreams themselves are not literary exercises. They are multimedia experiences or films rather than short stories or any other collection of mere words. They are polysensory experiences to which story-like narrations may be fit but from which they do not necessarily derive. Dream content – as in a dream report – is therefore not entirely irrelevant but neither is it a basic or foundational element. We do not write down dream stories, take them to a scenarist in the brain, then proceed to create a *mise en scène*. In the brain, it would appear to be quite the other way around. The scenario is the primary product and the report is derived from it. The report is therefore but a pale copy of the dream and we should not forget this important fact.

Dreaming is a holistic primary experience and verbal accounts of it are woefully inadequate and limited. Some dream observers become so frustrated by the inadequacy

of words to represent their experience that they resort to drawing. The drawing captures in a single sketch many descriptive facts that cannot be matched by words. Thus, to say that a picture is worth 1000 words is to suggest that a single drawing is worth 10 dream reports (the average length of which is less than 100 words). In **Figures 7 and 8**, we have drawn attention to some of these features in one work, the *Dream Drawings of the Insect Man*. These drawings not only help us to illustrate our formal analytic approach but they show dreaming to be both emotionally salient and creative as is particularly clear in **Figure 8**.

In addition to many drawings to illustrate bizarre dream architecture, such as buildings without doors, or incongruous dream objects, like the truck in a flower garden or the ingenious but impossible filing system for research notes (**Figure 8**), the Smithsonian insect scientist devoted over 50 of his drawings to describe anomalous trajectories such as running to third instead of first base, circling in his car in a parking place too narrow to allow even one turn, and two golf balls colliding improbably, while still aloft. All of these events can be described in words but they are more simply illustrated by drawings.

Why Do We Dream?

When we dream, we see, feel, imagine, and assess events in our minds. Our minds are thus theaters where we witness, with or without critical comment, a virtual reality of such enormous complexity as to match the complexity of the world itself. It is difficult to resist the idea that the mental experience of dreaming evolved to help us be aware, in a predictive way, of the reality of the world that we live in.

One of the major functions of dreams must thus be to create, maintain, learn from, and modify our mental model of the world. That we do as well as we do in adapting to that world is a testimonial to our power to create and recreate reality inside our heads. The same could be said to be consciousness itself. Waking, consciousness, and dreaming reveal internal models of our world which allows us to evaluate, to plan, to adjust our strategies for living in that world. It is not so much that we are protecting consciousness from invasion by our own desires as it is that consciousness is helping us to recognize and fulfill those desires.

We need, always, to be prepared for unforeseen and potentially harmful events. Hence, dreams are frequently fearsome. However, we also need to know a good thing when we see one so we must also learn to be impulsive enough to make possibly profitable personal decisions. Tentative liaisons are of obvious significance to our goal of procreation. Given those twin assumptions, it is not so surprising to find that dream emotion is predominantly

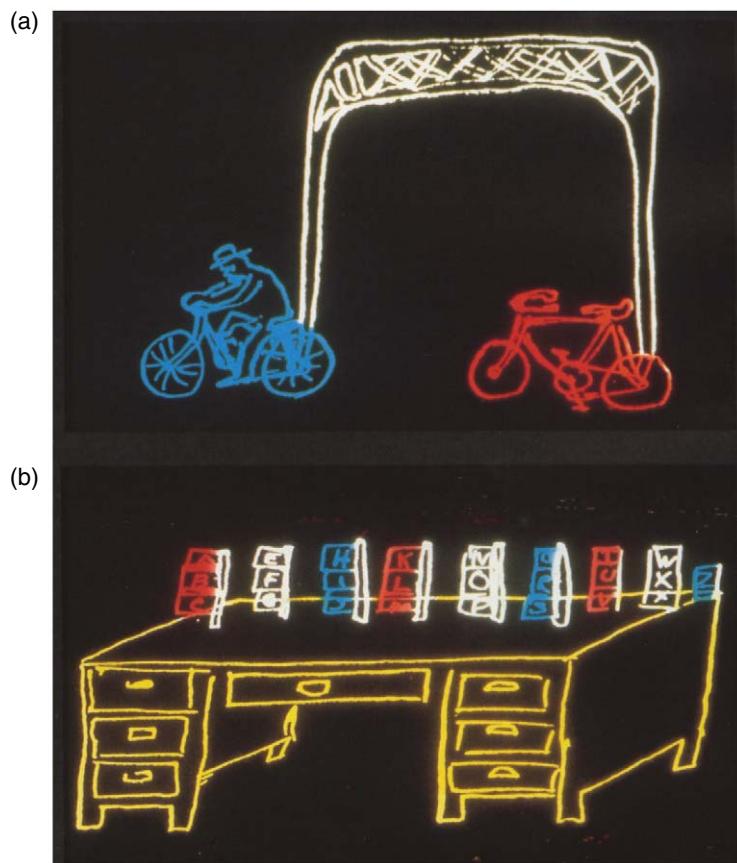


Figure 8 Two drawings from the journal of a Smithsonian insect specialist who recorded and illustrated 256 dreams in the summer of 1939. Both show content that is certainly bizarre but emotionally salient and even creative. Above is his dream bicycle built for two with the bachelor dreamer pedalling alone. Below is his dream desk-top computer whose alphabetized set of flags allows him to access notes and drawings stored in the drawers.

negative but occasionally ecstatically positive. We practice seeing and moving in a coordinated manner and we practice complex movement behaviors at least 1.5 h a day. This is not exercise for the muscles, but it certainly is exercise for the sensorimotor programs of the brain. Dreaming allows us to fraternize experimentally with a wide variety of social peers. Dreaming is thus safe, progressive calorie conservative, and compatible with other important procedures such as refurbishing thermal control.

Functional Implications

For the first 40 years of the sleep science revolution (1953–93), attention was so sharply focused upon the description of heretofore hidden phenomena and their mechanistic causation that functional questions were not even asked, let alone answered. It was not so long ago that many sleep scientists claimed that sleep function was entirely unknown. Now that sleep has many known functions whose scientific analysis is proceeding apace, we can anticipate a future, which will undoubtedly reveal many more. Our burgeoning

knowledge of sleep and dream function will eliminate forever the naive notions that sleep is a waste of time and is a behavior which can be done without.

Sleep Deprivation is Fatal to Rats

Allan Rechtschaffen is a psychologist who succeeded Nathaniel Kleitman as head of sleep research at the University of Chicago. While decrying our lack of understanding of sleep function, he designed experiments which indicate that sleep is essential to life. The death that accompanies the prolonged sleep deprivation of rats is characterized by defects in the regulation of both dietary and thermal homeostasis and by a loss of immune function, so that ultimately the sleep-deprived animal cannot prevent its own intestinal bacteria from invading its blood stream causing death by overwhelming sepsis.

Rechtschaffen's experiments were simply designed but heroically difficult to run. Two rats (one the index case and another, the control) were placed on opposite sides of the wall that divides a Lucite tube into two compartments, each

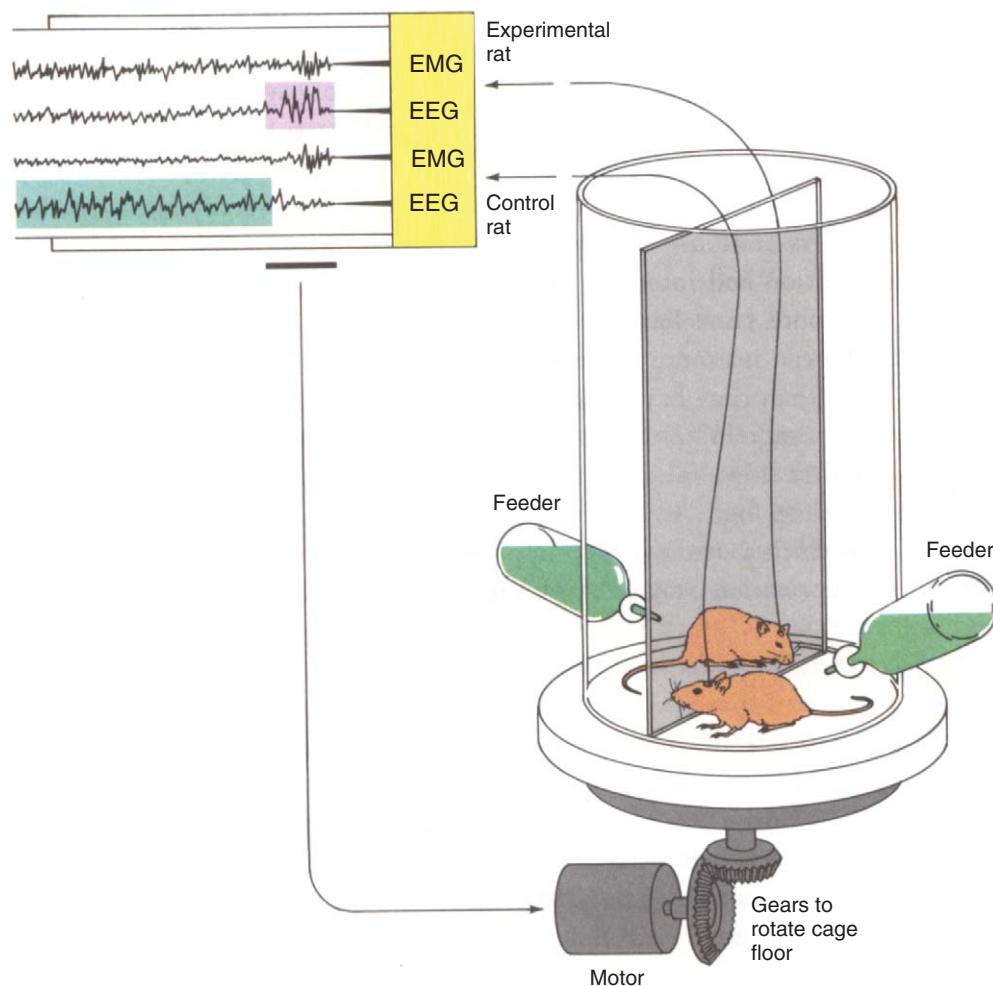


Figure 9 Experimental setup of Rechtschaffen experiments. Whenever the index rat tries to sleep, it is aroused by movement of the cylinder floor. The control rat can get plenty of sleep when the index animal is feeding or grooming.

supplied with *ad lib* food and water and a cable for recording physiological signals on a polygraph (Figure 9).

Whenever the index rat tries to sleep, cage floor movement wakes it up. The control rat can sleep whenever the index animal grooms or feeds. With this simple apparatus, the index rat loses 95% of its sleep while the control rat gets 95% of his.

The time course of degeneration is stereotyped. After 2 weeks, skin lesions appear in the index rat. By week 3, the index rat consumes increasing amounts of food in a vain effort to maintain body weight and keep its body temperature stable. Sleep deprivation has already produced a negative calorie drain. The index rat thereafter develops frenetic heat-seeking behavior and becomes susceptible to infection by intestinal flora to which it is normally immune. These infections and the associated starvation are universally fatal within 4 weeks of deprivation.

If only REM sleep is deprived, the same course of events is complete after 6 weeks. As REM takes up

only 15% of sleep time, this observation shows that REM has more than twice the functional power against deprivation than NREM sleep. This conclusion follows from the calculations of sleep deprivation in the two experiments: 440 h in total sleep deprivation as against only 136 h in the case of selective REM deprivation. The hypothesis that REM is super-sleep derives, in part, from this threefold difference. At the same time, REM is twice as effective in reducing NE and 5-HT output. This reduced expenditure could not only conserve neuromodulator but also allow much more effective aminergic receptor resensitization. Sleep deprivation, which is intrinsically stressful, drives the NE and 5-HT systems maximally and prevents any possible recovery of the efficacy of those systems.

Despite extensive experimental effort, Rechtschaffen was unable to specify the mechanism of the fatal dysfunctions it produced. From these studies, it can nonetheless be concluded that sleep serves thermal and dietary calorie control.

Sleep and Learning

Rechtschaffen's sleep-deprived rats were not cognitively impaired in any obvious way. However, they were not tested to see if their capacity to capture and incorporate new information was compromised.

The effort to establish a sleep effect on learning long-antedated Rechtschaffen's work has always had strong adherents despite the relatively weak effects of deprivation on learning capacity, and the relatively small gains in learning incurred through sleep. The most recent – and successful – program in this domain has been led by Robert Stickgold using the Karni–Sagi visual recognition task (VRT). In the VRT, subjects are asked to fixate the center of a computer screen and to signal recognition of an anomalous stimulus array as soon as possible after it appeared on the screen. Appeared is in quotes because subjects were not consciously aware of their quite accurate and rapid stimulus detection. As subjects get better at the task, the task is made more difficult until the learning curve becomes flat.

The timing, kind, and duration of subsequent sleep has small but consistent effects on subjects' skills on retest. If they have slept badly, they show no learning whatsoever. Fair sleep helps them perform at the level they attained with training. Good sleep actually improves their performance. This is true sleep learning and can only be explained by supposing that the subjects' brains were somehow running the learning paradigm during their subsequent sleep.

These results have been confirmed by others using a wide variety of experimental tests of sleep learning. Complimenting Stickgold's results of sleep favoring procedural learning have been Jan Born's studies, which suggest that semantic or narrative learning may also benefit from sleep. Sleep deprivation has been shown by Jim Horne to interfere with cognition when subjects attempt complex reasoning tasks.

Genetic research on sleep will soon confront the limitations of the genome to prescribe every neuronal address and every neuronal function. In that confrontation, the importance of developmental mechanisms, such as the early establishment and abundance of REM sleep *in utero*, will be investigated. It is almost a foregone conclusion that these studies will reveal sleep to favor certain aspects of the construction and operation of the mammalian brain. When this is done, we may be better able to appreciate the functional significance of sleep in the development and maintenance of the many cognitive abilities that, together, constitute consciousness.

The Neuropsychology of Sleep and Dreams

Since 1990, the field of sleep and dream science has been invigorated by the advent of brain imaging technology. This work has inspired an upsurge in the

long-dormant clinical study of sleep and dreaming. At the outset, however, it is now clear that the study of sleep and dreams is a mainstream endeavor of medical neuroscience.

Positron emission tomography studies

It is remarkable that the findings of the three original PET studies were so consistent. This consistency of findings by Pierre Maquet, Alan Braun, and Eric Nofzinger bespoke a combination of methodological power and phenomenologic robustness. The brain really does change state in sleep. It does so in a way that is easily observable and readily understandable, thanks to positron emission tomography (PET).

NREM sleep is associated with a global decrease in blood flow indicating a net fall in neuronal activity. Both conclusions fit with independent measures. When Kety and Schmidt measured cerebral blood flow in sleep using the Fick principle method about 30 years ago, they concluded that forebrain activity declined about 20% in NREM sleep. Microelectrode studies have confirmed a net decrease in neuronal activity. Although some cortical and thalamic cells increased their firing rate, most neurons throughout the brain decreased rate, on the order of 20–50%. NREM sleep is thus an unquestionably deactivated state of the brain.

It should be emphasized that even taking these data at face value, the brain remains 50–80% as active in NREM sleep, when consciousness is entirely lost or markedly obtunded. This means, therefore, significant information processing may go on even though consciousness is lost. The decline of cerebral activity in sleep is only relative and not absolute as Sherrington and Pavlov seem to have assumed. This recognition should keep us vigilant, especially with respect to functional hypotheses concerning the role of NREM sleep in learning and memory.

It was the magnitude and extent of brain activation in REM sleep revealed by PET studies that surprised the scientific world and moved dream theory to a new level of scientific credibility. With one important exception, the brain activation in REM sleep was global; with one exception being the dorsolateral prefrontal cortex, a brain region essential for executive ego functions. The failure of this region to reactivate correlates well with the cognitive deficiencies in dreaming. Executive ego functions such as: working memory, orientation; volition; self-reflection; and attention are all weakened as in REM sleep.

The brain regions that become more active in REM than in quiet waking were equally interesting from the psychophysiological point of view. These include the pontine brainstem (known to be important to REM through animal studies); the amygdala, the deep frontal white matter, and the parahippocampal cortex (consistent with the intensified emotional spectrum of dreams); and the parietal operculum (known to be important in spatial

sensory perceptions which is keenly sharpened in dreams). That the brain–mind is an integrated system with specific changes associated with its several states is now widely accepted.

Changes in dreaming following stroke

Mark Solms, a South African neuropsychologist working in London, asked stroke victims if they had noted any changes in their dreaming after their strokes. Complete cessation of dreaming was reported by patients whose strokes affected either the parietal operculum or the deep frontal white matter, two of the regions known to be hyperactivated in REM sleep. This correspondence between the brain imaging data and subjective experience strengthened the hypothesis that dreaming was correlated with differential activation of the brain compared to waking and that this differentiation helped to explain the changes in consciousness in two states.

Summary and Conclusions

Sleep serves survival by means of its energy conservation functions. However, sleep is functionally significant in other ways as well. Through its association with nesting and pair-bonding, sleep is also a constructive behavior: sleep facilitates reproduction. Ethologists need to take account of these obvious facts. It also facilitates development of the brain in the young and that provides lifelong opportunities for adaptive information processing.

The most recent and exciting theories see sleep and waking as not only mechanistically but also functionally reciprocal. In those higher animals which are conscious, many of the cognitive modules of conscious experience are activated in REM sleep as if dreaming were a virtual reality state allowing for practice of many important cerebral subroutines. Once considered inert or even deathlike, the richness of sleep can now be appreciated

as elaborate internal brain activity, which runs behind an only apparently quiescent façade provided by the quelling of motor output.

See also: Bioenergetics of Sleep; Neuropsychology of Sleep; Sleep Genetics; Sleep: Learning and Memory; Sleep: Medical Disorders.

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Stress and Arousal/Sleep

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Glossary

Adrenocorticotropic hormone (ACTH) – A peptide hormone synthesized in the anterior pituitary and released into the blood during the HPA-axis response to aversive stimuli. ACTH binds to receptors in the adrenal cortex and induces the release of glucocorticoid hormones.

Arousal – A complex physiological, sensory, behavioral, and emotional state that determines the physiological readiness for activity and the reactivity to internal and external stimuli, including emotional reactivity.

Corticotropin-releasing factor (CRF) (also known as Corticotropin-releasing hormone (CRH)) – A

neuropeptide that was isolated by Vale and colleagues in 1981 based on its ability to induce the release of ACTH from the anterior pituitary. In addition, CRF – via actions in extrahypothalamic regions – is an important mediator of autonomic and behavioral responses to aversive or stress-related stimuli.

Glucocorticoid hormones – Cortisol in the human and fish, corticosterone in most nonhuman vertebrates; these are steroid hormones that mobilize energy resources and, thus, prepare the organism for action during a stressful state. The synthesis and release of glucocorticoids are stimulated by ACTH.

Hypothalamic–pituitary–adrenal (HPA) axis – A neuroendocrine cascade, involving the hypothalamic CRF, pituitary ACTH, and adrenocortical glucocorticoid hormones, that is a hallmark of physiological responses to aversive or stress-related stimuli.

Stress – A physiological state, manifest by an adaptive syndrome that works in the direction of producing a return to homeostasis.

will use the term ‘arousal state’ to refer to a specific level of arousal, and the terms ‘arousal-promoting’ and ‘arousal-generating’ to refer to mechanisms that promote a higher state of arousal. Wakefulness is a form of arousal, but so is rapid eye movement (REM) sleep, albeit lower on the arousal continuum than wakefulness. Sleep is an arousal state that can be subdivided into non-rapid eye movement (NREM) and rapid eye movement (REM) sleep; both states are actively promoted by the brain via distinct mechanisms. Sleep is on the lower end of the arousal continuum with coma and death being the lowest levels of arousal.

Arousal-Promoting Mechanisms

Glutamatergic neurons within the brainstem reticular formation and several other neurotransmitter systems, some closely associated with the reticular formation, promote arousal. These neurotransmitters include the monoamines, serotonin (5-hydroxytryptamine; 5-HT), norepinephrine, dopamine, and histamine, the choline derivative acetylcholine (ACh), and the neuropeptides orexin-A and B, also called hypocretins, hypocretin-1 and 2. The activity of each of these systems is correlated with arousal states, with elevated activity during the active phase of the sleep–wake cycle (**Figure 1**).

Brainstem Reticular Formation

Arousal-generating systems include specialized groups of neurons in the brainstem and forebrain that increase cortical activity. A cornerstone of neural mechanisms underlying increased arousal, including wakefulness, is the brainstem reticular formation. Reticular formation neurons – located in the midbrain, pons, and medulla – have long, branched axons that project widely to regions of the hypothalamus, basal forebrain, thalamus, cerebellum, cortex, and spinal cord (**Figure 2**). The reticular formation inhibits sleep-promoting neurons and activates cortical neurons. Signals transmitted to the thalamus are relayed to broad regions of the cortex, where these signals maintain an alert, conscious state. This specific component of the reticular formation is referred to as the reticular activating system (RAS). Reticular formation neurons are responsive to every major ascending sensory modality, including somatosensory, thermosensory, nociceptive, vestibular, and auditory stimuli, and to visual stimuli, relayed via the superior colliculi. General

Introduction

Arousal is a dynamic state that exists in a continuum from low states of arousal to high states of arousal/wakefulness. An organism with high arousal is one that is more alert to sensory stimuli, more behaviorally reactive, and more emotionally reactive. Arousal can refer to general arousal, or to more specific forms of arousal, such as sexual arousal, playful states, anxious states, or fear states, which are defined by specific contexts and behavioral correlates. We

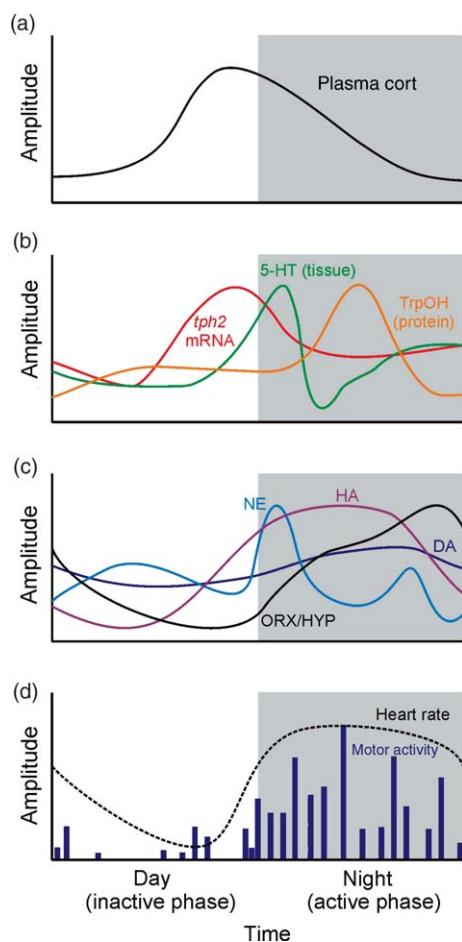


Figure 1 Schematic illustration of the diurnal rhythms of plasma corticosterone concentrations, arousal-promoting neuromodulators, motor activity, and heart rate in rats, a nocturnal species. The gray shading in each figure represents the dark, or active, phase, and the white area represents the light, or inactive, phase. (a) The circadian rhythm for plasma corticosterone concentrations. (b) The diurnal pattern of tryptophan hydroxylase mRNA (red) and protein (orange) expression in the dorsal raphe nucleus and tissue concentrations of serotonin in the striatum (green). (c) The diurnal rhythm of extracellular concentrations of the arousal-promoting neurotransmitters dopamine (striatum; dark blue), norepinephrine (paraventricular nucleus of the hypothalamus; light blue), histamine (anterior hypothalamus; purple), and orexin/hypocretin (lateral hypothalamus; black). (d) The diurnal rhythm of heart rate (black, dashed line) and locomotor activity (dark-blue bars). 5-HT, 5-hydroxytryptamine, serotonin; DA, dopamine; HA, histamine; NE, norepinephrine; ORX/HYP, orexin/hypocretin; *tpb2* mRNA, tryptophan hydroxylase 2 mRNA; TrpOH, tryptophan hydroxylase. Adapted from Castañeda *et al.* (2004) *Journal of Pineal Research* 36: 177–185; Lemmer *et al.* (2006) *Pharmacology and Therapeutics* 111: 629–651; Malek *et al.* (2007) *Neuroscience* 125: 749–758; Malek *et al.* (2007) *Endocrinology* 148: 5165–5172; Mochizuki *et al.* (1992) *Physiology and Behavior* 51: 391–394; Sanchez *et al.* (2008) *Molecular and Cellular Biochemistry* 317: 105–111; Spiga *et al.* (2007) *Journal of Neuroendocrinology* 19: 891–892; Stanley *et al.* (1989) *Life Sciences* 45: 275–282; and Yoshida *et al.* (2001) *European Journal of Neuroscience* 14: 1075–1081.

anesthesia, alcohol, sedatives, and sleep-inducing drugs inhibit RAS activity and result in a loss of alertness or consciousness. Extensive damage to the RAS can induce coma. The motor component of the reticular formation regulates somatic motor function, autonomic responses, heart rate, blood pressure, and respiration. Thus, increased excitability of the reticular formation can lead to facilitation of both sensory and motor function. In what will become a common theme discussed below, reticular formation neurons can be made more excitable by stress-related neuropeptides, such as corticotropin-releasing factor (CRF), and stress hormones, such as glucocorticoids. Thus activation of stress-related neural circuits or the hypothalamic–pituitary–adrenal (HPA) axis can co-opt normal arousal mechanisms, amplifying sensory responsiveness, vigilance, attention, and behavioral reactivity. The majority of neurons in the reticular formation use glutamate, an excitatory amino acid, as a neurotransmitter.

Serotonin

Serotonergic neurons are predominantly located in the brainstem raphe complex, a midline complex closely associated with the reticular formation within the mid-brain, pons, and medulla. A significant number of serotonergic neurons are located within the pontomesencephalic reticular formation itself. Although serotonergic neurons within the pontomesencephalic reticular formation may play a critical role in the regulation of arousal, their functional attributes have not been studied due to the highly scattered distribution of this cell group. The majority, but not all, of the serotonergic neurons have firing rates that are correlated with behavioral state, with high levels of neuronal firing during active-waking states, and progressively lower firing rates during behavioral quiescence, NREM and REM sleep. Similarly, the expression of the rate-limiting enzyme for 5-HT synthesis in the brain, tryptophan hydroxylase 2 (*tpb2*) varies in a diurnal manner as do tissue and extracellular concentrations of 5-HT in many brain regions (Figure 1(b)). *tpb2* mRNA expression rises just before the active phase of the diurnal cycle, while *tpb2* protein expression and tissue and extracellular 5-HT concentrations are elevated during the active period of the diurnal cycle. Diurnal variations in *tpb2* mRNA expression are dependent on the circadian variation in glucocorticoid secretion, with elevated glucocorticoids promoting *tpb2* expression (Figure 2).

Norepinephrine

Cortical-projecting noradrenergic (norepinephrine-producing) neurons are localized primarily in the pontine locus coeruleus (LC) and are involved in orientation,

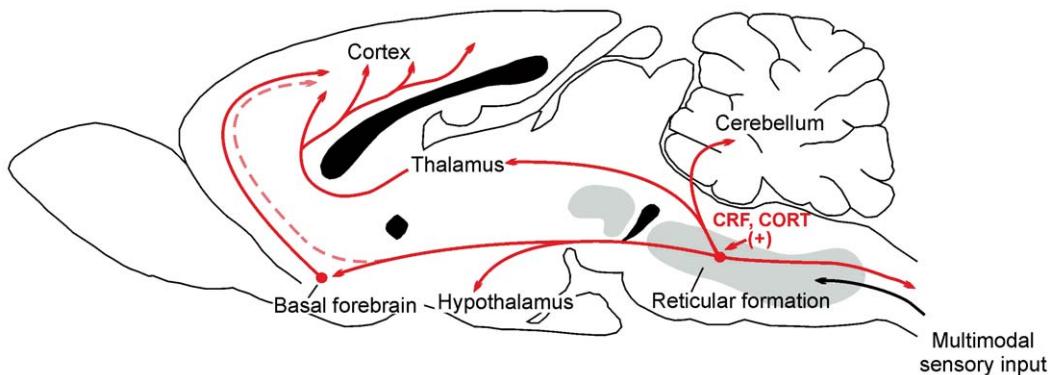


Figure 2 A highly simplified schematic representation of the brainstem reticular formation and its ascending and descending projections. The reticular formation is indicated by gray shading in the midbrain, pons, and medulla. Reticular formation neurons are responsive to every major ascending sensory modality, including somatosensory, thermosensory, nociceptive, vestibular, auditory stimuli, and visual stimuli. Ascending projections to the forebrain travel along a dorsal pathway to a thalamocortical projection system or along a ventral pathway to the lateral hypothalamus and basal forebrain. Although some reticular formation fibers continue along the ventral pathway to the cerebral cortex (pink dashed line), large numbers of cells in the reticular formation project to the basal forebrain where cholinergic neurons give rise to widespread projections to the cerebral cortex. Thus the basal forebrain acts as an extrathalamic relay system from the reticular formation to the cerebral cortex. Reticular formation neurons can be made more excitable by stress-related neuropeptides, such as CRF, and stress hormones, such as corticosterone. Thus activation of stress-related neural circuits or the HPA axis can co-opt normal arousal mechanisms, amplifying sensory responsiveness, vigilance, attention, and behavioral and emotional reactivity. For a comprehensive review of the chemoarchitecture, ascending projections and descending projections of the reticular formation, see Jones BE (1995) Reticular formation: cytoarchitecture, transmitters, and projections. In: Paxinos G (ed.) *The Rat Nervous System*, 2nd edn., pp. 155–171. New York: Academic Press.

attention, and maintenance and enhancement of cortical activation. Like the majority of the serotonergic neurons, the firing rates of LC neurons are highest during wakefulness, lower during NREM, and virtually absent during REM sleep. The expression of the rate-limiting enzyme for norepinephrine synthesis in the brain, tyrosine hydroxylase (TH) varies in a diurnal manner as do tissue and extracellular concentrations of norepinephrine in many brain regions. Extracellular concentrations of norepinephrine peak soon after dark onset (the active phase for rats, **Figure 1(c)**). Likewise, the activity of dopamine β -hydroxylase (DBH), which converts dopamine to norepinephrine, varies in a diurnal manner. Norepinephrine is an important modulator of multiple components of the arousal system. For example, noradrenergic fibers innervate the dorsal raphe nucleus, and activation of α_1 -adrenergic receptors there increases the firing rates of serotonergic neurons.

Dopamine

Dopaminergic neurons are located primarily in the substantia nigra (SN) and ventral tegmental areas (VTA), but are also present in the rostral part of the raphe complex and in specific regions of the hypothalamus and olfactory bulb. Dopaminergic neurons include the mesostriatal dopaminergic system projecting from the substantia nigra to the striatum and frontal cortex, and the mesolimbocortical dopaminergic system projecting from the VTA to limbic structures, including the nucleus

accumbens, and to the cortex. Although dopamine neurons are reported to show similar firing rates across the sleep–wake cycle, dopaminergic systems are important for behavioral arousal and motor activity, possibly via interactions with other arousal-promoting systems, or via actions of dopaminergic neurons that have not been widely studied, such as those in the rostral part of the raphe complex. Although the evidence suggests no change in the mean firing rates of dopaminergic neurons in the VTA and SN across the sleep–wake cycle, extracellular concentrations of dopamine and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) show changes across the diurnal cycle in the striatum and nucleus accumbens (**Figure 1(c)**). The increase in extracellular dopamine concentrations and dopamine metabolism, apparently without changes in firing rate, may represent diurnal variation in TH activity, dopamine synthesis and release, or the rate of dopamine clearance in the projection regions.

Histamine

Histaminergic neurons are localized in the posterior hypothalamus within the tuberomammillary nuclei from where they project to virtually the entire central nervous system. The firing rate of histaminergic neurons varies across the sleep–wake cycle, with the highest rates during waking and the lowest rates during REM sleep. Extracellular concentrations of histamine in the hypothalamus also vary across the diurnal cycle (**Figure 2(c)**).

Knock-out (KO) mice lacking the synthetic enzyme for histamine, histidine decarboxylase (HDC), show an altered circadian rhythm in wheel running and locomotor activity. Antihistamines can cause drowsiness. Histamine is an important modulator of multiple components of the arousal system. For example, histaminergic fibers innervate the dorsal raphe nucleus, and activation of histamine receptors there increases the firing rates of serotonergic neurons.

Acetylcholine

The cholinergic neurotransmitter system is also important in the regulation of behavioral and cortical arousal. The major cholinergic pathways in the brain arise from two populations of cholinergic neurons – one located in the brainstem, the other in the basal forebrain. Cholinergic neurons of the brainstem are located in the mesopontine tegmentum (including the pedunculopontine tegmental nucleus (PPTg) and laterodorsal tegmental nucleus (LDTg)) and in the medullary reticular formation. These neurons project to the SN, LC, and midbrain raphe complex and also project to the lateral hypothalamus and basal forebrain where signals are relayed to the cerebral cortex. This basal forebrain relay to the cerebral cortex is constituted of cholinergic neurons located in the magnocellular preoptic nucleus, substantia innominata (SI), medial septum (MS), and ventral and horizontal limbs of the diagonal band of Broca (VDB and HDB, respectively). Cholinergic neurons in the MS and VDB project to the hippocampus, and this pathway has been suggested to be involved in mechanisms underlying arousal associated with fear and anxiety-related states. In general, cholinergic neurons discharge at their highest rates during active waking and REM sleep, and virtually cease firing during NREM sleep. In the cortex, extracellular concentrations of acetylcholine change across the diurnal cycle; this effect is more pronounced in the sensory cortex than in the motor cortex. Although the extracellular concentrations of ACh are elevated during the dark (active) phase, converging lines of evidence suggest that in some brain regions (basal forebrain, hypothalamus, etc.) extracellular concentrations of ACh are greater during REM sleep than during waking.

Orexin/hypocretin

Orexin/hypocretin neurons are localized in the perifornical area of the lateral hypothalamus and are necessary for the maintenance of waking and behavioral arousal. An absence or reduction of the orexin/hypocretin peptide or receptors results in narcolepsy with cataplexy. Orexinergic neurons project widely to the forebrain, including the cerebral cortex, brainstem, and spinal

cord, and – like the ACh-basal forebrain system – orexinergic neurons project to the subcortical relays of the basal forebrain. Orexin/hypocretin modulates multiple arousal-promoting neurotransmitter systems, including noradrenergic, histaminergic, and serotonergic neurons. Orexinergic neurons fire selectively during waking and cease firing activity during both REM and NREM sleep. In the hypothalamus, extracellular concentrations of orexin/hypocretin (**Figure 2(c)**), and orexin/hypocretin mRNA expression also vary in a diurnal manner.

Sleep-Promoting Mechanisms

Sleep is often characterized by a common species-specific posture, reduced motility, higher arousal thresholds (i.e., stronger sensory stimuli are needed to induce a response), and reduced behavioral responsiveness to environmental stimuli. Sleep is most commonly defined by changes in the electroencephalogram (EEG), also referred to as brainwave activity. During active wakefulness, cortical EEG is predominantly a fast (15–25 Hz or cycles per second (cps)), low-amplitude/voltage waveform called beta EEG, whereas during quiet wakefulness cortical EEG is a synchronous waveform of 8–12 Hz called alpha EEG. Compared to wakefulness, the sleep EEG is generally slower and of higher amplitude. Sleep physiology can be divided into deep NREM and REM sleep stages with sleep-generating mechanisms varying between these stages. In the adult human, NREM and REM sleep alternate throughout the night approximately every 80–120 min.

NREM sleep

NREM sleep is dominated by slow (<8 Hz), high-amplitude (>20 μ V) synchronized EEG activity. In the human, NREM sleep is divided into stages 1, 2, and stages 3/4. Stage 1 is a light stage of sleep that occurs early during the wakefulness–sleep transition and lasts for a few minutes. Stage 1 sleep consists of a dominant theta (4–7 Hz) EEG waveform and the presence of vertex sharp waves – a fast negative deflection maximal at the vertex (apex). Stage 2 sleep makes up approximately 50% of total sleep time and occurs in equal amounts across the night. Stage 2 sleep consists of a dominant theta EEG waveform with the presence of sleep spindles and K-complexes. Sleep spindles are a synchronous sinusoidal EEG waveform of 12–14 Hz in the shape of a spindle with duration of at least 0.5 s. Spindles represent an inhibitory waveform between the thalamus and cortex that prevent sensory information transfer from subcortical to cortical areas. The K-complex is an EEG waveform with a well-delineated negative voltage-deflection immediately followed by a positive voltage-deflection with duration

of ~0.5 s. K-complexes occur in response to environmental sensory stimuli and are also endogenously generated. Arousal thresholds are higher in stages 1 and 2 sleep compared to wakefulness, yet behavioral responses to all forms of sensory stimuli often still occur. Many physiological processes slow down or are less active during NREM sleep when compared to wakefulness. For example, eye movements, respiration, muscle activity, heart rate, and whole body and brain metabolic rate are all lower and relatively stable during NREM sleep compared to wakefulness. Sleep is promoted by the inhibition of the RAS and is associated with a thalamocortical disassociation mediated by the inhibitory neurotransmitter γ -aminobutyric acid (GABA). Cholinergic neurons in the basal forebrain and brainstem, serotonergic neurons in the midbrain raphe nuclei and noradrenergic neurons in the LC decrease their activity during the wakefulness to sleep transition. The diminished release of ACh and norepinephrine affects the activities of thalamocortical and cortical cells, most notably to reduce responsiveness to afferent signals. GABAergic thalamic reticular nucleus neurons are responsible for the production of sleep spindles along thalamocortical pathways. Neurons in the ventrolateral preoptic nucleus (VLPO) of the hypothalamus are sleep active. Ventrolateral preoptic nucleus neurons promote sleep via GABAergic projections that inhibit excitatory histaminergic cells in the posterior hypothalamus and inhibit excitatory noradrenergic and serotonergic cells in the brainstem. NREM sleep is promoted by the activation of neurons in the VLPO, basal forebrain, nucleus of the solitary tract, and raphe nuclei, which project to and inhibit wakefulness-generating centers and the cortex. Stage 3/4 sleep (also referred to as slow-wave sleep (SWS)) is the deepest stage of sleep and is dominated by a slow, delta (0.5–2.5 Hz), high-amplitude (>75 μ V), synchronized EEG waveform. Slow-wave sleep occurs mostly during the first half of the night and is driven by homeostatic sleep drive. While neurobiological mechanisms of sleep homeostasis are not completely understood, there is strong evidence that the neuromodulator adenosine plays a role in homeostatic sleep drive. Adenosine levels build up with time spent awake and promote sleep by inhibiting wakefulness-promoting cholinergic basal forebrain neurons and by activating sleep-promoting neurons in the VLPO. The wakefulness-promoting drug caffeine exerts its effects primarily by blocking the effects of adenosine. Other endogenous sleep-promoting substances include immune factors such as prostaglandin D2 (PGD2), interleukin-1 (IL-1), and muramyl peptides.

REM sleep

REM sleep is defined by low-amplitude desynchronized theta EEG activity with the presence of saw-tooth waves

at the cortex. REM sleep is also characterized by the presence of pontine-geniculate-occipital (PGO) waves as well as hippocampal theta EEG activity. Compared to NREM sleep, REM sleep is considered the sleep state with the highest physiological arousal. Respiration, heart rate, and brain glucose utilization are more variable and activity can be as high as that observed during wakefulness. However, skeletal muscle activity is actively inhibited during REM sleep. REMs and other phasic activity such as middle ear muscle activity may be present during REM sleep.

Cholinergic neurons in the reticular formation are active during REM sleep, primarily those located in the pontine tegmentum. However, there are special populations referred to as REM-ON neurons that fire only during REM sleep, REM-OFF neurons that fire during wakefulness and are off during REM sleep, and a population of cholinergic neurons that fire during both REM sleep and wakefulness. GABAergic REM-ON neurons are located in the sublaterodorsal nucleus/peri-LC and the periventricular gray matter, and GABAergic REM-OFF neurons are located in the ventrolateral part of the periaqueductal grey matter and the lateral pontine tegmentum. REM-ON neurons inhibit REM-OFF neurons and thus promote REM sleep. Reciprocal inhibitory interactions between the REM-ON and REM-OFF neurons are thought to be involved in REM-sleep regulation. In addition stimulation of serotonergic, noradrenergic, or histaminergic cells will inhibit REM sleep. Orexin/hypocretin neurons also regulate REM-sleep timing via activation of the LC. Disinhibition of GABAergic neurons in the pons may also be involved in REM-sleep generation.

Stress Influences on Arousal/Sleep

There is currently no consensus in neurobiology on the definition of stress. However, the stress research pioneer Hans Selye suggested that "Stress is a state, manifest by an adaptive syndrome that works in the direction of producing a return to the unchallenged state of the body." The adaptive responses involved in restoring homeostasis during a stressful state include the activation of the autonomic nervous system and activation of the HPA axis, leading to the release of glucocorticoid hormones. This broad definition still has value, although in practice, the current usage of the term is often restricted to responses to aversive stimuli, and states with a negative valence. For example, voluntary exercise – which for many is not an aversive experience – activates both autonomic and HPA-axis responses that contribute to a return to homeostasis. Thus, according to Selye's definition, this would be considered a stressful state, but according to common usage, it would not. Here we will align more

closely with Selye's definition, as it is first and foremost the stress-related neuromodulators, such as CRF and CRF-related neuropeptides, and glucocorticoid hormones, that are acting on arousal circuits in the brain, although the cognitive appraisal of the situation (aversive or not aversive/controllable or uncontrollable) has important implications for physiological, behavioral, and emotional outcomes.

A general discussion of the HPA axis and neural mechanisms regulating responses to stressful or aversive stimuli can be found elsewhere in the encyclopedia. Overall, evidence suggests that stress-related neuromodulators, including CRF, CRF-related neuropeptides, and glucocorticoids, increase the excitability of arousal-promoting circuits. Thus, during a stressful state, the overall effect is to amplify arousal, leading to increased reactivity to sensory stimuli, increased behavioral reactivity, and increased emotional reactivity. The precise physiological, behavioral, and emotional consequences of this amplification depend on the context.

Brainstem Reticular Formation

Studies in vertebrates have shown that the stress-related neuropeptide CRF has predominantly excitatory effects on the neuronal firing rates of reticular formation neurons. One example of CRF modulation of reticular formation neuronal excitability is in the paradigm of fear-potentiated acoustic startle. Corticotropin-releasing factor released in the caudal pontine reticular formation increases tone-evoked neuronal activity and is critical for fear potentiation of acoustic startle. The source of the CRF acting on reticular formation neurons includes the descending CRF-containing projections arising from the central nucleus of the amygdala – an important structure controlling emotional behavior.

Consistent with the role of CRF, glucocorticoid hormones have direct actions at the level of the reticular formation. Corticosterone can act rapidly, presumably via nongenomic mechanisms, to either increase or decrease neuronal firing rates of reticular formation neurons. The effect of corticosterone is dependent on the context. Thus, the core of the arousal-generating circuitry is under direct control by stress-related neuropeptides and stress-related hormones, demonstrating how a stressful state can alter the baseline state of arousal. These mechanisms have likely evolved as adaptive mechanisms to create a higher level of arousal under conditions when the homeostatic state of the organism is threatened, leading to heightened sensory reactivity, behavioral reactivity, and emotional reactivity required to cope effectively with the stressful challenge.

Serotonin

Stressful stimuli increase serotonergic neurotransmission. CRF-containing fibers, possibly arising from the central nucleus of the amygdala and the bed nucleus of the stria terminalis, form synaptic contacts with serotonergic neurons in the dorsal raphe nucleus. Populations of serotonergic and GABAergic neurons in the raphe complex express CRF type 2 (CRF₂) receptors. A low level of CRF type 1 (CRF₁) receptor expression has also been reported in the raphe complex. CRF has biphasic effects on the neuronal firing rates of serotonergic neurons within the dorsal raphe nucleus. At lower concentrations, CRF inhibits serotonergic neuronal firing rates, while at higher concentrations, it increases them. The inhibitory effects of CRF are mediated by activation of CRF₁ receptors, while the excitatory effects are mediated by activation of CRF₂ receptors. These effects on neuronal firing rates are consistent with the effects of CRF on extracellular 5-HT concentrations in forebrain targets of serotonergic neurons. The endogenous ligands mediating these effects are not certain but could include CRF itself, which has high affinity for CRF₁ receptors and moderate affinity for CRF₂ receptors, urocortin 1 (Ucn 1), which has high affinity for both CRF₁ and CRF₂ receptors, or Ucn 2 or Ucn 3, which are both high-affinity ligands for CRF₂ receptors. Activation of the central nucleus of the amygdala increases extracellular 5-HT concentrations in forebrain targets of the dorsal raphe nucleus, and this can be prevented by blockade of CRF₂ receptors within the dorsal raphe nucleus. Stress and CRF also increase the activity of TPH – the rate-limiting enzyme in the biosynthesis of 5-HT – via phosphorylation-dependent mechanisms.

Serotonergic neurons also express glucocorticoid receptors (GRs). Glucocorticoids can acutely increase the neuronal firing rates of a subset of serotonergic neurons, and following prolonged treatment cause a desensitization of the normal 5-HT_{1A}-receptor-mediated negative feedback mechanisms, resulting in increased excitability of serotonergic neurons. Stress increases the expression of TPH mRNA, but the neurochemical mechanisms involved are not yet clear. However, as glucocorticoids are responsible for the diurnal variation in *tpb2* mRNA expression (**Figures 1(a) and 1(b)**), it seems likely that glucocorticoids may contribute to stress-induced increases in *tpb2* mRNA expression. Glucocorticoids are permissive for stress-induced increases in TPH activity, apparently via actions within the central nucleus of the amygdala, but have no effects on TPH activity by themselves. Thus, in conclusion, glucocorticoids play a critical role in the normal diurnal variation in *tpb2* mRNA expression (and, therefore, presumably, 5-HT synthesis and release), while stress neuromodulators, including CRF or CRF-related

neuropeptides acting at CRF₂ receptors, and stress-induced increases in glucocorticoids appear to further increase serotonergic neurotransmission via various, complementary mechanisms.

Norepinephrine

Like the serotonergic system, stressful stimuli also activate noradrenergic systems. CRF-containing terminals have been detected in apposition to noradrenergic neurons in the LC and retrograde tracing experiments have suggested that this CRF innervation comes from the nucleus gigantocellularis of the reticular formation and the paraventricular nucleus of the hypothalamus, among other sources. Almost all noradrenergic neurons in the LC express CRF₁ receptors. Direct administration of CRF into the LC increases the firing rates of noradrenergic neurons and this increase is associated with increased extracellular concentrations of norepinephrine in forebrain targets of the LC. Stress-related stimuli can increase TH mRNA and protein expression.

The LC contains a high density of GRs. As opposed to *tpb2*, evidence that glucocorticoids modulate TH mRNA expression or TH activity in adults is lacking.

Dopamine

Stress induces the release of CRF in the VTA and increases extracellular dopamine concentrations in limbic forebrain structures. CRF-containing nerve terminals in the VTA are made up of asymmetric synapses that are mostly glutamatergic and symmetric synapses that are mostly GABAergic. The synapses between CRF-immunoreactive axon terminals and TH neurons are asymmetric, and hence likely to be glutamatergic, suggesting that glutamatergic neurons containing CRF may be part of the stress-related neuronal circuitry that modulates dopaminergic neuronal activity. Dopaminergic neurons express CRF₁ receptors in both the VTA and SN and CRF₂ receptor mRNA has recently been detected in the VTA using reverse transcription-polymerase chain reaction (RT-PCR). Consistent with these findings, CRF increases the firing rates of dopaminergic neurons in the VTA.

Some, but not all, dopaminergic neurons in the VTA and SN appear to contain GRs. Stress increases the strength of excitatory synaptic transmission in midbrain dopamine neurons and this effect can be prevented by a GR antagonist.

Histamine

Although stress-related stimuli have been shown to activate histaminergic neurons and histaminergic neurons appear to play an important role in the stress response,

the interactions between CRF, glucocorticoids, and histaminergic systems are not well understood. This is an important subject for future studies.

Organic Cation Transporters

Organic cation transporters (OCTs) are a family of multi-specific, bidirectional, carrier-type permeases that transport organic cations, including 5-HT, norepinephrine, dopamine, and histamine. These transporters include OCT1, OCT2, and OCT3, also known as the extraneuronal monoamine transporter. As opposed to the presynaptic, high-affinity, low-capacity sodium-dependent transport mechanisms (5-HT transporter, norepinephrine transporter, dopamine transporter, etc.), OCTs are low-affinity, high-capacity transporters that are expressed widely in postsynaptic neuronal and glial cells. The transport activity of OCT3 is inhibited by corticosterone in the low nanomolar range, suggesting that inhibition of monoamine clearance by corticosterone may contribute to stress-induced increases in extracellular monoamine concentrations.

Acetylcholine

The cholinergic system plays an important role in the modulation of arousal systems by stressful stimuli. CRF-containing fibers innervate both brainstem and basal forebrain regions containing cholinergic neurons. Most of the cholinergic neurons in the brainstem and basal forebrain co-express CRF₁ receptors. Intracerebroventricular administration of CRF increases ACh release in the hippocampus and increases neuronal activity in brain regions associated with the cholinergic system and arousal including the MS and LDTg. It has been hypothesized that CRF (via CRF₁ receptors) activates cholinergic neurons in the LDTg projecting to the LC.

GRs are present (albeit at low concentrations) in regions of the basal forebrain and brainstem that contain cholinergic neurons including the MS, nucleus of the diagonal band of Broca, SI, and LDTg. However, it is unclear if GRs are present in cholinergic neurons. Stressful stimuli or the subcutaneous administration of corticosterone increases extracellular concentrations of ACh in the hippocampus. Nicotinic and muscarinic receptor agonists have been reported to increase plasma corticosterone concentrations via interaction with the noradrenergic system.

Orexin/hypocretin

Synaptic contacts between CRF-containing terminals and orexin/hypocretin perikarya have been observed in the hypothalamus. Orexin/hypocretin neurons

express CRF receptors, and there is some evidence for activation of orexin/hypocretin neurons during stressful states.

Thus, in summary, stress-related neuromodulators (CRF, corticosterone, etc.) can co-opt normal arousal mechanisms (the brainstem reticular formation, monoaminergic systems, cholinergic systems, and orexin/hypocretin systems) and potentially amplify sensory responsiveness, vigilance, attention, and behavioral and emotional reactivity.

Models of Insomnia

The psychophysiological model of insomnia provides strong support that insomnia is a disorder of 'hyperarousal.' Specifically, patients with insomnia compared to healthy controls show:

1. increased sympathetic arousal as indicated by reduced heart rate variability and higher circulating norepinephrine levels;
2. higher HPA-axis activity as indicated by higher cortisol levels immediately prior to sleep or during the beginning of the sleep episode;
3. higher functional magnetic resonance imaging (fMRI) brain activity of brainstem arousal systems during sleep;
4. higher cortical arousal as indicated by increased beta EEG activity during sleep, as well as reduced delta and theta EEG activity during NREM sleep;
5. increased processing of auditory stimuli during sleep as evidenced by changes in event-related brain potentials;
6. higher frontalis and mentalis muscle electromyography (EMG) activity during the sleep transition;
7. higher core body temperature,
8. higher whole-body metabolic rate, and
9. increased latency to fall asleep at all times of day.

The cognitive model for insomnia is complementary to the psychophysiological model in that stressful life-events and excessive worry lead to physiological and cognitive arousal, acute insomnia, and conditioned arousal associated with sleep. The physiological and cognitive arousal contributes to behaviors and thoughts that occur near or when patients think about the sleep. These include ruminations and worry about not being able to sleep and the consequences of insomnia for the next day. Often, many of these thoughts are considered dysfunctional and inaccurate. Regardless, these thoughts are considered to be precipitating events for acute insomnia and perpetuators for chronic insomnia. Over time, insomnia increases the worries about sleep and

physiological and cognitive arousal eventually lead to chronic insomnia.

Psychopathology

Chronic stress states, via effects of CRF-related neuropeptides, glucocorticoid hormones, and other stress-related neuromodulators, are likely to play an important role in disruption of sleep that is associated with a number of stress-related neuropsychiatric disorders, including generalized anxiety disorder, posttraumatic stress disorder (PTSD), panic disorder, and affective disorders.

See also: Animal Models of Bipolar Disorder; Animal Tests for Anxiety; Bioenergetics of Sleep; Circadian and Ultradian Clocks/Rhythms; Conscious and the Unconscious; Depression; Effects of Stress on Learning and Memory; Fear, Anxiety, and Defensive Behaviors in Animals; Fear Conditioning; Fear: Potentiation and Startle; Measuring Stress; Neural Basis of Attention-Deficit/Hyperactivity Disorder; Neural Substrates of Conditioned Fear and Anxiety; Neural Substrates of Unconditioned Fear, Defense, and Anxiety; Neuropsychology of Sleep; Neurotransmitters and Neuromodulators Regulating Sleep and Wakefulness; Psychoneuroendocrinology of Stress; Regulation of the HPA Axis by Acute and Chronic Stress; Sleep Genetics; Sleeping, Waking, and Dreaming; Sleep: Learning and Memory; Sleep: Medical Disorders; Stress and Brain Morphology; Stress and Drug Craving; Stress and Emotionality; Stress and Energy Homeostasis; Stress and Reward; Stress and Social Behavior.

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Stress and Social Behavior

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Glossary

Chronic variable stress (CVS) – This involves a combination of a number of different stressors that are usually applied in a random fashion over an extended period of time. CVS in animal models is used to mimic the effects of chronic stress while minimizing possible habituation to a given stressor.

Individual differences – For a given measure within a defined population, wide variation is often found that defines the normal distribution. An important new development in behavioral neuroscience and other disciplines has been to take advantage of this variance and separately analyze how different components of a population (e.g., those at one or both ends of the spectrum) respond differently to a given treatment or to stressors. This approach of using individual differences is often accompanied by an examination of the genes and environmental history of the individuals.

Sex differences – Within a given species, any detectable difference between male and females can be considered a sex difference. Body morphology and mechanisms of reproduction are overt examples of sex differences. In behavioral neuroscience, sex differences can include differences in brain neurochemistry, hormones, and reactivity to stressors between males and females.

Social behavior – Any intraspecies interactions between individuals can be considered a social behavior. General social behaviors can include communication, allo-grooming, and aggression. Reproductive social behaviors can include partner preference and mating behavior. Maternal social behaviors can include nursing, licking and grooming of offspring, and offspring protection. Within these secondary headings, a number of subcategories exist. For example, there are a number of forms of aggression, including territorial, mate guarding, fear induced, and dominance. The final forms of social behaviors can differ dramatically among species.

While exhibiting social behaviors an individual may experience a stressor that will alter social performance. A number of factors make stress and social behavior a highly complex topic. First, there is a wide range of possible stressors and social behaviors which can interact. Second, not all stressors uniformly inhibit social

behaviors, rather some social interactions can be enhanced. Third, some stressors are themselves a social behavior, such as aggression. Fourth, stressors can trigger the emergence of a social behavior, such as aggression or alarm calling, which involves vocalizations by individuals to alert conspecifics of an imminent threat. Fifth, some social behaviors can mitigate the effects of a stressor through a process called ‘social buffering’ whereby the process of performing social behaviors itself will dampen response of individuals to a stressor. Social buffering can be adaptive by minimizing the negative effect of a stressor on ongoing behaviors. Finally, individual differences and sex differences within a species can strongly determine the effect of a stressor on a behavior. One goal of behavioral neuroscience is to understand the complex interactions of stress and social behaviors.

Effects of Stress on General Social Behaviors

As shown in [Table 1](#), a number of studies have been conducted examining the effects of stress on a range of general social behaviors. Some of the most commonly studied general social behaviors and how those are affected by stressors are discussed. The relevance, or adaptive value of these responses, will also be provided, if it is known. The mechanisms by which stress modulates behaviors will be provided in some cases.

Communication

In humans, conversation is a social behavior, and changes in speech occur in response to stress. For example, voice pitch (specifically, the fundamental frequency floor) is elevated with stress, but this effect was only found in individuals either exhibiting high anxiety or reporting low anxiety. Further, for females reporting low anxiety, cognitive stress increases the precision of articulation, whereas emotional stress decreases this precision. A change in voice pitch or precision of articulation could be adaptive for communicating the stress state of an individual to others. Pitch changes would involve physiological changes in muscle tension, but how stress alters the nervous system pathways regulating pitch is not known.

Alarm calls are emitted by individuals of certain species to alert others of the group of impending dangers.

Table 1 Overview of effects of applied stressors on different social behaviors

Stressor (timing)	Effect	Social behavior	Species
Physical stress	↔	Communication	Bird males
Infection (juvenile)	↓	Communication	Bird males
Food restriction (juvenile)	↓	Communication	Bird males
Cognitive stress	↑	Articulation	Human females
Emotional stress	↓	Articulation	Human females
Cognitive/emotional stress	↑	Voice pitch	Human (subgroups only)
Predator stress	↓	Communication	Birds
Predator stress	↑	'Alarm calls'	Many species (incl. birds)
Restraint stress	↑	Allo-grooming	Rat
Crowding	↓	Allo-grooming	Chimpanzee
Rearing deficits	↓	Allo-grooming	Rhesus monkeys
Restraint stress	↓	Male aggression	Rat
Crowding	↓	Male aggression	Chimpanzee
Social isolation	↑	Male aggression	Mouse, rat
Footshock (acute)	↑	Male aggression	Mouse, rat
Electroconvulsive shock (acute)	↓	Male aggression	Rat
Electroconvulsive shock (chronic)	↑	Male aggression	Rat
Predator stress	↑	Mobbing	Many species
Conditioned defeat	↓	Male aggression	Hamster, mouse
Swim stress	↓	Partner preference	Prairie vole females
Swim stress	↑	Partner preference	Prairie vole males
Low social status	↓	Mating	Marmoset, tamarin
Restraint (acute)	↓	Lordosis	Rat female
CVS	↓	Ejaculation	Rat male
Social stress	↓	Mounting	Mouse male
Combat stress	↓	Mating	Human males
Social stress	↓	Mating	Meerkat
Restraint (chronic and acute)	↔	Pup retrieval	Mouse
CVS (pregnancy)	↑	Pup retrieval	Mouse
Social-defeat stress (pregnancy)	↑	Pup retrieval	Rat (high anxiety)
Male exposure (acute)	↓	Pup retrieval	Rat
Restraint (pregnancy and lactation)	↑	Pup licking/grooming	Mouse
CVS (pregnancy)	↓, ↑	Pup licking/grooming	Mouse
Social crowding (pregnancy)	↓	Pup licking/grooming	Rat
Limited nesting material	↓	Pup licking/grooming	Rat
CVS (pregnancy)	↓	Nursing	Mouse
CVS (pregnancy)	↑	Nursing	Rat
Social (acute)	↓	Nursing	Rat
Social (chronic)	↔	Nursing	Rat
Social defeat (pregnancy)	↑	Maternal defense	Rat (low anxiety)
Restraint (pregnancy)	↓	Maternal defense	Mouse
CVS (pregnancy)	↓, ↑	Maternal defense	Mouse
Restraint (acute)	↓	Maternal defense	Mouse
Restraint (chronic during lactation)	↔	Maternal defense	Mouse

↑ = elevates behavior; ↓ = decreases behavior; ↔ = no effect on behavior.

These are conserved in social species and are found in a range of taxa, including birds, rodents, and primates. Here, we see a unique social behavior emerge only with stress and in the context of a threat.

In songbirds, singing by males can be used to attract mates. Canaries injected with malaria as juveniles and zebra finches experimentally exposed to stress (including food restriction) as juveniles, both produce simpler songs as adults, indicating developmental stress can alter adult outcome. However, application of a weight to the tail feathers for 48 h does not alter song repertoire in Whitethroats, suggesting song can be resistant to some

stressors. While alarm calls described above are produced by some birds in response to predatory threat, a predator can similarly induce decreased singing. This decrease in singing (which can also be induced by human presence in a laboratory setting) may be adaptive to decrease detection from predators. The neuropeptide, corticotropin-releasing factor (CRF), can be released centrally in association with a response to stress, and indirect evidence suggests CRF mediates a decrease in singing with stress. For example, central injections of CRF decrease song produced by white-crowned sparrows in association with territory defense.

Allo-Grooming

The grooming of conspecifics occurs in a wide range of species. In rats, 1 day following a single restraint stress or repeated restraint stress, males show an elevation of grooming other males that they confronted in a neutral arena. This elevation of grooming coincides with a decrease in aggression, suggesting it is an appeasement behavior. In contrast, in captive chimpanzees, the social stressor of crowding decreases allo-grooming. The increased crowding occurred on cold days when the chimpanzees were housed indoors and the density increased, so behavior of the same group of animals could be compared when crowding and conditions differed. For chimpanzees, the decrease in allo-grooming also accompanied a decrease in aggression and was suggested to be a strategy for limiting the possibility for conflict. In rhesus monkeys, allo-grooming decreases if the animals were exposed to a rearing stress. In this case, 50% of the monkeys were reared by their mothers (considered the control) and the other 50% were reared using bottles and a nursery care staff. The lack of full maternal care as a stressor was employed for the first 3 months of the monkey's life and the monkeys were evaluated from 18 to 36 months of age. Norepinephrine (NE) and CRF are released centrally in association with a stress response and research suggests that both can promote self-grooming behavior. However, to what extent the circuitries of allo-grooming and self-grooming overlap, is not clear.

Aggression

Aggression is a highly variable social behavior when examined across species and within a given species. In general, aggression can be motivated by resource control (or a challenge to that resource) or by danger of harm or death to the individual. Resource control can include territorial aggression, parental aggression, dominance aggression, or mate acquisition. Aggression due to danger can include fear-induced aggression. In this latter case, the response to stress is adaptive because the emergence of this aggressive social behavior may be critical for self-preservation. Stressors can elevate, impair, or have no effect on the different forms of aggression. For example, in male rats, restraint stress reduces aggression when the males are tested in a neutral arena. However, social isolation (which is considered a stressor) is a common tool for elevating aggression in male rats and mice. While acute electroconvulsive shock impairs resident-intruder intermale aggression in rats, bidaily treatment for 7 days elevates this form of aggression. Here, we see an example where possible habituation to a treatment may alter outcome. Footshock can elicit increases in male aggression in rats. In some mouse strains, however, footshock elicits increased aggression

and this depends upon age, but other strains show no effects, regardless of when examined. This finding highlights that, even within a species, differences in how populations respond to a stressor are found. In chimpanzees, crowding stress results in decreased aggression. The act of aggression can be considered a trade-off because it can bring with it gains in different forms, and it also increases the risk of injury to the individual. A stressor may alter the perception of the gains/risks ratio and thereby have complex effects on the production of aggression.

Cortisol or corticosterone (CORT) is released peripherally from the adrenal gland in association with a stress response and physical exertion, such as aggression output. Exogenously applied CORT facilitates aggression in adrenalectomized rats, suggesting that increases in CORT could feed-forward to promote some forms of aggression. However, CORT can also be without effect on some forms of aggression or even enhance a defeat response (see below). Interestingly, CRF injected centrally promotes stress-induced aggression, but impairs resident-intruder aggression in rodents. NE has been found to promote some reactive forms of intermale aggression and antagonizing the beta-adrenergic receptor reduces aggression in humans. Inhibition of the alpha 2 adrenergic receptors can either promote or inhibit intermale rat aggression. Thus, it is likely that both CRF and NE mediate some of the complex effects of stress on aggression.

In response to a potential predator, a number of species act collectively to ward off the intruder in what is termed 'mobbing behavior.' For example, a group of crows may attack a larger raptor and this involves vocalizations, chasing, and swoop attacking the potential predator. Interestingly, birds from unrelated species can be found mobbing together to protect a region. In some squirrel species and in some nonhuman primates, such as tamarins, mobbing behavior can be triggered by snakes. Mobbing behavior is adaptive because it provides cohesion among a group and allows the group to deter a potential threat that could not be accomplished by any one individual. How a predator stress might elicit a mobbing behavior is not known; however, it would be expected that activation of fear-induced aggressive routines within individuals would be an important component.

Conditioned Defeat

Conditioned defeat is a model for social stress whereby a male rodent is repeatedly defeated by another (usually larger) male. The outcome of conditioned defeat is that the male shows decreased aggression when tested later, even if the opponent is smaller and the focal male is defending his territory. Defeated males can also show increased submissive behaviors and avoidance of

conspecifics. In association with defeat, a heightened stress response has been found that could correspond to elevated central release of CRF. Indeed, conditioned defeat in hamsters has been linked to CRF actions in dorsal raphe and bed nucleus of stria terminalis as antagonizing the CRF receptors in either region suppresses the effects of defeat. Injections of the γ -aminobutyric acid-A (GABA-A) agonist, muscimol, into amygdala also reduces the defeat response, suggesting a role for this region in the response to stress. CORT itself has little effect on submissive behavior, but CORT application along with the experience of defeat elevates levels of defeat behavior in mice, suggesting the timing of CORT release is important. Conditioned defeat can be considered adaptive because if an individual has almost no chance of winning an aggressive encounter, then submissive behavior may be the best way to avoid harm.

Effects of Stress on Reproductive Behaviors

A number of studies have examined the effects of stress on reproductive social behaviors (Table 1). Some of the most commonly studied reproductive behaviors and how those are affected by stressors are discussed.

Partner Preference

An interesting example of sex differences in response to a stressor is seen in prairie voles. Males usually do not form a partner preference when cohabited with females for 6 h, but females do form a preference in this time course. When a forced-swim-stress test (which involves placing a rodent in water without access to a platform) preceded the 6-h cohabitation period, males showed a preference for the females when they were examined later using a three-chambered choice apparatus. In contrast, females given a stressor before cohabitation lost their preference for the male. These results could reflect sex differences in life-history choices, whereby in a high-stress environment the female is inclined to delay reproduction, while the male is inclined to promote reproduction. For male prairie voles that showed elevated partner preference with stress, exogenously applied CORT also promoted partner preference in adrenalectomized males. For female prairie voles that showed decreased partner preference with stress, however, applied CORT reduced their preference. Further, if CORT is removed through adrenalectomy, then partner preference is elevated in females. Recent work indicates a role for dopamine acting in nucleus accumbens on partner preference, but how stress would differentially affect those pathways in males and females, is not known.

Mating Behavior

Mating behavior here indicates the ability to successfully mate and bring an offspring to term. Sexual behavior is a motivated behavior and the ability to reproduce successfully also involves functioning of the hypothalamic-pituitary-gonadal (HPG) axis. Of all the social behaviors evaluated here, the effect of stress on mating behavior across species is the most uniform. Stress has a predominantly inhibitory effect on mating behavior. Sites of stress impairing mating can either occur in terms of motivation or the HPG axis. The impairment of reproduction by stress may be adaptive because if environmental conditions do not favor reproduction, it may be valuable to wait until more favorable conditions exist. In humans, a number of studies have shown that exposure to a variety of stressors decreases reproduction. For example, for combat veteran males suffering from posttraumatic stress disorder (PTSD), sexual dysfunction was found, including up to 85% with some form of erectile dysfunction. In one study, sexual desire was found to be decreased with PTSD, but in another it was not. For couples in a stable relationship, an incremental negative effect of stressors on sexual behavior has been found, including induction of a hypoactive sexual desire in men and women. Interestingly, female sex drive was strongly impaired by 'internal stress' that originated within the couple such as conflicts, whereas male sexual dysfunction was strongly regulated by critical life events. Unemployment has been found to correlate with a low sex drive. Research suggests CRF, CORT, and serotonin signaling may mediate some of stress's effects on sexual behavior.

Decreased reproduction may also occur by an elevation of spontaneous abortions, especially in the first trimester. Anovulation in females can be elevated by a variety of stressors, while a decrease in semen quality in males can be induced by psychological stressors. Male medical students in Brazil exhibited significant decreases in sperm concentration and motility of spermatozoa in association with examinations. In a study in Denmark, negative effects of stress were only found for males in the lowest quartile for sperm concentration. In a third study, immediate death of a close family member correlated significantly with decreased sperm performance, but other psychological stress levels did not. The exact mechanisms by which stress alters this element of the HPG axis are not clear.

Studies in other animals are in line with the studies in humans. In marmosets and tamarins, nonhuman primates, low social status is associated with decreased reproduction. Anovulation is a common mechanism for decreased mating behavior. In rats, exposure to restraint stress reduced the number of oocytes and decreased the lordosis quotient when measured later in the same day. Male rats exposed to a chronic mild stress exhibited a decreased

capacity to ejaculate. Dominant male mice exposed to a social stressor, whereby they were allowed to observe the subordinate mouse interacting with a female for 9 days, showed a significant impairment in sexual behavior, including levels of mounting. In a field study on cooperatively breeding meerkats, it was found that dominant females could suppress reproduction in subordinate females by harassing them and socially isolating them. This finding suggests that members of a species may actively exploit the inhibitory effects of stress on mating behavior.

Effects of Stress on Maternal Behaviors

Maternal behaviors are important social behaviors between mother and offspring. They can also include interactions of mothers and conspecifics as the mother protects her offspring. An overview of how stress can alter maternal behaviors is provided in **Table 1**.

Pup Retrieval

In mice, the application of chronic variable stress (CVS) during pregnancy triggered a faster time to retrieve pups. Interestingly, these effects are only observed when mothers are presented with offspring produced by stressed mothers (as compared to offspring produced by nonstressed mothers). Thus, a complication of studies where stress is applied during pregnancy is that direct effects on offspring occur *in utero* and offspring interactions with rearing mother can affect behavioral outcome. In rats that were stressed during pregnancy by subjecting the females to restraint and exposing them to aggressive attacks by a lactating female, an increased retrieval speed was observed. In the latter case, however, only rats bred for high anxiety showed this response, whereas rats bred for low anxiety did not, suggesting individual differences contribute to how an animal responds to a stressor. In rats, acute exposure to a male prolonged the retrieval. In mice, neither acute nor chronic restraint stress applied during lactation altered the timing of retrieval. The enhancement of retrieval by a stressor may be adaptive because it would rapidly bring offspring together and improve the ability for the mother to buffer the offspring from stressful conditions.

Pup Licking and Grooming

Restriction of nesting material may act as a stressor and in rats this treatment triggered decreases in licking and grooming of pups. For mice that were restrained for 2 h per day for 10 days during pregnancy and also restrained during lactation, an increase in grooming of offspring was observed. Exposure to light and restraint during

pregnancy for 7 days, however, did not alter pup-grooming levels in rats in a separate study, indicating pup grooming is not always altered by stress. As for pup retrieval, a subset of studies indicates that exposure to stress *in utero* alters some aspect of pup physiology or behavior that then alters the maternal grooming behavior. In the same study in mice, CVS applied during pregnancy triggered both an increase (when control pups were used) and a decrease (when stressed pups were used) in time spent licking and grooming offspring. Social crowding of rats during pregnancy resulted in decreased grooming of pups and this effect was seen when control pups were being raised by stressed and nonstressed mothers. In addition, pups born to stressed mothers elicited lower grooming from both stress and control rat mothers. A decrease in pup grooming by stressors could be adaptive if the mother needs to reallocate time and resources away from the nest. However, elevated grooming by stressors may be adaptive if the grooming helps to buffer offspring from the effects of stress.

Nursing

During lactation, acute exposure to a male rat increased latency to nurse for female rats; however, longer-term chronic exposure to both males and other lactating females did not have this effect, suggesting some habituation to the stimulus. For CVS applied during pregnancy, decreases in nursing were found in mouse mothers, but in rats increases in nursing were observed. A decrease in nursing by stress could be adaptive if the female needs to alter her allocation of resources. However, keeping nursing levels buffered from stress effects or elevating nursing with stress would benefit offspring development.

Offspring Protection

The protection of offspring during lactation (also termed maternal defense or maternal aggression) is highly conserved among mammalian species. In mice, application of acute restraint or swimming stress significantly decreases the time a female spends protecting offspring. However, repeated application of restraint stress during lactation had no effect, suggesting a habituation to the stressor. Restraint stress applied daily during pregnancy or CVS applied during pregnancy decreased the maternal defense in mice. However, in a separate study, application of restraint and heat during pregnancy elevated the maternal defense. Similarly, in rats, application of a social-defeat stress during pregnancy elevated the offspring protection. However, this effect was only seen in rats bred for low anxiety and not in rats bred for high anxiety, suggesting that a pre-existing anxiety state can strongly affect the stress responses. In mice, centrally

administered CRF strongly impairs offspring protection, but peripheral CORT is without effect, suggesting that stress impairs this behavior through central, not peripheral, systems. The decrease in offspring defense by stressors may be adaptive in species with high rates of reproduction, such as mice, because offspring protection involves risks to the mother. If conditions are not optimal for rearing offspring, then deferring offspring protection to a second or third litter, when conditions improve, may result in improvement of the overall reproductive rates.

Individual and Sex Differences in the Reactivity to Stressors

As indicated above, in some studies whether stress alters a social behavior is only noted when populations are subdivided. For example, rats bred for low anxiety do not respond to stress in the same way as rats bred for high anxiety, although they were originally derived from the same strain. In humans, speech is altered by stress, but only within certain subgroups. Thus, a complexity of examining stress actions on social behaviors is that individual differences can strongly affect outcome. In one example that does not involve a social behavior, the size of the hippocampus inversely correlated with level of PTSD in war veterans. However, this correlation was also seen if the hippocampal size of the twin brother was substituted in the correlation, even though the twin had no military experience. This outcome strongly suggests that pre-existing size of hippocampus and/or other inherited features predispose some individuals to PTSD. As indicated above, PTSD can affect reproductive behaviors in humans. An understanding of individual differences in any study can strengthen conclusions about stress effects on social behaviors.

In addition to individual differences, sex differences in response to stress can be found. As described above for partner preference, male and female prairie voles have an opposite response to stress. During lactation there is a suppressed reactivity to stress in females, while a dynamic change in reactivity is not seen in most males during their life history. Thus, individual differences, sex differences, and stage of life history, all of these can influence response to a stressor and should be incorporated into analysis of stress actions on social behaviors.

Complexities of Stress

In addition to individual differences and sex differences that can affect outcome in response to a stressor, a number of other features add to the complexity of stress and social behavior relations. When attempting to examine the

effects of stress on social behaviors either across or within species, it is valuable to appreciate how variable stress can be. For example, restraint is a simple form of stress, but its application can be highly variable. Restraint can be brief (5 min) or much longer (hours); it can be used once or repeated many times before measuring a behavior. The timing at which animals are tested after the end of the stress application also can vary from immediate to hours or even days. In mice, a 30-min restraint significantly decreases the time a female will protect her offspring, but only if the female is tested immediately following the end of restraint; no effect is seen 15 min or 2 h after restraint. Restraint for rodents can involve placement in a clear-Plexiglas tube or involve Velcro straps and in many cases it has not been evaluated whether the form of restraint affects outcome. Thus, for a stressor as simple as restraint, a number of details must be considered when evaluating results across studies, including timing and form of restraint.

As described above, social conditions and behaviors can act as stressors. Crowding is commonly evaluated as a stressor, but this is complex among species and rearing environment. Isolation is considered a stressor in most rodents, but cohabitation can also elicit high levels of stress among individuals within the established hierarchy. For example, in the visible burrow system in rodents, a limited number of male and female rodents live together and establish hierarchies that play a role in access to food and mates. Subordinate animals in this system show signs of chronic stress. Understanding where an animal sits within a hierarchical system and how it perceives stress from that position is important for untangling how social stressors modulate behavior. As with any other stressor, the timing and duration of exposure to a social stressor is important.

A general question is how or why stress can both elevate and decrease some social behaviors. Classical studies demonstrated that shock can elevate learning at lower levels, but impair learning at higher levels, highlighting that a stressor can have an inverted U-shaped response on a dependent measure. For some social behaviors, such as pup grooming, increases and decreases in response to stress are found and it is possible that stress has an inverted U-shaped effect on these behaviors. If an inverted U-shaped response accounts for some of the social behavioral responses to stress, then it is important to understand an animal's baseline level of behavior (where it sits on the curve) and to what extent that individual perceives the stressor.

Different types of stress can also be applied to an animal during development (*in utero*) or during rearing. Again, the type of stressor and the duration of the stressor can vary dramatically. In these cases, often, the effect of stress is not evaluated until the animal reaches adulthood, which adds an even greater possibility for variability

among studies. Given that so many aspects of a ‘common stressor’ can be altered, it is rare that all parameters of a common stressor are identical in different studies. In addition, how the dependent social behavior is measured can vary greatly across studies. The dependent social behavior output across species will always differ to some extent, hence, any cross-species comparisons need to be done cautiously. Attention to the specifics of the stressor (timing, form, and duration), individual differences, sex differences, strain or species differences, and behavioral testing differences, may all contribute to alternate outcomes.

Role of Social Behaviors in Mitigating Response to Stressors

A number of studies across a wide range of taxa have shown that the presence of conspecifics of a species confer a ‘social buffering,’ whereby the individual shows a mitigated stress response when presented with a stressor. In most of these studies, CORT levels are used as the dependent measure to evaluate changes in stress reactivity. For example, for female guinea pigs living with a male and another female, a reduced CORT response is found if that female is exposed to a novel environment and a cage mate is also present. The presence of a novel individual does not confer the same effect. The isolation of social partners acts as a stressor to elevate CORT in marmosets, but exposure to vocalizations by the partner alone was sufficient to significantly decrease the CORT response and this has been termed ‘vocal buffering.’ Young goats showed a suppressed CORT response when they were with their mother and exposed to a human. Moreover, normally shy young goats display a suppressed CORT response to humans if they are accompanied by conspecifics that display a bold behavior. In human males, support from their opposite sex partner prior to a public-speaking stress test resulted in a significant buffering of the CORT response. Interestingly, for females, support from male partners did not have this effect. When given a demanding computer test to induce CORT increases, both men and women showed suppressed CORT responses if they were exposed to a video that relayed supportive commentary. Laboratory rodent species showed decreased basal CORT levels and a decreased CORT reactivity to stressors when they were group housed (relative to isolated); however, as indicated above, crowding can have the opposite effect. For male rats, co-housing for as little as 24 h can reduce aspects of the stress response to footshocks. Furthermore, being tested while with the cohort reduces the stress response.

A dramatic example of social buffering is found in lactating females, whereby the process of being maternal

confers a protection from stressors. For example, in breast-feeding women, a decrease in CORT is found in response to an exercise stress test and a psychological stress test. In lactating rats, contact with offspring during lactation, including nursing, supports both decreased CORT release and suppressed anxiety when exposed to a stressor. Interestingly, no suppression of CORT is found if an intruder male is the stimulus, suggesting the female responds to certain stressors during lactation, but not to others.

Social buffering is reciprocal in mothers and offspring. Heightened licking and grooming of pups by mother rats result in a decreased CORT response in the offspring when tested with a stressor as adults. An increased expression of glucocorticoid receptors in hippocampus, possibly due to demethylation of the receptor gene, has been linked to the suppressed CORT response in offspring.

With evidence of increased stress pathologies in humans, it may be especially important to focus studies on the neural basis of social buffering to identify ways to improve reactivity to stressors.

See also: Animal Models of Sexual Function; Mating Behavior; Neural and Pharmacological Substrates of Aggression; Offensive and Defensive Aggression; Parental Behavior; Personality, Temperament, and Behavioral Syndromes; Regulation of the HPA Axis by Acute and Chronic Stress; Social Bonding and Attachment; Social Communication; Stress and Arousal/Sleep; Stress and Emotionality.

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Regulation of the HPA Axis by Acute and Chronic Stress

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Glossary

ACTH (adrenocorticotrophic hormone) – Pituitary hormone promoting synthesis and release of glucocorticoids by the adrenal cortex.

Corticotropes – ACTH-producing cells in the anterior pituitary gland.

Corticotropin-releasing hormone (CRH) – Hypothalamic neuropeptide responsible for release of ACTH by the pituitary.

Facilitation – Enhanced hypothalamo-pituitary-adrenocortical (HPA) axis responsiveness to novel stressors in chronically stressed individuals.

Glucocorticoid negative feedback – Glucocorticoid inhibition of hypothalamic neurons and pituitary corticotropes, limiting activation of the hypothalamo-pituitary-adrenocortical (HPA) axis. It can occur in fast (seconds to minutes) and delayed (hours to days) time domains.

Glucocorticoids – Cholesterol-derived steroid hormones responsible for the modulation of gene expression and cellular activity in brain and periphery. It is commonly released following stress.

Habituation – Reduced HPA responsiveness caused by repetitive exposure by a given stimulus.

Hypothalamo-pituitary-adrenocortical (HPA) axis – Neuroendocrine system responsible for the release of glucocorticoids.

Limbic system – Set of interconnected telencephalic and diencephalic structures that collectively regulate memory and emotion.

specific response of the organism to any challenge” has since gone through numerous revisions, due to the emerging discovery of stimulus-specific aspects of physiological responses. In general, current thinking holds that stress is a real or anticipated threat to homeostasis or an anticipated threat to well-being. Real physiological threats (e.g., blood loss) trigger largely reflexive responses that do not require higher-order cognitive processing. In contrast, the ability to anticipate threat requires the organism to interpret the significance of multi-modal sensory stimuli with respect to previous experience or innate response programs.

This article focuses on neuroregulatory processes governing activity of the hypothalamo-pituitary-adrenocortical (HPA) axis following stress. The HPA axis is one of the critical systems involved in the stress response. A summary of the organization of the HPA axis is presented elsewhere in this encyclopedia. Whereas the HPA axis is a key component of the stress response, it is important to note that activation of this system represents but one manifestation of stress. Stress also engages the sympathetic nervous system and adrenal medulla, which trigger cardiovascular and respiratory responses (among others) and the so-called ‘adrenaline surge’. Sympathoadrenal activation comprises the classic fight-or-flight response, enabling rapid reactivity to stressors. The peak HPA-axis response is engaged slightly later in time (on the scale of tens of minutes), and should not be considered part of the fight-or-flight reaction. In fact, some believe the HPA response is critical for recovery from the initial sympathetic response, acting to limit the disruptive effects of the initial stress response.

The ‘Stress’ Concept

The origin of the stress concept, first articulated by Hans Selye, was based on observed similarities between the physiological consequences of a wide variety of noxious stimuli. Selye’s original conclusion that stress is “the non-

Excitation of HPA-Axis Stress Responses

Paraventricular nucleus corticotropin-releasing hormone (CRH) neurons are activated by neural inputs from a number of possible sources (Figure 1). Stressors signaling homeostatic challenge (commonly referred to as systemic

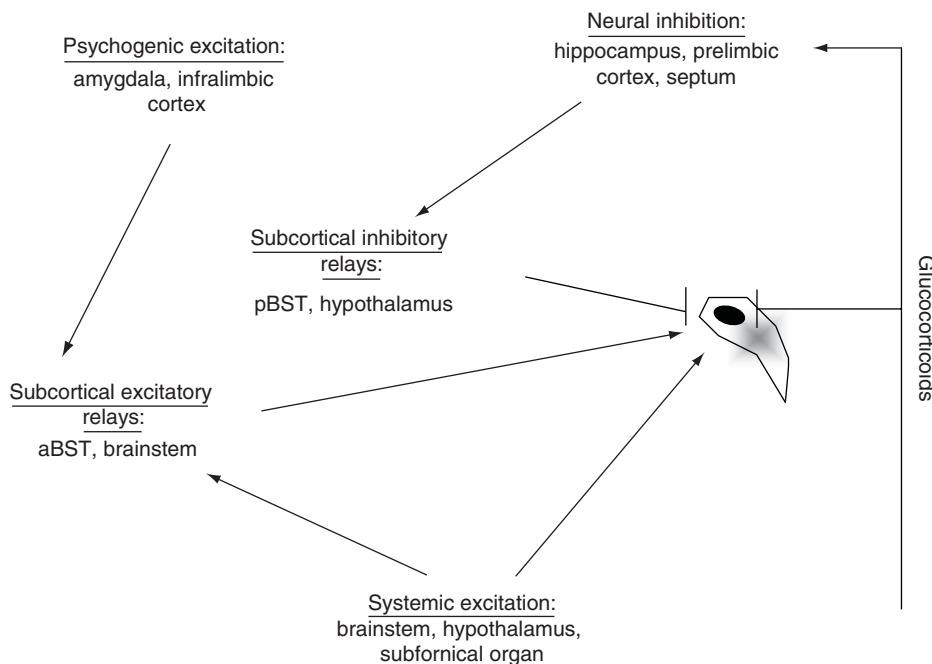


Figure 1 Neural regulation of stress responses: mechanisms. PVN neurons can be activated by multiple pathways. Reflexive activation occurs in response to homeostatic perturbations and are communicated to the PVN by brainstem, hypothalamic or circumventricular organ (subfornical organ) feedforward input. Anticipatory stressors are initiated by limbic interpretation of multimodal sensory input, and involve relays in subcortical sites that in turn project to the PVN, such as the anterior bed nucleus of the stria terminalis (aBST). Some excitatory input may be channeled through brainstem pathways also used for reflexive responses. Inhibition of PVN neurons is mediated by direct, fast feedback actions on PVN CRH neurons as well as input from other brain regions, including the hippocampus, prelimbic cortex, and lateral septum. These upstream structures are in receipt of glucocorticoid information and are involved in delayed negative feedback inhibition of the HPA axis. As was the case for limbic excitatory inputs, neural inhibitory information is channeled through subcortical relays, including the posterior BST (pBST) and several hypothalamic nuclei, including the preoptic area and dorsomedial hypothalamus. Limbic inhibitory regulation occurs largely by transsynaptic inhibition, i.e., excitation of inhibitory PVN-projecting neurons. Excitatory and inhibitory limbic input may overlap in these subcortical sites, providing an opportunity for summation upstream of the PVN. Arrows denote excitation, bars inhibition.

stressors) are mediated by neurocircuits providing direct excitation of paraventricular nucleus (PVN) neurons. For example, HPA-axis activation by blood loss (hemorrhage) is mediated by activation of brainstem norepinephrine neurons that project directly to the PVN. Activation of the HPA axis by fluid imbalance appears to be triggered by angiotensinergic neurons of the subfornical organ, which sense increases in circulating angiotensin II. Negative energy balance signals are relayed to the PVN by the arcuate nucleus, which activates CRH neurons by release of excitatory peptide messengers (such as neuropeptide Y and agouti-related peptide). Responses to systemic stress are thought to be reflexive, that is, they do not require engagement of cognitive processes.

In contrast, anticipatory HPA-axis responses are mediated by multisynaptic limbic forebrain circuits. The amygdala and ventral divisions of the medial prefrontal cortex (e.g., infralimbic cortex) appear to be critical for anticipatory release of glucocorticoids. These brain structures are involved in memory and emotional processing. Thus, the HPA-axis response may be thought of as a systemic mediator of limbic system action. In general,

the amygdala and prefrontal cortex have no direct inputs to the PVN, and require synaptic relays in subcortical structures such as the bed nucleus of the stria terminalis, preoptic area, and lateral hypothalamus. In many cases, subcortical relays also receive homeostatic information, providing a means for the integration of descending limbic input with ongoing physiological state.

Inhibition of the HPA Axis: Glucocorticoid Negative Feedback

Activity of the HPA axis is controlled by negative feedback, a process whereby the end-products of the stress response (glucocorticoids) inhibit their own release. Glucocorticoid feedback involves both genomic and non-genomic mechanisms. Fast feedback inhibition occurs within minutes, and is responsible for rapid termination of PVN activation and adrenocorticotrophic hormone (ACTH) release following stress. Rapid inhibition of the HPA axis is believed to be mediated by membrane receptors in the hypothalamus and/or pituitary, and is almost

certainly nongenomic. Delayed feedback occurs over a more extended time frame, and is thought to be mediated in large part by genomic actions of the nuclear adrenocorticoid receptors (glucocorticoid receptor (GR) and mineralocorticoid receptor (MR)). Both GR and MR act as ligand-activated transcription factors, modifying expression of a large number of genes. The MR has a very high affinity for endogenous glucocorticoids and is thought to regulate circadian secretory rhythms. In contrast, the GR is extensively bound by high levels of glucocorticoids (e.g., during HPA-axis stress responses), and appears to be the main factor in delayed glucocorticoid inhibition of stress responses.

Feedback effects are evident at the level of the brain, pituitary, and adrenal (Figure 1). The brain is the most sensitive mediator of negative feedback. Glucocorticoid receptors are expressed in multiple brain regions, many of which contribute to integration of the feedback signal. In particular, hippocampal and medial prefrontal cortex GRs appear to be required for inhibition of anticipatory HPA-axis responses. As was the case for the amygdala and stress excitation, hippocampal and medial prefrontal cortex neurons do not project directly to the PVN. Inhibition of the HPA axis is mediated by connections between excitatory neurons of the hippocampus and medial prefrontal cortex with inhibitory neurons in the bed nucleus of the stria terminalis, medial preoptic area, dorsomedial hypothalamus, and lateral septum.

Fast feedback inhibition occurs at the level of primary secretory cells. Glucocorticoids rapidly inhibit PVN CRH neurons by way of membrane actions, involving mobilization of endocannabinoids and presynaptic inhibition of glutamate release. Similarly, glucocorticoids inhibit pituitary ACTH release by inducing annexin 1 translocation to the cell membrane of folliculostellate cells, which then bind to corticotropes and inhibit secretion.

Glucocorticoids are not the only source of HPA-axis feedback inhibition. There is ample evidence for glucocorticoid-independent inhibition of PVN neurons by neural mechanisms. For example, peripheral metabolic signals (e.g., sucrose load) can reduce HPA-axis activation in the absence of glucocorticoid signaling.

HPA-Axis Regulation by Chronic Stress

Most organisms undergo periodic exposure to prolonged stress. Prolonged (or chronic) stress can cause long-term modifications at all levels of the HPA axis (see Table 1). In the brain, experimental chronic stress regimens increase biosynthesis of CRH and other ACTH-stimulatory factors (e.g., arginine vasopressin) in the PVN, reflecting increased drive of the central limb of the HPA axis. Chronic stress regimens promote synthesis of pituitary proopiomelanocortin, the precursor molecule for ACTH, and increase

Table 1 Effects of acute and chronic stress on the HPA axis

Measure	Acute stress	Chronic stress
<i>Brain</i>		
PVN CRH mRNA	↑	↑↑
PVN CRH peptide	↑	↑↑
PVN AVP mRNA	↑↑	↑
PVN Fos	↑↑	-
<i>Pituitary</i>		
POMC mRNA	↑	↑↑
ACTH content	↓	↑↑
<i>Adrenal</i>		
Corticosterone content	↑↑	↑
Zona Fasciculata cell #	-	↑↑
Zona Fasciculata size	-	↑↑
ACTH sensitivity	-	↑↑
<i>Plasma</i>		
ACTH	↑↑	-
Corticosterone	↑↑	↑

pituitary ACTH stores. In addition, chronic stress increases adrenal size and causes enhanced sensitivity to ACTH, resulting in increased resting glucocorticoid levels as well as increased cumulative exposure to steroids over time. The latter effects are evident in glucocorticoid-sensitive peripheral systems. For example, the thymus gland undergoes significant atrophy under chronic stress, a pathology that is directly mediated by glucocorticoid secretion.

The net impact of chronic stress on the HPA axis varies along dimensions of predictability, intensity, and duration. Mild, predictable stress regimens (e.g., repeated short restraint in rats) cause significant HPA-axis habituation, reflected in decreased responses to stressors over time and minimal long-term change in HPA-axis endpoints (Figure 2). Habituation is most evident in paradigms that employ repeated exposure to the same stressor. In contrast, prolonged increases in HPA-axis output are most pronounced when stress exposure is unpredictable (e.g., regimens using random exposure to multiple stressors), persistent (e.g., social instability), or severe (e.g., chronic pain). Some extremely severe stress regimens (e.g., prolonged social stress) can even produce HPA-axis hyporesponsiveness, a phenomenon attributed to stress-induced adrenal exhaustion.

In most stress regimens, the capacity of the HPA axis to respond to a novel stress experience is maintained throughout periods of chronic stress. Thus, during chronic stress, the HPA-axis response to a new stimulus matches or exceeds that observed in controls that were not exposed to chronic stress (Figure 2). This phenomenon is referred to as HPA-axis facilitation, and usually occurs in the context of increased glucocorticoid secretion. Thus, chronic stress appears to inhibit glucocorticoid feedback inhibition of HPA-axis responses to previously unexperienced stressors. Chronic stress facilitation ensures that the organism can still mount an adaptive

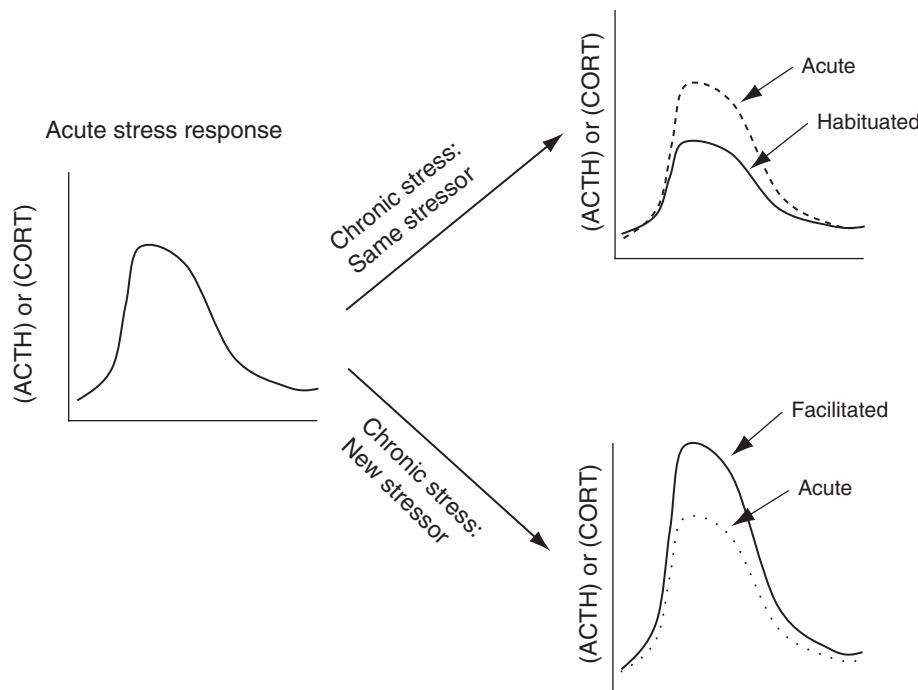


Figure 2 Habituation and facilitation of HPA responses after chronic stress. Typically, repeated exposure to the same stressor produces significant habituation, resulting in a progressive decrease in the HPA-axis response (ACTH or glucocorticoid (GC)) to the repeated stressors. When animals are exposed to chronic stress and given a new stressor, HPA-axis responses are typically augmented, consistent with response facilitation.

response to a novel threat, and is an important aspect of HPA-axis function.

Long-Lasting Effects of Chronic Stress

Chronic stress can affect HPA-axis function long after cessation of stressor exposure. Long-term effects of stress are most notable when exposure occurs in early life. For example, maternal deprivation causes persistent enhancement of HPA-axis (as well as behavioral) stress reactivity into adulthood. The profound impact of early stress is associated with remodeling of stress-regulatory brain circuits, consistent with glucocorticoid effects on neural development. One of the early-life effects of stress on HPA-axis regulation may be mediated by epigenetic modification of DNA. In rodents, good mothering is associated with alterations in DNA methylation in the promoter region of the glucocorticoid receptor, resulting in permanently elevated receptor expression, increased feedback signaling, and decreased HPA-axis stress responsiveness later in life.

In adults, long-term effects of chronic stress are more subtle, but remain physiologically relevant. For example, chronic social stress causes persistent increases in body adiposity, a consequence with implications for long-term health. Moreover, chronic unpredictable stress produces a late-emerging, long-lasting hypofunction of the HPA

axis, wherein responses to stressors are significantly blunted. The latter observation is of relevance to diseases that are accompanied by decreased HPA-axis activity (e.g., posttraumatic stress disorder).

Glucocorticoids negatively affect processes governing dendritic plasticity, neurogenesis, and neuronal cell death. Many of the brain regions subject to negative neuromodulatory effects of glucocorticoids (prefrontal cortex, hippocampus, and amygdala) regulate HPA-axis function. These data have led some to suggest that stress-induced neural impairments lead to further increases in glucocorticoid secretion, producing a feed-forward loss of HPA-axis control. Progressive loss of HPA-axis control is thought to contribute to age-related HPA-axis dysregulation and decreased neuronal viability.

Individual Differences, Stress and Health

Individual differences in HPA regulation are common. Genetic background, social status, and early background/experience contribute to the overall impact of stress on the HPA axis in both acute and chronic time domains. For example, colony housing of rats produces a dominance hierarchy, resulting in a single dominant rat and several subordinate animals. Despite housing under identical conditions, subordinates exhibit elevated HPA-axis function,

whereas dominants do not. Similarly, animals selected for low behavioral reactivity to novelty have decreased HPA-axis reactivity relative to high-responding counterparts. These individual differences likely affect the overall perception of acute or chronic stress severity, which then determines the net output of the HPA axis.

Stress is believed to be a major factor in diseases such as depression and posttraumatic stress disorder, both of which can be accompanied by HPA-axis dysfunction (hyper- and hyposecretion, respectively). However, only a subset of chronically stressed individuals develops these disorders, suggesting that genetic, social, and/or experiential determine individual vulnerability to HPA-axis dysfunction and mood disorders. Additional research efforts need to elucidate factors that are responsible for increased vulnerability in some individuals, and stress resistance and resilience in others.

Acknowledgments

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See also: Effects of Stress on Learning and Memory; Maternal Deprivation; Measuring Stress; Motor Learning in the Vestibulo-Ocular Reflex; Stress and Emotionality.

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Stress and Energy Homeostasis

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Glossary

Ghrelin – A 28-amino-acid peptide that is produced predominantly by gastrointestinal endocrine cells and is released during periods of negative energy balance resulting in increased feeding.

Homeostasis – It refers to a state of equilibrium within a system, or the tendency toward such a state among components of an internal system.

Hypothalamic-pituitary-adrenal (HPA) axis – Neuroendocrine system that responds to stress. The interactions among the hypothalamus, pituitary glands, and adrenal glands regulate a variety of physiological processes, including energy storage and expenditure, immunity, and reproduction.

Melanocortins – Posttranslational products that are derived from proopiomelanocortin prohormone, including adrenocorticotropic hormone (ACTH), α -melanocyte-stimulating hormone (α -MSH), and β -endorphin. Melanocortins are involved in the regulation of the stress response, energy balance, inflammation, learning and memory, thermoregulation, and analgesia.

Metabolic syndrome – It generally refers to a collection of metabolic abnormalities, including glucose intolerance (type 2 diabetes, impaired glucose tolerance, or impaired fasting glycemia), insulin resistance, central or abdominal obesity, dyslipidemia, and hypertension.

Neuropeptide Y (NPY) – A 36-amino-acid peptide neurotransmitter found in the brain and autonomic nervous system. NPY is secreted in the hypothalamus and is associated with physiological processes, including regulation of energy balance, learning and memory, and epilepsy. Its main effect is increased food intake and decreased physical activity.

The field of stress research has generated growing interest in response to the escalating number of psychopathologies associated with chronic stress in humans. One of the major physiological effects of stress is on food intake and body weight ultimately resulting in anorexia or obesity. A better understanding of the mechanisms through which chronic stress can lead to these pathologies will enhance the development, effectiveness, and efficiency of rational clinical therapies.

Stress, the HPA Axis and Body Weight

Stress activates the hypothalamic–pituitary–adrenal (HPA) axis and is associated with increased activity of a spectrum of physiological systems. When an organism is challenged, it reacts by initiating a stress response. Briefly, the stress response involves two main physiological systems, the sympathetic branch of the autonomic nervous system and the HPA axis, which mediates the effects of perceived threat or stress in the periphery. The sympathetic response responds rapidly and exerts immediate influence over a wide range of organ systems, including the cardiovascular, respiratory, gastrointestinal, renal, and endocrine systems. Details about the activation of the HPA axis is discussed elsewhere in this encyclopedia.

The secretions of the HPA axis are normally maintained within strict limits to circumvent problems, because chronic activation or dysregulation can be associated with the development of disease states in animals and humans. As an example, the effects of acute and chronic stimulation of the HPA axis on the cardiovascular system have been examined in various animal models of social stress. The main conclusion of those studies is that if the stressful stimulation is either severe and acute or sufficiently long and chronic (even if it is milder stimulation), the same consequences result. That is, intense acute stress can result in cardiac dysfunction leading to coronary artery disease, hypertension, and even death in previously healthy animals. In contrast, if the stimulation is milder but lasts for a longer period of time, similar pathophysiological consequences may ensue even after the stimulus is terminated. The same principle may be applied to other stress-related pathological states, such as obesity.

Humans face social stress to varying degrees on an almost daily basis. Interpersonal relationships are dependent upon the interactions among people and on an individual's position in society. Social stress derived from these interactions can be one of the most important sources of stress in human life and play a critical role in the development of stress-related disorders and disease. Human studies indicate that psychosocial and socioeconomic challenges are related to the development of hypertension, osteoporosis, and depression. In addition, increased cortisol levels have been associated with obesity-related conditions, including visceral obesity, insulin

resistance, dyslipidemia, and cardiovascular disease. A recent study of young healthy men exposed to long-term stress revealed that chronic stress results in increased abdominal obesity and early signs of metabolic syndrome, suggesting that stress plays an important role in the genesis of metabolic abnormalities. In that study, subjects lost both fat and lean body mass during the first stressful episode and subsequently regained body weight as fat resulting in an overall decrease in protein mass.

Although epidemiological studies document associations between stress and changes in feeding behavior, body weight, body composition, and overall energy homeostasis, the specific mechanisms through which these changes are produced remain unknown. Further complicating the matter are the conflicting reports of weight loss or weight gain in response to stress. The response to stress is complex and involves many known, and likely many unknown, players that together modulate feeding, weight regulation, and overall energy homeostasis. The type, duration, and intensity of the stressor as well as individual differences in responding also impact the behavioral and physiological effect of the stressful experience. Animal models provide a valuable means to study the effects of stress in a controlled environment and have produced more data that more strongly implicate a critical role of the HPA axis in the regulation of food intake and body weight. The remainder of this article focuses on information derived from animal models and the current state of our knowledge regarding stress and energy homeostasis.

Animal Models of Stress and Influences on Food Intake

The central and peripheral mechanisms that mediate the effects of stress on body weight and food intake are complex and are currently the topic of active research. Animal models facilitate exploration of the metabolic and feeding changes that occur with stress. It is clear that the stressor type, duration, and intensity all play a role in determining an animal's response to stress. In addition, the developmental window during which stress occurs influences the short- and long-term consequences. We first discuss stress occurring during critical developmental periods followed by a summary of adulthood stress and its effects on energy homeostasis.

Early Life Stress

Events during fetal life or during the early postnatal period and adolescence have significant effects on developing offspring. The development of the HPA axis is influenced by both prenatal and postnatal factors and can essentially program offspring for altered feeding

behavior and obesity as adults. Glucocorticoids are associated with the regulation of body weight, food intake, and blood glucose levels, and exposure to glucocorticoids during early development alters function of the HPA axis that can lead to later vulnerability to obesity or other eating disorders. Other neural circuits that regulate energy homeostasis are also sensitive during early development and can have adverse consequences for the offspring.

Prenatal stress

Maternal stress during pregnancy has significant effects on offspring and those effects depend on the type of stress used and timing of stress during gestation. Repeated restraint stress has been widely used in rodent models of prenatal stress, but the stress is usually applied in a predictable manner. Thus, the animal comes to predict the stressor and stress responses diminish over repeated trials. A model of chronic variable stress (CVS) involves several different stressors applied in a random order and at random times over several days. This is more akin to the unpredictable nature of stressors normally encountered by an organism, including humans and is the focus of the discussion in this section.

The influence of prenatal stress on offspring could depend on species as well as on the timing of the stress. For example, CVS in rats predisposes to hyperphagia, greater weight gain, and increased adiposity in adulthood, particularly when provided with high fat diet. Impaired glucose tolerance and abnormalities in structure and function of the cardiovascular system also result. These outcomes are usually observed when the stress occurs during the last week of gestation. However, in mice, prenatal stress administered during the first or third weeks of pregnancy appears to be critical time windows in rodents in affecting body weight, food intake and glucose tolerance in offspring. These studies indicate that there are differences in the timing of stress and the metabolic parameters that they affect. This is not surprising as each period during gestation involves development and maturation of different organ systems or neural pathways which may be more vulnerable during different time windows. Thus, the specific mechanisms that are responsible for the effects on body weight and food intake during different periods during gestation are unclear but may involve very different pathways.

The important aspect of studies of prenatal stress is how those specific maternal responses translate to the fetus and program metabolic outcomes. The mediators involved in metabolic programming or predisposition to altered energy homeostasis include among others glucocorticoids, insulin and leptin. These hormones can cross the fetal-placental barrier from maternal circulation to the fetus. Stress and excess maternal glucocorticoids may have an effect prenatally by programming increased

susceptibility to stress or altered energy homeostasis in adulthood. Placental 11β -hydroxysteroid dehydrogenase (11β -HSD) serves to buffer the developing fetus from excess glucocorticoids, others have found that while this is true in response to acute stress, the upregulation of 11β -HSD is insufficient during chronic stress. Future studies are required to explore the role of 11β -HSD in the phenotypes observed. Similarly, both leptin and insulin are trophic factors that act during the pre- and postnatal periods and can significantly disrupt normal development of neural systems important in the maintenance of energy homeostasis.

Early postnatal and adolescent stress

Maternal separation (MS) is a postnatal stress paradigm that disrupts the mother–pup interaction has long been used since Seymour Levine established it in the 1950s. Brief separation from mother is viewed as a psychological stressor, whereas longer separations are considered to add a physiological aspect due to the lengthy disruptions of maternal regulatory influence on the pup, such as body temperature maintenance, physical contact, and excretion processes. MS has differential effects dependent upon the duration of separation and the age at which stress is applied.

Longer periods of MS from 2 to 24 h during the first week of life attenuates body weight gain during development and decreases food intake. These parameters may be influenced by the alterations in mother–pup behavioral interactions and the reduction in maternal milk secretion due to decreased suckling stimulation from the pups. It is important to point out that MS can also produce physiological endocrine changes in the rodent pup that could impact development of the neural circuits that regulate body weight and food intake independently of the psychological aspects of MS stress itself. As an example, plasma leptin and glucose are reduced during MS while ghrelin increases. The postnatal leptin surge that occurs between postnatal weeks 1 and 2 has an important role in programming the development of hypothalamic feeding circuits such as the neuropeptide-Y (NPY) system and thus interruption of that surge by MS could program greater food intake and obesity in offspring.

In contrast, shorter periods of MS from 5 to 15 min during the first 2–3 weeks of life reduce HPA axis responsiveness to stressors in adulthood. Literature suggests that the long-term effect of short periods of MS attenuates the adult HPA axis response to stress by increasing maternal care to the pup when they are returned to the nest. Greater maternal care behavior is associated with decreased ACTH and corticosterone response to acute stress, increased hippocampal glucocorticoid expression, enhanced glucocorticoid feedback sensitivity, and decreased CRH expression. Since CRH is a well-known hormone that decreases food intake, lower amounts of

CRH in short-term MS pups would not suppress food intake to the same degree as a control rat, thus resulting in greater food intake and body weight gain.

Currently, it is not known whether exposure to early life stress in and of itself causes increased food intake and body weight gain. Stress during early development has also been associated with development of behaviors reminiscent of those observed in depressed, anxious or schizophrenic patients. These neuropsychiatric conditions themselves impart a high risk for obesity and metabolic disease in humans.

Social Stress in Adulthood

Social stress induces a greater hormonal and cardiovascular response compared to other models of stress, such as foot shock or restraint, and thus has greater face validity in modeling stressors experienced by humans. Chronic social subordination stress in rats and mice typically involve social hierarchies (e.g., visible burrow system or VBS) or social interactions between conspecifics resulting in dominant–subordinate relationships (resident-intruder or social defeat stress). Social subordinates typically exhibit submissive behaviors, show less reproductive behavior and endocrine function, and generally have higher plasma corticosterone levels, all parameters indicating that they experience a greater degree of stress in these paradigms.

Intermittent acute social stress increases plasma corticosterone and ACTH and raises resting body temperature. Body weight gain and food intake in the socially subordinate animals is attenuated. In contrast, with repeated episodes of social stress in a resident-intruder paradigm, food intake increases specifically during the light cycle. However, this increase in food intake *per se* is not sufficient to affect body weight.

It appears that social stress affects feeding behavior and body weight dependent on a multitude of factors, including the chronicity of stress as discussed above and post-stress housing environment. For example, stress has different effects on animals that maintain contact with conspecifics, whether it is constant group housing or maintaining sensory, but not physical, contact with dominant animals. Group housed rats form dominance hierarchies and can remain co-housed for up to 2 weeks. Socially subordinate rats in this situation lose significant amount of body weight. The changes in body mass are associated at least in part to decreased food intake. Analysis of meal patterns suggests that meals taken by subordinate rats are smaller and spread throughout the dark and into the light cycle. Thus, in addition to a significant impact on the amount of food consumed, chronic stress also disrupts regular meal patterns and feeding behavior that in turn contributes to altered energy homeostasis in socially stressed subordinate rats. In contrast, dominant rats maintain their body weight, but alter

their body composition by losing adipose tissue and increasing lean tissue. These observations suggest that social dominance, which is also associated with some degree of stress as determined by elevated glucocorticoid levels albeit significantly less than that of the subordinate, also has an impact on energy homeostasis.

Allowing social subordinates to recover from stress demonstrates that effects of stress on food intake suppression are persistent in some models while others report a rapid escalation in food intake to compensate for lost body weight. Hyperphagia during the poststress period concurrent with an endocrine environment of high glucocorticoids and low androgen levels favors deposition of fat rather than lean tissue. Furthermore, this condition results in subordinates having a higher proportion of adipose tissue concentrated in the visceral fat depot compared to controls and dominants after recovery. Visceral adiposity is a risk factor for the development of symptoms of metabolic syndrome such as cardiovascular disease and diabetes. Consistent with increased body fat, social subordinates become hyperinsulinemic and hyperleptinemic compared to dominants and controls after recovery. Together these data suggest that cumulative effects of chronic social stress may result in the development of symptoms related to the metabolic syndrome.

Stress effects on diet choices

Palatable food (i.e., foods that are calorically dense and have high amounts of carbohydrates or fat or both) consumption is increased following stress. Although intake of standard chow decreases such that total food intake remains the same, the proportion of calories derived from fat is increased and can lead to the development of visceral obesity. Indeed, increased sensitivity to stress in humans has been associated to obesity and binge-eating disorder. Evidence that stress-related factors participate in diet choices following stress come from, for example, CRH-R2-deficient mice which are more vulnerable to stress and have a higher preference for high-fat diet when exposed to chronic variable stress.

Subordinate rats exhibit anhedonic behavior that persists after social stress and do not increase their consumption of a carbohydrate solution even when in negative energy balance, although this behavior may be specific to the solution's macronutrient composition. However, subordinate behavior may change if they are presented with diets composed of different macronutrients to consume, such as high fat diet, or if they are provided with a choice of macronutrients. It has been suggested that individuals consume more palatable food during stress in an attempt to reduce the negative effects of a chronic stressor. It has been suggested that having a choice of which macronutrient to consume can play a significant role in macronutrient influences on the

negative effects of stress. These are intriguing considerations that warrant future study.

Potential Mediators

The connection between stress and energy mobilization for appropriate responses is well established. However, with sustained activation of the stress axis the very responses that are meant to be adaptive, such as increased energy consumption, can become maladaptive to produce pathophysiological consequences. Obesity is one consequence that results from greater energy consumption, decreased activity and leads to more severe disease conditions such as cardiovascular disease and diabetes. The regulation of stress and energy homeostasis share some of the same neuroendocrine pathways, thus suggesting important interactions among these two systems and may lead to better management of stress to avoid negative consequences.

CRH-containing neurons are located in the paraventricular nucleus of the hypothalamus, one of the major centers that control food intake and energy balance. Central (intracerebroventricular, ICV) administration of CRH decreases food intake in rats and has also been implicated in the anorectic effects of leptin. Thus, increased CRH during stress not only activates the HPA axis to initiate the stress response, it also functions to suppress food intake. CRH receptor-1 (CRHR1) and receptor-2 (CRHR2) are involved in the stress-induced inhibition of food intake in rodents. CRHR2 knockout mice exhibit greater nocturnal food intake and this is manifest through increases in meal size rather than meal frequency suggesting a deficit in satiating value of food in these mice. Thus, the CRH system is likely intimately involved in the stress-induced changes in food intake and meal patterns. Glucocorticoids, in contrast, generally exert an opposite effect on food intake. During the stress response it may be the case that CRH initially functions to inhibit food intake. However, glucocorticoid secretion may then be involved in stimulating food intake once its inhibitory effects suppress CRH release.

The central melanocortin system is well studied in its function in regulation of food intake and body weight. Evidence also suggests that the melanocortin system interacts with the stress system as well. This system is activated by acute restraint and forced swim stress, both considered to be emotional stressors of a processive nature. Alpha-melanocyte stimulating hormone (α -MSH), a cleavage product of proopiomelanocortin (POMC), is expressed in the arcuate nucleus (ARC) of the hypothalamus and inhibits food intake. Emotional stress activates POMC neurons in the ARC and coincides with expression of anxiety-like behavior arising from acute restraint or forced swim stress. ICV administration of SHU9119, a

Table 1 Summary of potential players that mediate the effects of stress on feeding behavior and energy homeostasis

	Influence of stress	Effect on food intake
Corticotropin-releasing hormone (CRH)	Increase	Decrease
Ghrelin	Increase	Increase
Glucocorticoids (corticosterone, cortisol)	Increase	Increase
Leptin	Decrease	Decrease
Neuropeptide Y (NPY) – peripheral	Increase	Increase
Orexin	Increase	Increase
Proopiomelanocortin (POMC)	Increase	Decrease

melanocortin receptor-3 and -4 antagonist, can block the activation as well as stress-induced anxiety-like behavior. Agouti-related peptide (AgRP) is also expressed in the ARC, but was not affected by stress. Thus, POMC activation may be a mechanism through which acute stress-induced anorexia could occur. This was an acute stress study and patterns of activation and behavioral responses to chronic stress remain to be explored.

Some of the systems that are well known to maintain energy homeostasis have been discussed above and are likely to be affected by social stress. However, other central and peripheral neural systems are likely involved and may have an even greater impact on observed changes in appetite, food preference, and body composition following psychosocial stress. The association between mood disorders and obesity is high and new research suggests that some of the same systems that are known to regulate food intake are also affected by psychosocial stress (**Table 1**).

Ghrelin is a hormone produced predominantly by gastrointestinal endocrine cells and is released during periods of negative energy balance resulting in increased feeding. Chronic social stress (resident-intruder stress) increases levels of acylated ghrelin (active form) which correlates with greater food intake and body weight in mice. Ghrelin receptor knock-out mice did not show the hyperphagic response when subjected to social stress, thus implicating ghrelin as one mediator of stress effects on feeding. Ghrelin receptor-deficient mice also exhibited greater depressive-like behaviors. The actions of ghrelin appear to be mediated, at least in part, by activation of orexin neurons in the lateral hypothalamus. Other areas that may potentially be involved include the ventral tegmental area and hippocampus, two areas that have been implicated in mood regulation. These data suggest that ghrelin has a secondary, possibly adaptive role, during stress that may help the animal cope with stress via anxiolytic- and antidepressant-like behavioral adaptations. However, this benefit comes with the caveat of increased food intake and body weight and, perhaps over the long-term, having adverse metabolic consequences.

The peripheral sympathetic NPY system has been implicated in the development of metabolic syndrome

following stress. Stressors of differing intensity and duration have varying effects on body weight and adipogenesis. For example, while physical restraint increased plasma norepinephrine levels and decreased appetite and body weight, cold swim stress is apparently mild enough that mice adapt to the cold water within a few days and exhibit no change in weight. However, intense cold and resident-intruder stressors produce a sustained level of stress to which the mice do not habituate. It is at this more intense level of stress that NPY levels increase. The dietary environment was also a determining factor in the metabolic response to stress. That is, stressed mice gained weight, primarily in the mesenteric fat depot, but only when fed a high fat, high sucrose diet. Furthermore, mice subjected to stress and fed high fat diet gained about 50% more than those mice that were only fed a high fat diet suggesting a stress-related potentiation of diet-induced obesity in this animal model.

Conclusion

Stress strongly influences feeding behavior resulting in long-term effects on body weight, body composition, and food selection. Nonhomeostatic effects of pathways initially implicated only in energy homeostasis have received more attention in mediating the effects of stress on depressive or anxiety-like behavior and food preferences. The range of effects of these pathways have opened up intriguing lines of research that may point to more effective clinical solutions to stress-associated obesity and anorexia.

See also: Control of Food Intake; Feeding; Regulation of the HPA Axis by Acute and Chronic Stress; Stress and Brain Morphology; Stress and Emotionality; Stress and Reward; Stress and Social Behavior.

Further Reading

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