CHAPTER

56

Learning and Memory

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OUTLINE

BRIEF HISTORY OF MEMORY RESEARCH IN HUMANS

The ability to learn and to remember is one of the most fundamental features of the brain. Understanding how learning and memory work is important because what we learn and what we remember determine, to a great extent, what we are and who we are. Memory, not merely facial and physical appearance, defines an individual, as everyone who has known someone with Alzheimer's disease understands all too well. Furthermore, the impact of learning and memory reaches far beyond the individual, and they form the very foundation for transmitting knowledge through generations, consequently serving as the major forces in driving cultural and social evolution.

By definition, learning is the acquisition of new information, whereas memory is the retention of acquired information. The concept of memory of mind has existed since the time of Aristotle. It is only during the past 50 years or so that scientists have begun to unravel some of the anatomical and cellular bases underlying such a complex mental process.

Most neuroscientists regard the ideas and observations of Santiago Ramón y Cajal at the end of the 19th century as the beginning of the cellular exploration of just how memory is retained in the brain. Upon his original observation of synaptic conjunction between neurons, he immediately entertained the idea that the modification of these conjunctions could form the anatomical basis responsible for the persistence of memory.

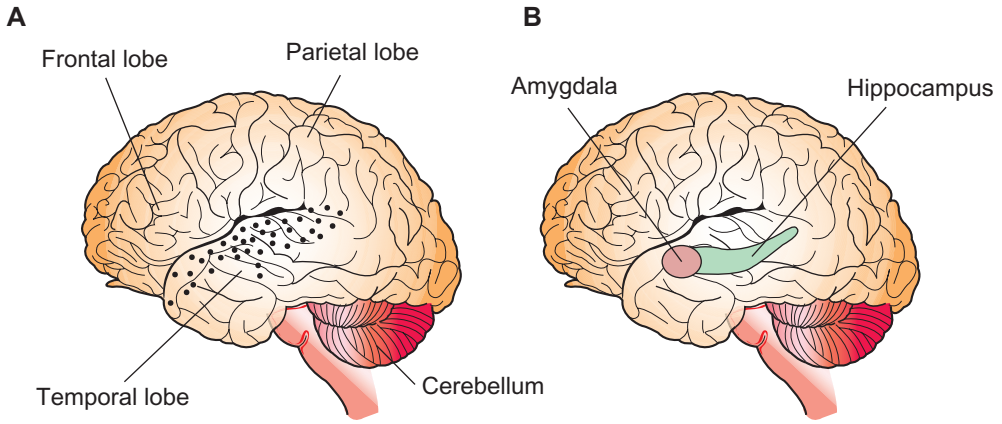


FIGURE 56-1 **Illustrative drawing of the temporal lobe system in the human brain. (A)** Anatomical sites, marked by black dots, within the temporal lobe where electrical stimulation evoked experiential responses in Penfield's patients. **(B)** The location of the hippocampus and amygdala inside of the temporal lobe.

The Penfield studies

So where should one look for such changes? The answer to this seemingly simple question lies at the core effort of modern neuroscience. Scientists must first find out where memories reside in the brain. A breakthrough was made in the mid-1950s by Wilder Penfield, who had the opportunity to stimulate the cortical surface of over a thousand of epileptic patients in the course of neurosurgery for removing epileptogenic tissue (Penfield & Jasper, 1954). Since the brain does not have pain receptors, the operation only required local anesthesia on the scalp, and thus the patients remained fully conscious during surgery. Some patients reported that they were hearing voices and music or seeing images and having other mental perceptions uniquely when electrical stimulation was delivered to a brain area known as the temporal lobe (Figure 56-1). Those electrical stimulation–triggered mental experiences often had a dream-like quality, which suggested that they reflected flashbacks to past experiences.

Further studies have shown that electrical activation of specific limbic structures within the temporal lobe system, such as the hippocampus and amygdala (Figure 56-1), are capable of generating experiential responses (Halgren et al., 1978). These fascinating reports were the first indications that the temporal lobe system may play a crucial role in representing memories and thoughts. In fact, we now know that memories are processed in many regions of the brain, far beyond the temporal lobe system (Fuster, 1994). The results of many studies suggest that memory is both distributed and localized. In other words, no single memory center exists in the brain, but rather memory is encoded along many pathways in the brain by a set of specific circuits.

Amnesia patients and the role of the temporal lobe in memory

Almost at the same period of Penfield's studies, Brenda Milner of the Montreal Neurological Institute examined a patient, known by his initials as H. M., who had undergone bilateral surgical removal of the temporal lobe (medial temporal cortex, amygdala and two-thirds of the hippocampus). The surgery was apparently a success in terms of relieving his severe epilepsy, but left him with a devastating loss of his ability to form memories (Scoville & Milner, 1957). For example, although H. M. recognized his childhood pictures and remembered well his childhood events, he had trouble recalling major personal and social events that had taken place a couple of years before his operation. This inability to remember things that happened several years preceding the surgery is called *retrograde amnesia*.

More strikingly, in H. M.'s case, the surgery also produced severe *anterograde amnesia*—the inability to form new memories about events, people and places he encountered after the operation. For example, H. M would not recognize Dr. Milner even though, following the surgery, she continued examining him frequently for more than 40 years. His anterograde amnesia was so severe that H. M. could not even recognize current photos of himself, despite the fact that he viewed himself in a mirror every morning.

Interestingly, H. M. preserved his ability to form short-term memory. For example, with rehearsal and as long as he was not interrupted, he could remember for several minutes a string of six-digit numbers. His intact short-term memory suggests that the temporal lobe is crucial for the conversion of new memory into long-term memory, a process termed *memory consolidation*. Even more amazingly, H. M. exhibited normal learning and memory for perceptual–motor tasks such as mirror drawing (learning to draw pictures by looking at his hand in a mirror) despite his inability to remember the repeated daily learning sessions. Thus, it seemed clear that H. M.'s ability to learn and retain new motor skills and procedural operations remained intact.

H. M. was the first human case in which specific amnesia could be linked to selective regions of the brain. Since then, many patients have been identified as having selective lesions to the temporal lobe system, especially within the hippocampus. They exhibited amnesias to H. M.'s. For example, *amnesic patient R.B,* who had a specific lesion in the CA1 region of hippocampus, showed profound loss of ability to form new memories of people, places, and events (Zola-Morgan et al.,

1986). R.B. also lost memories regarding public and personal events that he had experienced two years before his CA1 lesion. Such clinical observations have established the view that the hippocampus system, especially the CA1 region, is critically involved in memory processes.

DIVISIONS OF MEMORY

Declarative memory vs. procedural memory

Based on the types of memory selectively affected in those amnesic patients, memory can be divided into two major classes: declarative memory and procedural memory. *Declarative memory*, also termed *explicit memory*, is memory of events, places, facts, and people, and is dependent on the temporal lobe system. Retrieval of these memories requires conscious recollection. This type of memory tends to form easily and many specific details of memories can be easily forgotten.

Declarative memory can be divided further into two subclasses: episodic memory and semantic memory. *Episodic memory* refers to memory of episodic events that contain "what," "where" and "when" information. This is the major type of memory encoded in our daily life. *Semantic memory* refers to memory of facts and knowledge that are no longer ascribable to any particular occasion in life. (In other words, one cannot necessarily remember where and when one acquired this information.) Thus, semantic memory, created through either single or repeated experience, represents a more abstract generalization of experience that may give rise to concepts and categorization. Lesions in the temporal lobe, such as the hippocampus, are known to greatly impair patients' ability to learn new facts, concepts, vocabulary and knowledge about the world.

Procedural memory, also termed *non-declarative memory* or *implicit memory,* is the counterpart of declarative memory and encompasses a variety of perceptual–motor learning skills and mental operations. This type of memory does not depend on the structural integrity of the temporal lobe system. For example, H. M. showed good ability to reduce the time required for completing a 10-choice tactile maze after 80 trials despite the fact that he never remembered the correct sequence of turns during the maze test. Those amnesic patients with temporal lobe lesions retain other learning capacities such as eye-blink conditioning, habituation, sensitization, priming (the facilitation of performance by prior exposure to words) and mental operations such as how to put together a jigsaw puzzle and how to apply a numerical role. It should be mentioned that priming is a short-lived phenomenon, whereas learned motor skills (such as how to ride a bike) can endure for a long time. Currently, the brain regions involved in encoding procedural memory are not clear, at least in humans. In rodents, lesion studies suggest that the striatum may be engaged.

Short-term memory vs. long-term memory

The classification of memories into declarative memory and non-declarative memory is based on the anatomy of the temporal lobe system. However, memory is often classified temporally: *short-term memory* has a time course on the order of seconds to hours, whereas *long-term memory* has a time course of weeks, months, or years. The basis for temporal division of the memory process is revealed by observations that newly formed memories (short-term memories) are more vulnerable to interferences and disruptions. For example, if someone were involved in a car accident lapses into a coma from head trauma, after regaining consciousness he or she very likely will not remember the events leading up to and including the accident. Similarly, someone who receives electroconvulsive treatment will lose memories of events immediately preceding the convulsion. However, these same treatments have no effects on old memories that were generated long ago (long-term memories). These phenomena have led to the notion that memories are initially created in a much more labile status, and are gradually converted into a more stable state (*memory consolidation*).

MOLECULAR MECHANISMS OF LEARNING

Hebb's rule and experimental models for synaptic plasticity

How is memory formed and stored in those brain regions? Ramòn y Cajal was the first to hypothesize that modification of the physical structures of individual signaling units, i.e., synapses, may account for the cellular event of memory storage. In 1949, Canadian psychologist Donald O. Hebb came up with a simple yet profound idea to explain how memory is represented and stored in the brain. According to Hebb's postulate (Hebb, 1949),

When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.

These 49 words have formed what now is known as *Hebb's Learning Rule*. Although some minor modifications have been required over the years, the essence of Hebb's rule remains unchanged: a memory is produced by coincident neural activity; when two connected nerve cells are active simultaneously, the strength of their synaptic connection increases; this confers a basis for the persistence of memory.

In the 1970s, Timothy Bliss and Terji Lomo observed that the perforant-dentate gyrus pathway in the anesthetized rabbit hippocampus exhibits increased synaptic responses after a train of high-frequency stimulation. The increase in synaptic transmission, as often measured by either the amplitude or slope of the EPSP, can last for hours *in vitro* and days and weeks *in vivo*. This phenomenon is called *long-term potentiation* (LTP) (Bliss & Collingridge, 1993). Later studies by many scientists have shown that high-frequency stimulation of every pathway within the hippocampus, including the CA3–CA1 Schaffer-collateral pathway, can produce LTP (Figure 56-2). In fact, we now know that LTP exists in many animal species and in many brain regions including the neocortex, amygdala, and striatum. Moreover, LTP occurs in several distinct forms.

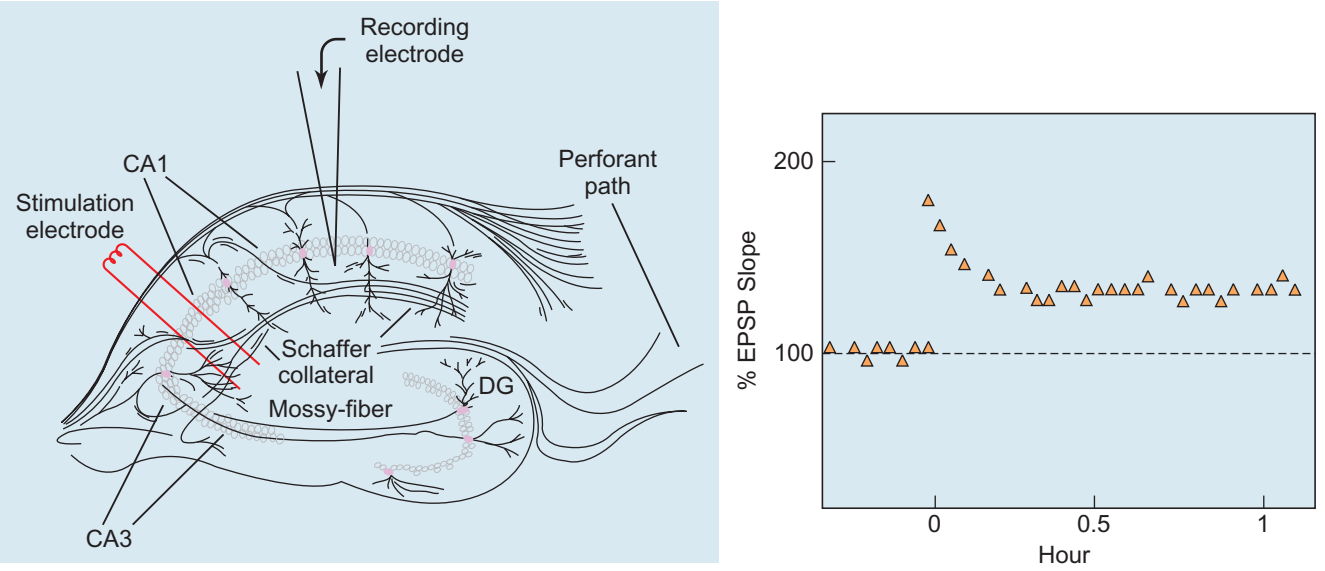


FIGURE 56-2 A drawing of the major pathways and synaptic plasticity of the hippocampus. The hippocampus is made of three major synaptic pathways (left panel). The first one is called the perforant pathway, and transfers information from the entorhinal cortex to the granule cells of the dentate gyrus. The second relay is from the dentate gyrus to the CA3 pyramidal cell, and it is called the mossy fiber pathway. The third pathway, from the CA3 cells to CA1 pyramidal cells, is termed the Schaffer collateral pathway. This pathway is one of the most studied systems *in vitro*. Long-term potentiation, as measured by increase in EPSP of CA1 cells in response to stimulation of the CA3 axon bundle, is induced by high-frequency stimulation in the Schaffer collateral pathway (right panel). This increased potentiation can last for several hours *in vitro* and days and weeks *in vivo*.

A series of investigations suggests that LTP generally possesses four basic characteristics: (1) temporal specificity, (2) cooperativity, (3) associativity and (4) input-specificity. *Temporal specificity* means that the strengthening of synaptic efficacy requires the presynaptic cell(s) to fire *before* the postsynaptic cell. This temporal requirement resembles the temporal feature of associative Pavlov conditioning in which the conditioned stimulus (i.e., bell-ring) must precede the unconditioned stimulus (e.g., food) if the conditioning is to be successful. *Cooperativity* refers to the fact that many synapses are required to produce enough depolarization to induce LTP. *Associativity* refers to the special scenario in which strong activation of one set of synapses can facilitate LTP induction at a set of recently and weakly activated adjacent synapses of the same postsynaptic cell. Finally, *input-specificity* of LTP means that potentiation is only induced at the synapses receiving stimulation, and not at unstimulated synapses on the same cell. This property is crucial since it guarantees the specificity of altered connections, and may also increase the capacity of individual neurons to process and store information.

The NMDA receptor and LTP induction

What molecular machinery controls these forms of synaptic plasticity? One of the most studied brain regions is the CA1 region of the hippocampus. The CA1 region is not only crucial for memory formation (profound amnesia in patient R.B with selective CA1 lesion), but also exhibits a well-organized laminar structure ideal for electrophysiological recording.

It is well established that the *induction mechanism of LTP* at the CA3-to-CA1 synapse of the Schaffer collateral pathway requires postsynaptic activation of the NMDA receptors. The NMDA receptor seems to be a perfect cellular device to detect the synaptic coincidence between presynaptic and postsynaptic neurons and to associate two events at the cellular level (Wigstrom & Gustafsson, 1985). The NMDA receptor is a ligand-gated channel protein that sits in the postsynaptic membrane (Figure 56-3). The electrical stimulation of a presynaptic neuron releases glutamate, which binds to the postsynaptic AMPA and NMDA receptors. Glutamate binding to the NMDA receptor alone is not sufficient to activate the channel because at the usual resting membrane potential the Mg^{2+} blocks the channel of the NMDA receptor, thereby preventing cations from flowing through the channel. The relief of the Mg^{2+} blockade comes when the postsynaptic site is sufficiently depolarized by the repetitive activations of the AMPA receptors, which cause Na^+ influx and the consequent EPSP. Thus, the opening of the NMDA receptors is both ligand-dependent (release and binding of glutamate) and voltage-dependent (the depolarization of post-synaptic neurons). Because the NMDA receptor is permeable not only to Na^+ and K^+ but also to Ca^{2+}, calcium influx into a dendritic spine triggers activation of protein kinases, which initiate a cascade of biochemical events (Figure 56-3). These biochemical cascades are believed to modify synaptic strength. Thus, the NMDA receptor is a gating switch for the induction of synaptic plasticity. See further details of NMDA receptors in Ch. 17.

The mechanistic properties of the NMDA receptor help account for the properties of temporal specificity, cooperativity, and associativity of LTP. They can also explain why

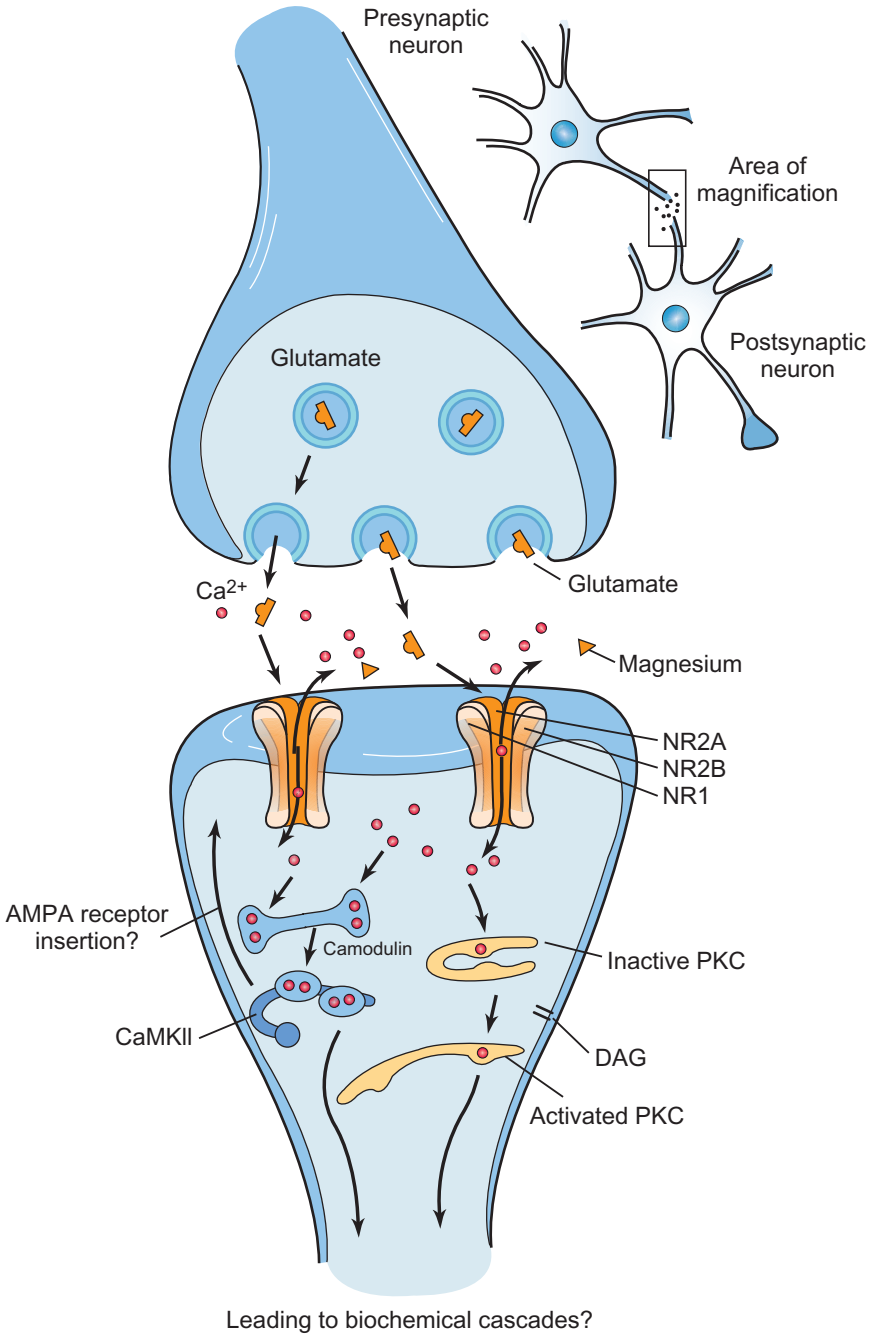


FIGURE 56-3 An illustration of a synapse between the presynaptic and postsynaptic neurons. The glutamate released from presynaptic terminal activates both AMPA and NMDA receptors. While the AMPA receptor is responsible for basal synaptic transmission, the NMDA receptor acts like a volume controller, regulating the efficacy of synaptic transmission. Synaptic transmission is enhanced if the NMDA receptor detects the co-activity of the presynaptic (release and binding of glutamate) and postsynaptic neuron (enough depolarization to expel Mg^{2+} from the channel pore). When such a coincidence event occurs, the NMDA receptor is activated, which opens the channel pore and allows Na^{+} and Ca^{2+} to rush in and K^{+} to rush out. The influx of Ca^{2+} then activates biochemical cascades that eventually strengthen the synapse. It is believed that some of these kinases bind directly to the C-terminus of the NR2B subunit, allowing efficient signal detection and amplification.

both high-frequency stimulation (100 Hz) and pairing low-frequency stimulation with postsynaptic depolarization can induce LTP. The occurrence of presynaptic activity followed by postsynaptic activity determines a temporal sequence and specificity. To generate sufficient depolarization in the postsynaptic cell to expel Mg^{2+} from NMDAR channels usually requires contemporaneous depolarization of many synapses. Moreover, the requirement of postsynaptic depolarization also underlies associativity since the depolarization caused by the strongly activated synapses can relieve the Mg^{2+} blockade of the NMDA receptors on weakly activated synapses.

Molecular mechanisms underlying the early- and late-phase expressions of LTP

While opening the NMDA receptors is crucial for coincidence detection, the sensitivity and robustness of coincidence detection is not solely determined by the opening time window. It is dependent on at least three other features: summation of the opening duration, the peak amplitude and proper intracellular signal transduction.

Among many signaling molecules involved in the relevant biochemical cascades, Ca^{2+}/calmodulin-dependent

protein kinase II (CaMKII) plays a key role in mediating the early-phase expression of LTP (Nicoll & Malenka, 1999). Ca^{2+} entry through NMDA receptors promotes binding of calcium/calmodulin to CaMKII, which causes physical translocation of α-CaMKII to the post-synaptic density zones (PSD) by binding to the C-terminus of the NMDA receptor NR2B subunits at synapses (Figure 56-3). Autophosphorylation at Thr^{286} of α-CaMKII further enhances the Ca^{2+}/CaM binding affinity with the CaMKII and prolongs the association of the CaMKII holoenzyme at PSDs. It is believed that the activated CaMKII at the PSD zone is responsible for potentiating synapses, probably by causing synaptic insertion of AMPA receptors and/or increasing their single-channel conductance. Several other kinases, such as PKC and MAP kinase, may also be involved in the expression of LTP. This phosphorylation-dependent modification of synaptic potentiation is believed to be capable of supporting LTP for one to three hours. This period is termed *the early phase of LTP*.

For maintaining synaptic potentiation beyond the initial three hours, protein kinase A (PKA) and ERK pathways may be involved. This is termed *late-phase LTP* and appears to require gene transcription and protein synthesis (Nguyen et al., 1994). While the nuclear transcription factor CREB (cAMP response element-binding protein) has been suggested to be important for turning on gene expression and for long-term memory (Kandel, 2001), recent experiments, including forebrain- or hippocampus-specific CREB knockout, failed to reveal any significant effect on hippocampal LTP or LTD (Perazzona et al., 2004) or contextual fear conditioning and spatial water maze memory (Balschun et al., 2003). Over the past decades, differential cloning techniques have successfully identified a set of immediate-early genes and effector genes. These genes include tissue plasminogen activator (tPA), Arc (also known as BAD1 or Arg 3.1), homer-1a (also known as Vesl-1S), BDNF, etc. (Qian et al., 1993; Bramham et al., 2008).

Since the transcription occurs in neuronal soma, the complex morphological specialization of the neuron and the large number of synapses mean that newly synthesized proteins have to be selectively targeted to these activated synapses without altering the function of all other synapses in the neuron. One hypothesis to deal with this issue is that synaptic plasticity may be partially mediated via local production of new proteins only at specific subsets of synapses or individual spines (Steward & Schuman, 2001). Another hypothesis is that the activated synapses may create some types of "synaptic tagging" signals by which the newly synthesized proteins can find their ways to the supposed sites (Frey & Morris, 1998). A significant amount of effort is currently directed identifying at "synaptic tagging" molecules. Emerging studies indicate that molecules such as CaMKII (Wang et al., 2008) and homer-1a (Vesl-1S) (Okada et al., 2009) may be part of this NMDA receptor–dependent reinforcement process (Wittenberg & Tsien, 2002).

Other forms of synaptic plasticity: Long-term depression (LTD) and NMDA receptor-independent LTP

In addition to its ability to produce LTP, a synapse also possesses the ability to decrease its synaptic efficacy. For instance, it has been shown that low-frequency (~1 Hz) stimulation of the hippocampal Schaffer-collateral pathway for 15 minutes produces decreased ESPS responses at CA3-CA1 synapses. This type of synaptic plasticity can last at least one hour, and is called *long-term depression* (LTD). Similar to the typical form of LTP at the CA1 region, the induction of LTD also requires NMDA receptor activation (Bear & Abraham, 1996). Pharmacological studies suggest that in contrast to the involvement of α-CaMKII in the expression of LTP, Ca^{2+}/calmodulin-dependent phosphatase plays a major role in the expression of LTD. It is interesting to note that although both LTP and LTD induction are dependent on the activation of the NMDA receptor and Ca^{2+} influx, experiments indicate that differential levels of Ca^{2+} influx distinguish which of these signaling pathways will be activated. High Ca^{2+} influx, produced by tetanic stimulation, leads to activation of CaMKII-mediated cascades, whereas lower Ca^{2+} elevation, produced by low-frequency stimulation, favors activation of protein phosphatase cascades.

It is now known that synapses in other regions can produce the NMDA receptor-independent LTD following the same low-frequency stimulation. This, in fact, was true of the first reported case of LTD: the original observation of synaptic weakening after stimulation was made by Masao Ito and his colleagues, who recorded in the cerebellum. In the cerebellar cortex Purkinje cells receive input from two major pathways, namely climbing fibers from the inferior olive nuclei of the brainstem and the parallel fiber of the cerebellar granule cells. Interestingly, a single climbing fiber makes hundreds of excitatory synapses on a Purkinje cell, whereas a single parallel fiber makes only one synapse (but a single Purkinje cell receives as many as 100,000 passing parallel fibers). Ito and his colleagues found that the parallel fiber-Purkinje synapses become weaker after the low-frequency pairing of climbing fiber stimulation with stimulation of the parallel fibers. Induction of this *cerebellar LTD* requires activation of a metabotropic glutamate receptor (mGluR), which is coupled to a G protein that activates phospholipase C. The activation of this enzyme leads to the production of a second messenger, diacylglycerol (DAG), which in turn activates protein kinase C.

Like NMDA receptor–independent LTD, at least two types of LTP can occur independently of NMDA receptor activation. First, in the CA1 Schaffer-collateral pathway and some pathways in the visual cortex, extremely high-frequency stimulation (200–250 Hz) can lead to slow development, over 20–30 minutes after stimulation, of synaptic potentiation that is dependent on the activation of voltage-gated calcium channels (VGCC) (Teyler et al., 1994). Thus, this form of plasticity is also known as *the voltage-gated calcium channel–dependent LTP*, or VGCC-LTP. Another interesting feature of VGCC-LTP is that in contrast to the synaptic localization of the NMDA receptors, voltage-gated calcium channels are situated at the base, or around the base, of dendritic spines. This suggests that this type of LTP may be able to spread to nearby synapses.

A second type of NMDA receptor-independent LTP exists in the mossy-fiber pathway at the dentate granule cell-to-CA3 pyramidal cell synapse (Zalutsky & Nicoll, 1990). This form of LTP, termed *mossy fiber-CA3 LTP*, is believed to involve PKA activation in the presynaptic cell, which leads to increased neurotransmitter release. However, the exact induction mechanism is not yet clear.

Doogie mice: a smart way to validate Hebb's rule for learning and memory

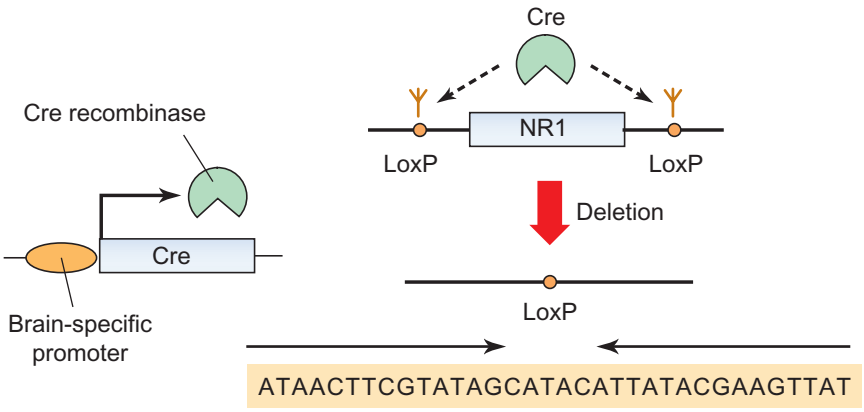
As the unique receptor in the brain with the coincidence-detection property, the NMDA receptor seems to be an ideal candidate to gate the formation of memory at the synaptic level. Early observations reported that infusion of NMDA receptor blockers into brain ventricles resulted in animals' poor performance in the hidden-platform water maze. Unfortunately, such drugs typically come with significant side effects, such as sensorimotor disturbances, which greatly complicate the data interpretation.

While gene knockout provides a powerful method to study the role of a gene *in vivo*, deletion of the NMDAR 1 (NR1), which encodes the core subunit of the NMDA receptor, results in neonatal lethality. In the mid-1990s, Joe Tsien and colleagues developed *conditional gene knockout technology* (Tsien et al., 1996) and knocked out the NR1 gene selectively in excitatory pyramidal neurons of the CA1 region (Tsien et al., 1996) (Figure 56-4). These CA1-specific NMDA receptor knockout mice indeed lacked NMDA receptor-dependent forms of LTP and LTD in the CA1 region, and these mice exhibited significant deficits in a variety of spatial and nonspatial memory tasks (Tsien et al., 1996; Rampon et al., 2000).

While the CA1-specific NMDA receptor knockout experiments support the role of the NMDA receptors in memory, they are less than fully conclusive in linking the synaptic coincidence-detection feature of the NMDA receptor to memory formation. In the theory, CA1-specific gene knockout experiments could have produced memory impairment via a mechanism independent of the coincidence-detection function of the NMDA receptor. For example, one may imagine that the physical absence of the NMDA receptor channels may cause subtle structural reconfiguration at the synapse, thereby altering normal synaptic plasticity.

To fully demonstrate the NMDA receptor's unique property for learning and memory, scientists devised an unconventional experiment: They enhanced its coincidence-detection feature to see if this would lead to enhanced memory. To achieve this feat, researchers manipulated the other subunits of the NMDA receptor, which is composed of tetrameric complexes consisting of the NR1 subunit and NR2 (NR2A, 2B, 2, and NR2D) or NR3 subunits. In the cortex and hippocampus, NR2A and NR2B are the main subunits forming the NMDA receptor channels, and both of them impart strong Mg^{2+} dependency, a feature ideal for coincidence detection. In comparison, NR2C and NR2D, which exhibit greatly reduced Mg^{2+} dependency (and are thereby less suitable for coincidence function), are mainly expressed in the cerebellar granule cells and interneurons in the midbrain regions, respectively (Monyer et al., 1994).

There are crucial differences in NR2A vs. NR2B: NR2B prolongs the duration of channel opening, whereas NR2A



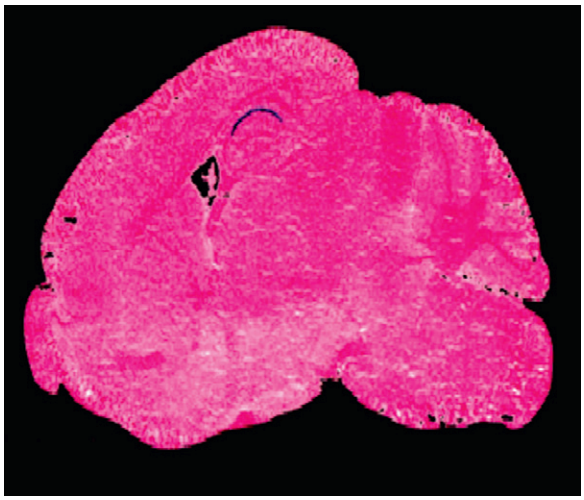


FIGURE 56-4 The strategy for achieving brain region–specific gene knockout. This second generation of genetic technique employs a trick used by bacteriophages to infect host cells: the *Cre/loxP* recombination system. The Cre recombinase acts like a DNA scissor that cuts specifically at the loxP sites. By expressing the *Cre* gene on in a specific region of the brain, any gene flanked by loxP sites, which are inserted by embryonic cell homologous recombination, can be deleted. The bottom picture illustrates the efficient deletion restricted to the CA1 region of the mouse hippocampus.

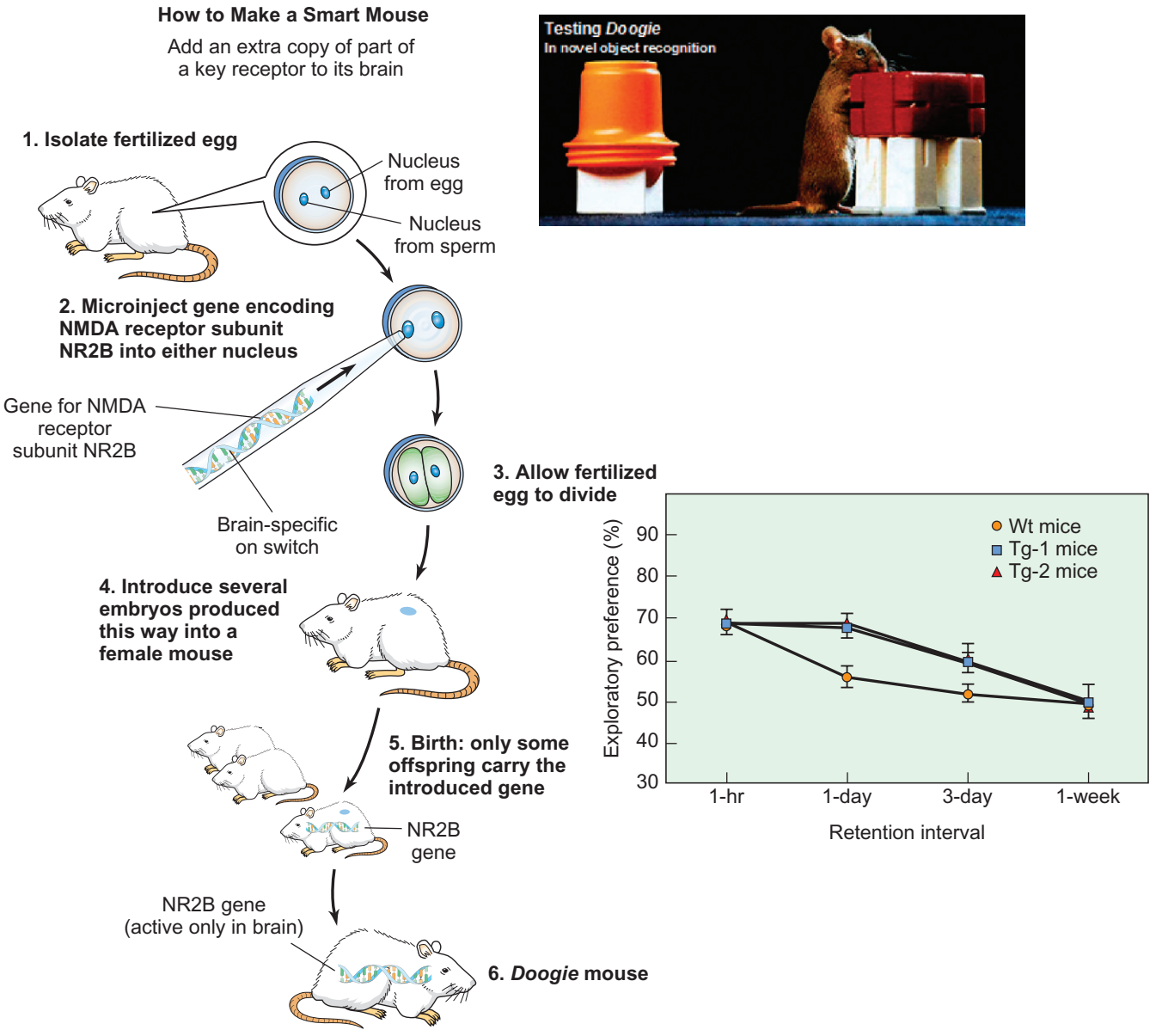


FIGURE 56-5 **The procedure to make "smart mice" through transgenic microinjection technique.** A genetically engineered smart mouse is performing the novel object recognition task. This task allows researchers to measure the amount of time the animal spent on exploring either the old toy (the orange one on the left) or the new one (the red one on the right). If the mouse remembers the old toy, he tends to spend more time playing with the new one. The graph shows the transgenic NR2B mice are capable of remembering for at least three days, whereas the wild-type littermates retain the memory for only one day. This task assesses only one type of memory. In other tests, the transgenic mice also showed greater learning ability. (Modified from Tsien, ZJ, *Scientific American*, 282: 62–68, 2000.)

shortens it (Monyer et al., 1994). Moreover, NR2B expression is higher in the postnatal developing and immature brain and becomes highly restricted to the cortex and hippocampus in the adult brain, whereas NR2A is lower in the postnatal developing brain but becomes the predominant form when animals enter adulthood. In species ranging from birds to rodents to primates, the NMDA receptor channel almost invariably stays open longer in young brains than in adult or aged brains. These age-dependent physiological changes are accompanied by changes in the molecular composition of NMDA receptors. Recent studies suggest that this change in the NR2b-to-NR2A ratio may be regulated by casein kinase 2 and histone methylation by Setdb1 histone methyltransferase (Sanz-Clemente et al., 2010; Jiang et al., 2010).

In the mid-1990s, Joe Tsien postulated that the natural switch with age of NR2B to NR2A could explain why the NMDA receptor in adult brain has a much narrower window of time for cellular association to occur, and it might explain why adult animals find it harder to learn and register new information. Therefore, Tsien and his colleagues devised a genetic experiment in which they produce forebrain-specific transgenic NR2B mice, nicknamed *Doogie* mice (Tsien 2000; Tang et al., 1999) (Figure 56-5).

As a result of making more NR2B as a partner for NR1 in the adult cortex and hippocampus in the *Doogie* mice, these NMDA channels in the transgenic brain retain some juvenile receptor properties, which make them better at detecting synaptic coincidence and more easily forming stronger synaptic connections between coactivated cells. *Doogie* mice exhibit a broad range of learning and memory enhancement (at least in seven different memory tests including novel object recognition memory, contextual and cue fear memory, fear extinction learning, spatial maze, spatial working memory, etc.) (Figure 56-5). Several follow-up studies not only confirmed the original findings, but also showed that *Doogie* mice continue to outperform age-matched controls even at advanced ages, indicating that long-term expression of NR2B is beneficial to brain's cognition during the aging process too. Therefore, the creation of *Doogie* mice has not only identified the NR2B as a key subunit of the NMDA receptor, but has also clearly validated Hebb's coincidence-detection rule for learning and memory. The latest experiments have revealed that a variety of other means that can increase NR2B expression in the brain, such as increasing the transport of NR2B to synapses or slowing down the degradation of NR2B at synapses, have consistently enhanced memory function. These concerted efforts may also lead to potential new ways for novel therapeutic intervention of brain aging such as Alzheimer's disease or mild cognition impairment (see Box 56-1).

MOLECULAR MECHANISMS OF MEMORY CONSOLIDATION AND STORAGE

Retrograde amnesia and post-learning consolidation by the hippocampus

Researchers have sought to understand the biological mechanisms underlying the formation of long-term memory since Muller & Pilzecker at the turn of the 20th century (Muller & Pilzecker 1900). The hippocampus is involved in the conversion of short-term memory into long-lasting memory even long after learning has occurred. This post-learning process is termed *memory consolidation*. Memory consolidation requires the hippocampus to continue its engagement for an additional period of time after learning (Kim & Fanselow 1992). Those observations have led to the general notion that the memory process is not an instant and unitary one, but rather a gradual and continuous process that can be divided into four distinct temporal stages: acquisition (learning), consolidation, storage and retrieval.

It is widely accepted that long-term memory may be ultimately stored in the form of changes in synaptic structure. The initial hypothesis, termed the *single-cascade hypothesis,* is that such structural changes result from a learning-triggered molecular cascade, (Kandel, 2001). However, emerging evidence suggests that the single-cascade hypothesis is insufficient to account for the formation and consolidation of long-term memory in the mammalian brain. Rather, memory consolidation is a continuous process and dependent on multiple rounds of post-learning NMDAR reactivations so that those synaptic changes (or synaptic memory traces) created by initial learning can be repeatedly reinforced offline through repeated post-learning reinforcement of synaptic modifications, termed synaptic reentry reinforcement (SRR) (Wittenberg & Tsien, 2002). It can account for the time scales of memory consolidation as well as deal with the destabilizing effects caused by metabolic turnovers of synaptic proteins (e.g., synaptic receptors and associated proteins can turn over in days). SRR-mediated offline strengthening or stabilizing of synaptic connections, either during a wakeful state or during sleep, requires only pair-wise reactivation between coherently coactivated neurons. However, the cellular reactivation producing SRR during subconscious consolidation is not the same as the conscious reactivation of memory traces, in the sense that it does not require sequential activation of coding assemblies at the circuitry level. In theory, consolidating synapses only require pair-wise coreactivation between the connected neurons to maintain their existing synaptic efficacy.

The most recent evidence shows that a similar SRR process also seems to be required for long-term storage of remote memories in the brain (Wang et al., 2006). It is further postulated that sleep may serve a major process in which SRR can occur. In this sense, sleep is not merely for memory consolidation as proposed in the literature, but rather plays a much more fundamental role in creating systematic conditions for initiating SRR to dynamically preserve the entire brain's circuit stability.

NEURAL POPULATION-LEVEL MEMORY TRACES AND THEIR ORGANIZING PRINCIPLES

In search of memory's neural code

One fundamental goal of neuroscience is to understand the organizing principles and the neural network mechanisms by which the brain encodes and processes information in real time. Although valuable information can be obtained either by using EEG or field recording to map global brain responses or by recording the activity of one or few neurons at a time, neither approach provides a direct means to investigate the network encoding mechanisms underlying information processing. In EEG or field recording experiments, one can only study the summed neural responses across one or multiple areas. In single neuron studies, recorded activity of single neurons typically needs to be averaged over many trials or even using different animals in order to overcome its firing variability and to identify its event-related responses and encoding properties. However, the brain is unlikely to accomplish its processing through many repetitions in order to seek out statistically meaningful results.

To explain how the brain might achieve its neural coding, Hebb proposed (1949) that information processing in the brain may involve the coordinated activity of large numbers of neurons, or cell assemblies. This notion, although rather vague, makes good sense both from the computational and cellular perspective. However, little is known regarding the actual organizing principles and network architecture at the population level. Therefore, the major challenge to date has been to identify the actual patterns of activities of a large

TARGETING NR2B FOR MEMORY IMPROVEMENT

Joe Z. Tsien

The NMDA receptor is the central molecular device for controlling synaptic plasticity and memory function, and so understanding the control and action of the NMDA receptor at central synapses may provide clues to therapeutic strategies for treating memory disorders (Li & Tsien, 2009).

The creation of *Doogie* mice has demonstrated that it is possible to manipulate a single NMDA receptor subunit for a broad range of learning and memory enhancement (Tang et al., 1999; Tsien, 2000; Tang et al., 2001; White & Youngentob, 2004; Cao et al., 2007). Since then, scientists have further generated NR2B transgenic rats, nicknamed Hobbie-J, which also exhibited larger LTP and similar enhancement in learning and memory (Wang et al., 2009). This cross-species validation adds to the notion that NR2B may act as a universal key switch for gating memory enhancement in various mammalian brains. On the other hand, conditional knockout of NR2B in the mouse forebrain or hippocampus results in decreased NMDA receptor–mediated charge transfer, reduced cellular LTP, and impaired spatial performance (von Engelhardt et al., 2008).

Proteomics analysis suggests that the core NMDA receptor tetramer associates with a multiprotein complex that includes more than 70 associated proteins, many of which influence trafficking, stability, subunit composition, or function of NMDARs (Husi et al., 2000; Sanz-Clemente et al., 2010). Studies so far have shown that facilitating transport of NR2B to synapses or slowing down the degradation of NR2B at synapses can also be a quite effective means to elevate synaptic NR2B levels and subsequently improve memory function. For example, transgenic mice with the overexpression of KIF-17, a kinesin motor protein involved in transporting NR2B protein from soma to dendrites, showed a higher NR2B expression at synapses, and these mice possessed superior memory (Wong et al., 2002). Another study reports a significant role for tissue plasminogen activator (tPA) in regulating NR2B trafficking and NMDA receptor complex stability in the hippocampus (Norris & Strickland, 2007). Transgenic mice overexpressing tPA also had better performances in spatial orientation learning tasks (Madani et al., 1999).

In addition, recent studies suggest that the degradation of NMDA receptors is regulated by the Ca^{2+}-dependent protease calpain by rapidly cleaving NMDAR subunits and resulting in a decrease in the number of functional NMDA receptors in the postsynaptic density (Simpkins et al. 2003). This calpain-dependent proteolysis of NR2B is regulated by cyclin-dependent kinase 5 (Cdk5) (Hawasli et al., 2007). A recent study reports that conditional knockout of Cdk5 in the adult mouse brain reduces NR2B degradation, which causes elevation in total surface and synaptic NR2B subunit levels and stronger LTP. These Cdk5 knockout mice also showed better contextual fear conditioning memory, faster fear extinction, and more flexible learning in the reversal water maze task (Hawasli et al., 2007). It is also noteworthy that silencing Cdk 5, a major kinase associated with tau hyperphosphorylation in Alzheimer's disease (AD), has been reported to reduce neurofibrillary tangles in transgenic Alzheimer's mice (Piedrahita et al., 2010).

Interestingly, another transgenic mouse that overexpresses tau-tubulin kinase-1 (TTBK1), another kinase for tau, had increased tau phosphorylation, a higher level of p25 and p35 (both are Cdk5 activators), enhanced calpain I activity, and reduced levels of hippocampal NR2B subunit (Sato et al., 2008). Therefore, it seems that NR2B is also a target for AD-associated changes via calpain, Cdk 5 and tau pathways. On this note, a recent study provided a suggestive association between a polymorphism in the NR2B promoter region, reduced NR2B expression levels and increased risk of Alzheimer's disease (Jiang & Jia, 2009).

More recently, researchers identified another synaptic transmembrane protein associated with NMDAR protein, Neto1. Neto1 is an interesting molecule because its intracellular domain binds a PSD-95 that is known to directly interact with NMDAR, and its extracellular domain interacts with NR2A and NR2B (Ng et al., 2009). Neto1 knockout mice had diminished synaptic NR2A (but not NR2B) in the hippocampus. Interestingly, administering the ampakine CX546, an AMPA receptor agonist, leads to secondary increase of NMDA currents by relieving the Mg^{2+} blockade of the NR2B-containing NMDARs, subsequently rescuing both LTP deficits and spatial learning deficits in the mutant mice. This was the first report of a pharmacological rescue of inherited plasticity defects and restoration of memory functions by pharmacologically enhancing NR2B-containing NMDA receptor.

Other researchers are actively exploring additional strategies to boost NR2B-containing NMDA receptor functions, such as by transcriptional modification of NR2B/NR2A ratio (Jian et al., 2010) or via optimizing a proper Mg^{2+} in the CSF by supplemental diet (Slutsky et al., 2010). The latter approach can be interesting since the majority of American adults consume less than the estimated average requirement of magnesium, a deficiency in which may have a detrimental effect on memory function.

All together, the above several examples represent current ongoing translational efforts that may one day provide a much-needed solution for treating AD and memory impairments. However, because memory processing is vastly more complicated in the human brain and memory disorders often have diverse causes, much work and many challenges may lie ahead.

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neuronal population during cognition, and then to extract the network-level organizing mechanisms that enable the brain to achieve its real-time encoding, processing and execution of cognitive information.

The neural code is the set of rules and syntax that transform electrical impulses emitted by the neurons into perceptions, memories, knowledge, decisions, and actions. Neuroscientists try to decipher the brain's neural codes by searching for reliable correlation between firing patterns of neurons and behavioral functions (Abbott & Sejnowski, 1999; Sanger, 2003). As early as 1920s, Edgar Adrian in his pioneering recording showed that the firing rate of a frog muscle's stretch receptor increases as a function of the weights on the muscle (Adrian, 1926), suggesting that information is conveyed by specific firing patterns of neurons. Two leading neural coding theories can be found in the literature: namely a '*rate code*' and a '*temporal code*'. In the *rate code*, all the information is conveyed in the changes of the firing of the neuron. In the *temporal code*, information is also conveyed in the precise inter-spike intervals.

Changes in discharge frequency of neurons upon learning or electrical stimulation are well known. Some of the earliest experiments came from *in vivo* recordings made by Richard Thompson in the hippocampus using classical eye-blink conditioning (Thompson, 2005) or during the investigation by John O'Keefe of the place cells (O'Keefe & Dostrovsky, 1971). Place cells in the hippocampus show "location-specific" firing when an animal navigates through familiar environments, indicating that these cells may be involved in encoding spatial navigation or self-location of the animals (O'Keefe & Dostrovsky, 1971).

Over the course of investigations many neurophysiologists came to realize that response variability at the single neuron level, even to an identical stimulus, is a prevalent phenomenon. In another word, at any given trial, a neuron may or may not change its firing upon the stimulus presentation, which in turn makes it difficult for researchers to use the firing changes of the neuron to predict the stimulus identity (Bialek & Rieke, 1992; Fenton & Muller, 1998).

The traditional way to deal with the response variability of single neurons is to average spike discharge of the neurons over repeated trials (Eggermont, 1998). The data averaging across trials permits the identification of tuning properties of the individual neurons, but unfortunately this practice invariably loses crucial information regarding real-time encoding process in the brain. Therefore, the major challenge is to elucidate what real-time memory traces are in the brain.

Visualizing network-level real-time memory traces

To examine the real-time encoding mechanisms underlying the network-level representation of memories in the brain, researchers have recently developed a large-scale ensemble recording technique in mice, capable of recording activities of several hundreds of individual neurons simultaneously. They used a 128-electrode array, which was capable of simultaneously monitoring the conversations of 200 to 300 neurons as mice learned the trace fear conditioning (to associate a neutral tone with a mild foot shock 20 seconds later). A computational algorithm, based on multiple discriminant analysis (MDA) (a statistical pattern classification method), translated the neuronal chatter into a discernable and dynamic activity pattern that provided scientists a trace or picture of what the memory looked like as it was formed and recalled. Scientists successfully identified a variety of distinct memory traces, in forms of transient neural ensemble dynamics, including memory traces for conditioned tone, foot shock and the time interval between the tone and foot shock (Chen et al., 2009). Interestingly, these memory traces tend to reverberate or replay during the post-training periods. Moreover, the number of real-time memory traces detected during post-learning consolidation and recall correlated tightly with the mice's performance scores, such as freezing upon hearing the tone or when they were returned to the chamber where the foot shock occurred. One amazing feature is that during recall, various memory traces reappeared in tandem, at a rate of 6 to 14 times per minute in the CA1 region (Figure 56-6). This ability to provide exquisite descriptions of various real-time memory patterns in the brain could have far reaching implications for advanced brain-machine-interface applications, disease model diagnoses and drug validation.

Identification of neural cliques as real-time memory coding units

Our understanding of neural representation of memories in the brain requires not only the ability to decode real-time memory traces, but also the elucidation of the organizing principles at the neuronal population level. Since the brain is well known to produce vivid and long-lasting memories about emotional events such as devastating earthquakes, high-speed roller coaster rides, or attacks by predators, the researchers have designed similar versions of these fearful episodes for mice, such as laboratory-version earthquakes caused by unexpectedly shaking the mouse cage, or a sudden blast of air to a mouse's back (mimicking an owl attack from sky), or a brief vertical free fall inside a plunging small elevator (simulating the precipitous drop of a cookie jar holding the mouse) (Lin et al., 2005) (Figure 56-7). Large-scale recording and decoding analyses reveal that various fearful events form distinct CA1 ensemble encoding patterns. Further analyses reveal that the encoding power at the population level is actually derived from a set of network-level functional coding units termed *neural cliques*—groups of neurons in the CA1 cell population with similar response property and selectivity (Lin et al., 2006) (Figure 56-8). For example, a *general startle neural clique* consists of individual cells capable of responding to all types of startling stimuli including the elevator drop, earthquake, and air blast, whereas the *subgeneral startle cliques* are neural groups that respond to a combination of two types of, but not all, startling events. In addition, there are neuron groups that exhibit high specificity towards one specific type of fearful events, such as elevator drops (*the drop-specific neural clique*), earthquakes (the *earthquake-specific neural clique*), or sudden air-blow events (the *air-puff–specific neural clique*).

One crucial feature of neural cliques is that the individual neurons belonging to a given clique exhibit "collective co-spiking" temporal dynamics (Figure 56-9). The collective co-spiking dynamics among neural clique members enable the memory-coding units to achieve real-time network-level encoding robustness by overcoming the response variability of individual neurons (Figure 56-9). Moreover, based on the temporal dynamics, neurons within each clique can be further subgrouped into the four major subtypes: (1) *transient increase*, (2) *prolonged increase*, (3) *transient decrease* and (4) *prolonged decrease*. The existence of four types of neurons can greatly enhance the real-time encoding robustness as well as provide potential means for modifying clique membership via synaptic plasticity. Finally, neural cliques, as network-level functional coding units, should also be less vulnerable to the death of one or a few neurons, and therefore exhibit graceful degradation should such conditions arise during the aging process or disease states.

General-to-specific feature-encoding neural clique assemblies

Through examining the overall organization of neural clique assembly involved in startle memory encoding, it is clear that the internal CA1 representations of any given startle episode involves a combinatorial set of neural cliques, invariantly consisting of the *general startle clique*, *subgeneral startle clique, startle identity–specific clique, and context-specific startle clique* (Lin et al., 2006) (Figure 56-8). Thus, each clique assembly is organized in a categorical hierarchy manner and invariantly consists of a *feature-encoding pyramid* (Figure 56-8) that starts with the neural clique representing the most general features (common to all categories) at the bottom layer, followed by neural cliques responding to less-general features (covering multiple, but not all, common categories), then moving gradually up towards more and more specific and discriminating features (responding to a specific category), and eventually culminating in the most discriminating feature clique (corresponding to context specificity) on the top of the feature-encoding pyramid.

According to this hierarchical structure of network-level memory encoding (Figure 56-8), the *general startle neural clique* represents the neurons engaged in the extraction of the common features among various episodes (e.g., encoding abstract and generalized knowledge that "such events are scary and dangerous" by integrating neural inputs from the amygdala). The *subgeneral neural cliques* are involved in identifying subcommon features across a subset of startling episodes (e.g., perhaps, the *earthquake and drop–specific clique* for encoding the semantic memory of the fact that "those events involve shaking and motion disturbances" by integrating inputs from the vestibular system), whereas the *startle identity–specific cliques* encode discriminative information about startle types (defining "what type" of events has happened) and the *startle context-specific cliques* provide even more specific features, such as contextual information about "where" a particular startling event happened (Lin et al., 2006; Tsien, 2007).

This invariant *feature-encoding pyramid* of neural clique assemblies reveals four basic principles for the organization of memory encoding in the brain (Figure 56-8). First, the neural networks in the memory systems employ a categorical and hierarchical architecture in organizing memory coding units. Second, the internal representation of external events in the brain through such a feature-encoding pyramid is achieved not by recording exact details of the external event, but rather by re creating its own selective pictures based on the importance for survival and adaptation. Third, the "feature-encoding pyramid" structure provides a network mechanism, through a combinatorial and self-organizing process, for creating seemingly unlimited numbers of unique internal patterns capable of dealing with potentially infinite numbers of behavioral episodes that an animal or human may encounter during its life.

Forth, in addition to its vast memory storage capacity, these neural clique–based hierarchical extraction and parallel binding processes also enable the brain to achieve abstraction and generalization, cognitive functions essential for dealing with complex, ever-changing situations.

The finding that the memory-encoding neural clique assembly appears to invariantly contain the coding units for processing the abstract and generalized information is interesting. It fits well with the anatomical evidence that (1) virtually all of the sensory input that the hippocampus receives arises from higher-order multimodal cortical regions and (2) the hippocampus has a high degree of subregional divergence and convergence at each loop. This unique anatomical layout supports the notion that whatever processing is achieved by the hippocampus in the service of long-term memory formation should have already engaged with fairly abstract, generalized representations of events, people, facts and knowledge.

Concept cells in the hippocampus: nest cells and Halle Berry cells

In fact, recent studies in both rodents and humans have confirmed the existence of concept cells in the hippocampus. In the mouse hippocampus, researchers have discovered a small number of hippocampal neurons that appear to respond to the abstract concept of "nest." These cells react vigorously to all types of nests, regardless of whether they are round or square or triangular or made of cotton or plastic or wood (Lin et al., 2007) (Figure 56-9). Place a piece of glass over the nest so the animals can see it but can no longer climb in, and the nest cells cease to react. Thus, these cells are responding not only to the specific physical features of the nest—its appearance or shape or material—but to its functionality: a nest is some place to curl up to sleep.

In human epileptic patients requiring electrophysiologic studies in preparation for surgery, neurosurgeon Itzhak Fried of the UCLA has also found concept cells. What he and his team observed is that some hippocampus cells seem to fire in response to a specific person, thereby encoding that person's identity. For example, a cell increased its firing to "actress Halle Berry" whenever the patient viewed her photo portraits, her Cat-woman character and even a string of a string of beads arranged to spell her name (Quiroga et al., 2005). In addition to such highly specific concept cells, they also found more general concept cells such as neurons that responded to categories of objects, such as animals, outdoor scenes or faces in general. Therefore, the successful identification of these types of cells in the human hippocampus provides a broad support for the notion that the general-to-specific feature–encoding pyramid offers a key framework for organizing memory information at the neural population levels. Such a general-to-specific feature–encoding pyramid makes it obvious that episodic memory is intimately linked with and simultaneously converted to semantic memory and generalized knowledge.

Differential reactivations within episodic cell assemblies underlying selective memory consolidation

The hippocampus plays a crucial role in converting short-term memory into long-term memory, a process termed as memory consolidation. While our brain can recall a great amount of detail in an accurate manner immediately after an event (in the time domain of short-term memory), there appears to be a gradual loss of many specific details in the domain of long-term memory. In other words, long-term memory contains only partial information about the original experiences, usually retaining general and more abstract information better than those about specific details.

How does the brain perform such differential memory consolidations? To investigate the neural network mechanism that governs this selective consolidation process, researchers used a set of distinct fearful events, such as earthquake, free fall, or sudden air puff, to study whether and how hippocampal CA1 cells would engage in selective memory consolidation. Again, these distinct episodes activate a unique assembly of CA1 episodic cells whose response selectivity ranges from general to specific features. A series of parametric analyses further reveals that post-learning CA1 episodic pattern replays or reverberations are mostly mediated by cells exhibiting event intensity–invariant responses, not by the intensity-sensitive cells. More importantly, intensity-invariant cells encoding general episodic features tend to display stronger reactivation cross-correlations during the immediate post-learning period than those invariant cells encoding specific

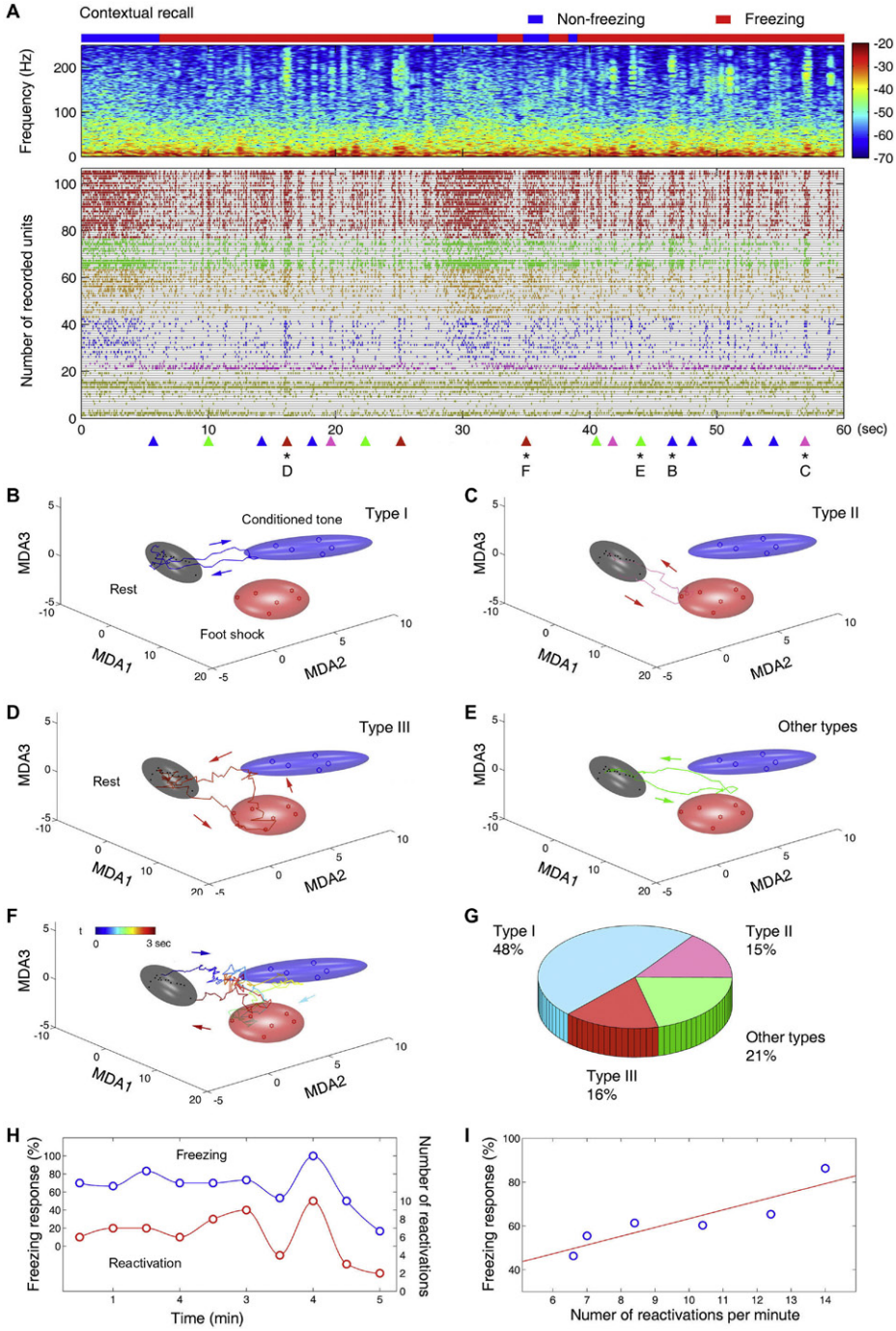

FIGURE 56-6 **Real-time memory traces in the mouse hippocampus during contextual fear memory recall.** (**A**) A trained mouse was brought back to the fear-conditioning chamber. The animal exhibited fear as indicated by the amount of freezing (the blue bar on the top indicates the non-freezing state, whereas the red bar indicates the freezing state of the animal). The spectrogram of local field potentials during the 60-sec epoch of the contextual recall test in this mouse is shown. The colored triangles at the bottom indicate the various moments at which memory traces reappeared in tandem. (**B**) The blue trajectory shows that a representative conditioned tone trace (Type I) was retrieved, indicated by the star marked with B at the bottom of the raster in (A). This transient dynamic lasted about one second. (**C**) The pink trajectory shows a representative foot shock memory trace that lasts about 0.7 seconds (marked by the pink triangle with C in (A), Type II). (**D**) The red trajectory is a representative foot shock–tone association trace (the red triangle with D in (A), Type III). This trajectory occurred for about 0.8 seconds. (**E**) The green trace shows a type of different trace often seen during recall testing. It visited the space between the foot shock and conditioned tone clusters and lasted about one second. (**F**) The colored trajectory shows a Type III trace that was retrieved at the time indicated by the red triangle with F in (A). This trajectory occurred in the non-freezing state and, interestingly, had a reversed directionality, namely, moving from the CS ellipsoid to the US ellipsoid. It lasted about three seconds. (**G**) The percentages of different types of ensemble patterns during contextual recall in six mice. (**H**) Freezing responses and the total numbers of pattern retrievals were calculated and compared during the entire 5-min contextual recall test (mouse #1). The blue circles are the freezing responses counted in every 30 seconds; the red circles show the averaged numbers of three major types of ensemble traces counted in the same 30 seconds. Cross-correlation analysis shows that the correlation between freezing responses and occurrences of pattern retrievals was significant ($r = 0.8840$, $p < 0.001$). (**I**) Averaged freezing responses in six mice are also tightly correlated with their averaged numbers of total pattern retrievals during the contextual recall tests ($r = 0.8956$, $p < 0.05$). Each circle represents the data from a single mouse. This plot indicates that the numbers of patterns retrieved is almost in linear proportion to behavioral performances as measured by the amounts of contextual freezing.

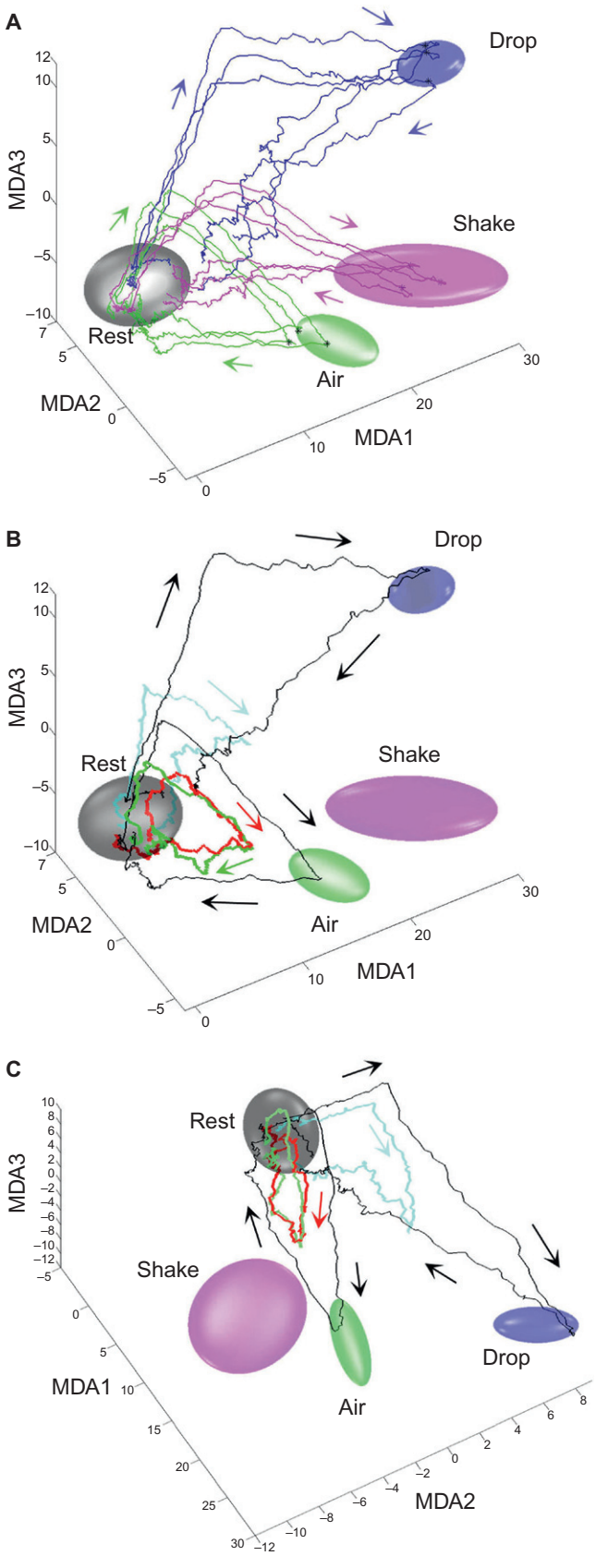

FIGURE 56-7 Classification, visualization, and dynamical decoding of CA1 ensemble representations of fearful episodes by multiple-discriminant analysis (MDA) method. (**A**) Ensemble patterns during awake rest (dots, yellow ellipsoid), Air-blow (circles, green ellipsoid), Drop (triangles, blue ellipsoid) and Earthquake (stars, magenta ellipsoid, labeled as shake) epochs are shown in a three-dimensional subencoding space obtained using MDA for a mouse in which 260 CA1 cells were simultaneously recorded; MDA1-3 denote the discriminant axes. Three representative dynamical trajectories of network patterns during the encoding of each type of startling events are shown. (**B**) Dynamical monitoring of post-learning spontaneous reactivations of network traces during and after the actual startling events. 3-D subspace trajectories of the ensemble encoding patterns during Drop and Air-blow episodes in the same mouse are shown. The initial response to an actual Air-blow or Drop event (black lines) is followed by spontaneous reactivations (red and green lines for two air-blow reactivations, and purple line for Drop pattern reactivation), characterized by co-planar, geometrically similar lower-amplitude trajectories (directionality indicated by arrows). (**C**) The same trajectories of reactivation traces from a different orientation after a 3-D rotation show that the trajectories are highly specific toward their own startle clusters. These post-learning dynamical trajectories are typically smaller in amplitude and take place without any time compression, and the numbers of reactivations within the initial several minutes seem to be in the range of one to five, with random intervals.

features. These differential reactivations within the CA1 episodic cell populations, thus, can provide a hippocampal selection mechanism to consolidate preferentially more generalized information for long-term memory storage.

The generalization function of the hippocampus

While it is fairly intuitive to see the significance of general and subgeneral cliques, it is useful to realize the potential of the event-specific cliques or event/context-specific cliques in the processing of semantic knowledge. That is, while these cliques in CA1 may carry specific features about the event, their participation may not be only limited to encoding of specific episodic features. For example, on three separate visits to California, if earthquakes strike every time, the repeated activations of the context-specific clique in CA1 and their downstream projection sites may readily contribute to the encoding of the semantic aspect of memory, such as that "California is the place with a lot of earthquakes." As such, the generalization function by the hippocampus should be viewed as an intrinsic encoding process that can integrate incrementally various shared features across different experiences.

In supporting this prediction, lesioning of the hippocampus in both humans and animals not only greatly reduces their brains' ability to remember new episodic experience and new knowledge, but also impairs their generalization capacity (Wild & Blampied, 1972; Freeman et al., 1973; Mastroianni, 1979). A recent study further suggests that generalization may require the hippocampus to work closely together with the other brain regions such as the ventral tegmental area (VTA) and substantia nigra (Shohamy & Wagner, 2008).

The ability to generalize can vary from individual to individual, depending on how many overlapping connections were genetically laid down during development within the

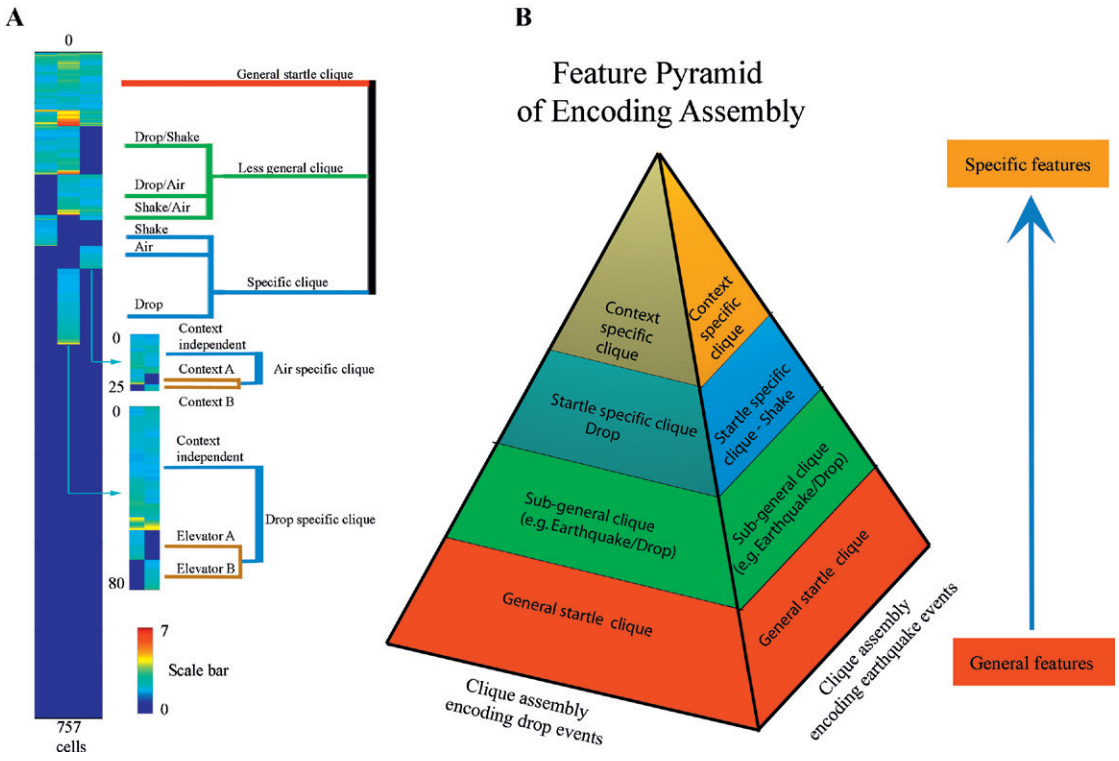

FIGURE 56-8 **Categorical and hierarchical organization of the memory-encoding neural clique assembly. Memory coding units, termed neural cliques, are organized in a categorical and hierarchical manner.** The hierarchical clustering analysis of responses of a total of 757 CA1 neurons from four mice to the three different types of startling episodes reveals the existence of seven major neural cliques (Panel A): General startle clique, subgeneral startle cliques (Drop–Shake clique, Air blow–Drop cliques, Shake–Air blow clique), startle type–specific cliques (Drop-specific clique, Shake-specific clique, and Air blow-specific clique), and startle context–specific clique (Air-blow in context A–specific clique, Air-blow in context B–specific clique, Drop in Elevator A–specific clique, and Drop in Elevator B–specific clique). Nonresponsive units are grouped in the bottom half. The color scale bar indicates the normalized response magnitude (1 to 7).

memory circuits. Nonetheless, generalization is an ongoing, accumulative process, subject to continuous modifications by new experiences. This constant updating process allows the brain to build complex networks for vast amounts of knowledge as more and more life experiences accumulate over years and decades. This, in turn, would predict that a significant fraction of the hippocampal cells should be highly convergent and readily responsive to a variety of episodic experiences.

Imagination of the hippocampus

Since memory retrieval usually contains a rich recollection of episodic imagery and multimodal details as well as semantic information, imagining future events or fictitious experiences is likely to engage the same neural circuits that process these various features (Shohamy & Wagner, 2008). This notion is supported by the recent observations that amnesic patients have difficulty envisioning themselves in the future and often lack rich details and emotions (Addis et al., 2007; Atance & O'Neill, 2001) and has led to a set of fMRI studies of memory using the imagination as a paradigm (Rosenbaum et al., 2005; Addis et al., 2007; Szpunar et al., 2007). These new studies suggest that imagining new experiences is part of the functions of memory circuits that include the hippocampus, parahippocampal gyrus and retrosplenial cortex, as well as the brain regions such as anterior prefrontal cortex, posterior cingulated cortex and precuneus, which may play a role in supporting self-schema and the familiarity process important for distinguishing real from imaginary memories (Okuda et al., 2003; Hassabis et al., 2007).

Mechanistically, the neural process for imagination within the hippocampus can be explained by dynamic interactions between various feature-coding units and combinatorial interplays between various neural assemblies within the neural population (Figure 56-4). The combinatorial activation among a set of coherently organized neural cliques, arranged in a novel manner, would allow the generation of various network-level patterns representing fictitious experiences in the imagination. For example, while keeping the other general and subgeneral cliques but only replacing the context clique of an San Francisco earthquake with a new context clique coding New York City, a brain can now "imagine" a fictitious earthquake taking place in the Big Apple. This imaginative ability, arising out of the combinatorial and hierarchical organization of memory coding assemblies (Figure 56-4), would also enable memory circuits to participate in the assessment and prediction of possible future events and actions (Tsien, 2007). The ability to predict is essential for our intelligence

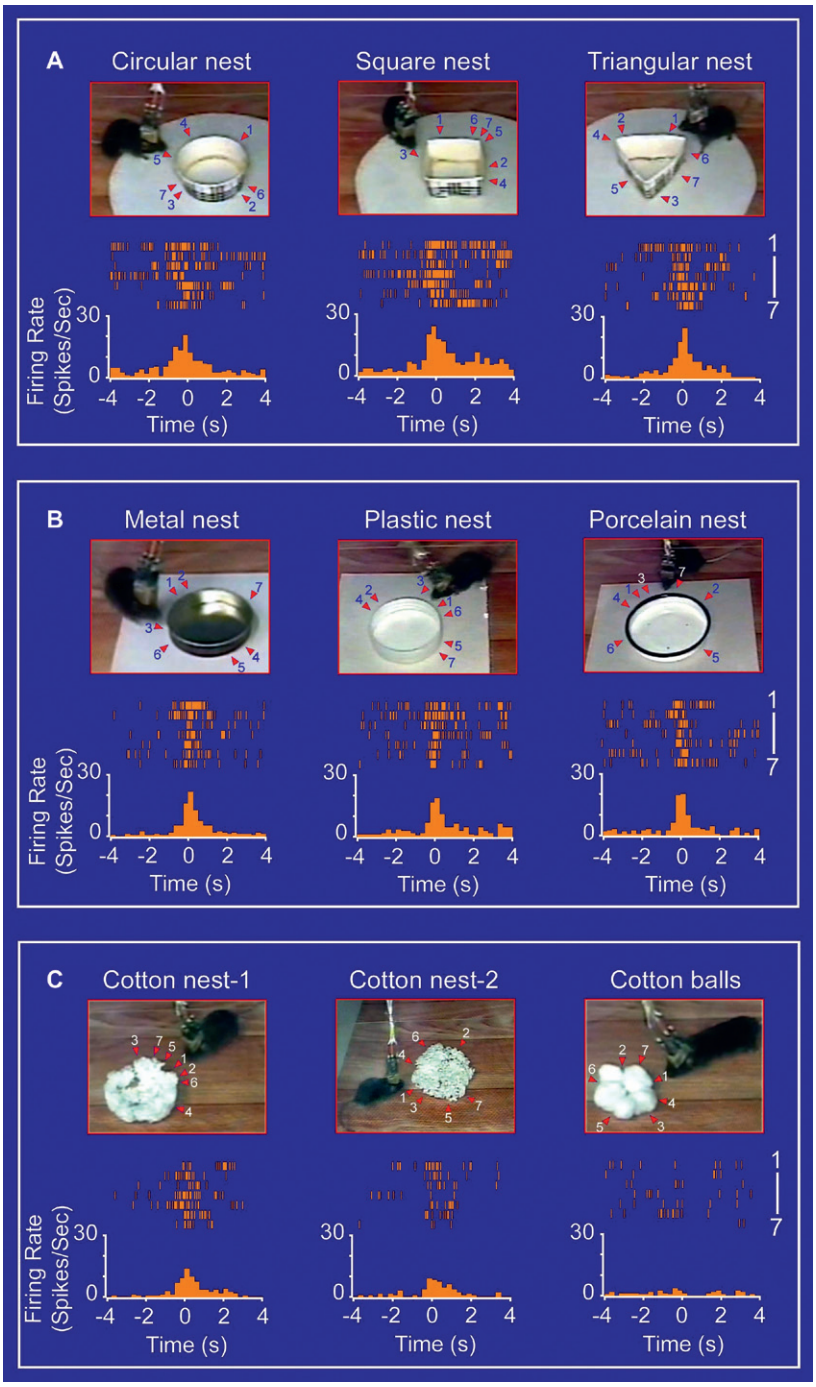

FIGURE 56-9 **Nest concept cells exhibiting invariant responses over the geometric shapes, physical appearances, colors, constructing materials, etc.** (**A**) Invariant responses of a nest cell to various geometric shapes of nests (e.g., a circular nest, a square cardboard nest (middle column), and a triangular cardboard nest (right column). (**B**) This cell also responded to nests made from different materials such as metal (left column), plastic (middle column), and porcelain (right column). (**C**) The cell further responded to natural cotton nests (left and middle columns), but not to five cotton balls that were simply lumped together (right column). The bin width in the peri-event spike histogram is 250 msec.

and logic processes, and allows us to efficiently anticipate problems in the ever-changing world and subsequently generate corresponding solutions.

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