MOLECULAR BASIS OF LONG-TERM PLASTICITY UNDERLYING ADDICTION

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Studies of human addicts and behavioural studies in rodent models of addiction indicate that key behavioural abnormalities associated with addiction are extremely long lived. So, chronic drug exposure causes stable changes in the brain at the molecular and cellular levels that underlie these behavioural abnormalities. There has been considerable progress in identifying the mechanisms that contribute to long-lived neural and behavioural plasticity related to addiction, including drug-induced changes in gene transcription, in RNA and protein processing, and in synaptic structure. Although the specific changes identified so far are not sufficiently long lasting to account for the nearly permanent changes in behaviour associated with addiction, recent work has pointed to the types of mechanism that could be involved.

Addiction continues to exact enormous human and financial costs on society, but available treatments remain inadequate for most people. By analogy with other medical disorders, an improved understanding of the biological basis of addiction will lead to more effective treatments and eventually to cures and preventive measures.

Addiction can be best defined as the loss of control over drug use, or the compulsive seeking and taking of drugs despite adverse consequences. Addiction is caused by the actions of a drug of abuse on a vulnerable brain and generally requires repeated drug exposure. This process is strongly influenced both by the genetic makeup of the person and by the psychological and social context in which drug use occurs. Once formed, an addiction can be a life-long condition in which individuals show intense drug craving and increased risk for relapse after years and even decades of abstinence. This means that addiction involves extremely stable changes in the brain that are responsible for these long-lived behavioural abnormalities.

Here, I review recent progress in identifying the types of molecular and cellular mechanisms that underlie the long-lasting behavioural plasticity associated with addiction. As will be seen, many of the mechanisms that have been identified so far are similar to those implicated in other stable changes in brain and behaviour, such as memory storage. This indicates that there are a finite number of ways in which the brain responds and adapts over time to diverse types of perturbation. Studies of addiction might provide a unique contribution to solving the molecular nature of such stable neural and behavioural plasticity, given the availability of increasingly sophisticated animal models of addiction.

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Neurobiology of addiction

To understand addiction, one must comprehend how the effects of a drug during an initial exposure lead progressively to stable molecular and cellular changes after repeated exposure. We now know what the target proteins for most drugs of abuse are[1] (TABLE 1). For example, opiates are agonists at opioid receptors, whereas cocaine binds to and inhibits the nerve terminal transporters for dopamine or other monoamine neurotransmitters.

Although drugs of abuse are chemically divergent molecules with very different initial activities, the resultant addictions share many important features.

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Table 1 | **Acute actions of some drugs of abuse**

Drug	Action	Receptor signalling mechanism
Opiates	Agonist at μ-, δ- and κ-opioid receptors*	G_i
Cocaine	Indirect agonist at dopamine receptors by inhibiting dopamine transporters‡	G_i and G_s§
Amphetamine	Indirect agonist at dopamine receptors by stimulating dopamine release‡	G_i and G_s§
Ethanol	Facilitates $GABA_A$ receptor function and inhibits NMDA receptor function‖	Ligand-gated channels
Nicotine	Agonist at nicotinic acetylcholine receptors	Ligand-gated channels
Cannabinoids	Agonist at CB_1 and CB_2 cannabinoid receptors¶	G_i
Phencyclidine (PCP)	Antagonist at NMDA glutamate receptors	Ligand-gated channels
Hallucinogens	Partial agonist at 5-HT_{2A} serotonin receptors	G_q
Inhalants	Unknown	

*Activity at μ- (and possibly) δ-receptors mediates the reinforcing actions of opiates; κ-receptors mediate aversive actions.
‡Cocaine and amphetamine exert analogous actions on serotonin and noradrenaline systems, which may also contribute to the reinforcing effects of these drugs.
§G_i couples D_2-like dopamine receptors, and G_s couples D_1-like dopamine receptors, both of which are important for the reinforcing effects of dopamine.
‖Ethanol affects several other ligand-gated channels, as well as voltage-gated channels at higher concentrations. In addition, ethanol is reported to influence many other neurotransmitter systems, including serotonin, opioid, and dopamine systems. It is not known whether these effects are direct or indirect through actions on various ligand-gated channels.
¶Activity at CB_1 receptors mediates the reinforcing actions of cannabinoids; CB_2 receptors are expressed in the periphery only. Proposed endogenous ligands for the CB_1 receptor include anandamide and 2-arachidonylglycerol, arachidonic acid metabolites. (GABA, γ-aminobutyric acid; NMDA, *N*-methyl-D-aspartate; 5-HT, serotonin.)

This can be explained by the fact that each drug, despite its many distinct actions in the brain, converges in producing some common actions. Prominent among these actions is the activation of the mesolimbic dopamine system (FIG. 1). This activation involves increased firing of dopamine neurons in the ventral tegmental area (VTA) of the midbrain and a subsequent increase of dopamine released into the nucleus accumbens (NAc) (also called the ventral striatum) and other regions of the LIMBIC forebrain (for example, the prefrontal cortex). Several drugs of abuse also activate the mesolimbic dopamine system by mimicking (opiates) or activating (alcohol, nicotine) endogenous opioid pathways that innervate the VTA and NAc. Other drugs seem to act directly in the NAc through other mechanisms (for example, CANNABINOIDS and PHENCYCLIDINE). These various actions seem to produce some similar net effects (generally inhibition) on MEDIUM SPINY NEURONS of the NAc. This occurs in part because opioid, cannabinoid, and certain dopamine receptors, all of which are G_i-coupled (SEE TABLE 1), are expressed by some of the same NAc neurons. There is compelling evidence that these various mechanisms are central in mediating the acute reinforcing properties that are shared by all drugs of abuse[1–5]. However, we still have a very limited understanding at a neural circuit level of precisely how such actions on NAc neurons actually lead to reinforcement.

The mesolimbic dopamine system and its forebrain targets are very old from an evolutionary point of view and are part of the motivational system that regulates responses to natural reinforcers such as food, drink, sex and social interaction. Drugs of abuse affect this pathway with a strength and persistence probably not seen in response to natural reinforcers. One likely mechanism of addiction, then, is that repeated, strong stimulation of these neurons changes them in a way that leads to marked alterations in reinforcement mechanisms and motivational state. Several types of functional alteration have been described[1–5]. TOLERANCE might contribute to the escalation of drug intake seen during the development of an addiction. DEPENDENCE might contribute to the DYSPHORIA and high rates of relapse seen during early phases of withdrawal. SENSITIZATION might contribute to the increased risk of relapse after longer withdrawal periods. Some investigators use the term 'protracted abstinence' to describe these various changes in drug reward and the persistent dysregulation of the reward circuitry that underlies them.

Drug addiction probably involves changes in many brain structures in addition to the VTA and NAc. In particular, persistent drug craving and relapse to drug use can be triggered markedly by exposure to contextual cues associated with past drug use (for example, drug paraphernalia or locations of past drug use) and by stress[6,7]. The effects of cues and stress, although partly mediated by the VTA–NAc pathway, seem to involve plasticity in structures that mediate learned or conditioned responses, such as the amygdala, the hippocampus and the cerebral cortex[3–7].

Each of these various types of behavioural phenomena can be modelled with increasing accuracy in rodents, which has made it possible to identify some of the molecular and cellular mechanisms involved. Most progress so far has focused on the VTA and NAc, but some recent evidence indicates that analogous mechanisms might operate in other brain regions.

Transcriptional mechanisms

Regulation of gene expression is one mechanism that should lead to relatively stable changes within neurons[8,9]. According to this scheme, repeated exposure to a drug of abuse would eventually lead to changes in nuclear function and to altered rates of transcription of particular target genes by causing repeated perturbation of intracellular signal transduction pathways[10,11] (BOX 1). Altered expression of these genes would lead to altered activity of the neurons in which those changes occur and, ultimately, to changes in the neural circuits in which those neurons operate. The result would be stable changes in behaviour.

Whereas neural genes are probably regulated by hundreds of distinct types of transcription factor, two transcription factors in particular have so far been implicated in addiction: the cyclic-AMP response-element-binding protein (CREB) and ΔFosB.

CREB and upregulation of the cAMP pathway. CREB regulates the transcription of genes that contain a CRE site (cAMP response element; consensus sequence TGACGTCA) within their regulatory regions[12,13]. CREs

LIMBIC SYSTEM
A collection of cortical and subcortical structures important for processing memory and emotional information. Prominent structures include the hippocampus and amygdala.

CANNABINOIDS
Derivatives of 2-(2-2-isopropyl-5-methylphenyl)-5-pentyl-resorcinol, a molecule found in the plant *Cannabis sativa*. Cannabinoids are responsible for the psychoactive effects of marijuana.

PHENCYCLIDINE
A potent psychoactive drug also known as angel dust, which has anaesthetic and analgesic actions. It blocks the NMDA receptor channel.

MEDIUM SPINY NEURONS
The main cell population of the ventral and dorsal striatum; these GABA-mediated projection neurons form the two main outputs of these structures, called the direct and indirect pathways.

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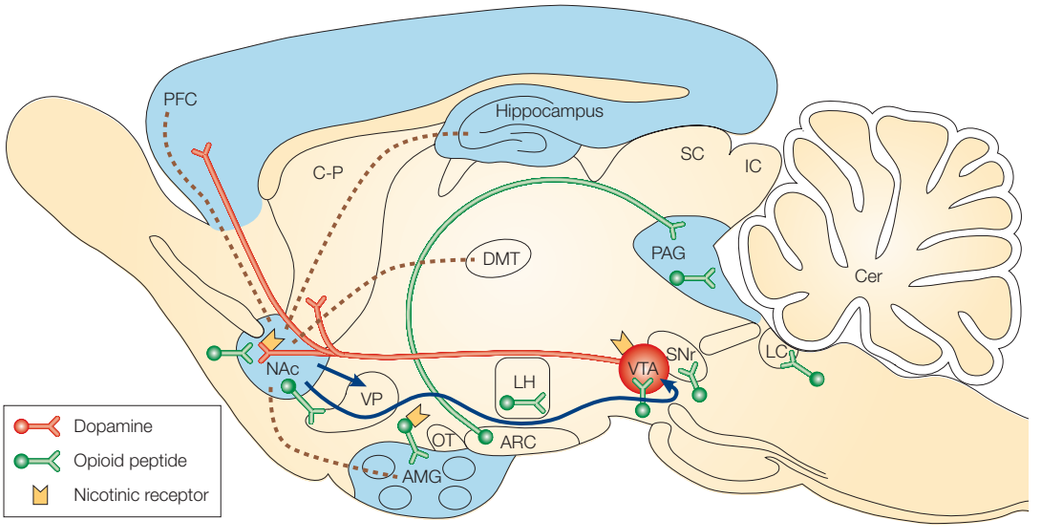

Figure 1 | **Key neural circuits of addiction.** Dotted lines indicate limbic afferents to the nucleus accumbens (NAc). Blue lines represent efferents from the NAc thought to be involved in drug reward. Red lines indicate projections of the mesolimbic dopamine system thought to be a critical substrate for drug reward. Dopamine neurons originate in the ventral tegmental area (VTA) and project to the NAc and other limbic structures, including the olfactory tubercle (OT), ventral domains of the caudate-putamen (C-P), the amygdala (AMG) and the prefrontal cortex (PFC). Green indicates opioid-peptide-containing neurons, which are involved in opiate, ethanol and possibly nicotine reward. These opioid peptide systems include the local enkephalin circuits (short segments) and the hypothalamic midbrain β-endorphin circuit (long segment). Blue shading indicates the approximate distribution of $GABA_A$ (γ-aminobutyric acid) receptor complexes that might contribute to ethanol reward. Yellow solid structures indicate nicotinic acetylcholine receptors hypothesized to be located on dopamine- and opioid-peptide-containing neurons. (ARC, arcuate nucleus; Cer, cerebellum; DMT, dorsomedial thalamus; IC, inferior colliculus; LC, locus coeruleus; LH, lateral hypothalamus; PAG, periaqueductal grey; SC, superior colliculus; SNr, substantia nigra pars reticulata; VP, ventral pallidum.) (Adapted from REF. 1.)

TOLERANCE
Reduced drug responsiveness with repeated exposure to a constant drug dose.

DEPENDENCE
Altered physiological state that develops to compensate for persistent drug exposure and that gives rise to a withdrawal syndrome after cessation of drug exposure.

DYSPHORIA
Negative or aversive emotional state usually associated with anxiety and depression.

SENSITIZATION
Enhanced drug responsiveness with repeated exposure to a constant dose.

RAS PROTEINS
A group of small G proteins involved in growth, differentiation and cellular signalling that require the binding of GTP to enter into their active state.

LOCUS COERULEUS
Nucleus of the brainstem. The main supplier of noradrenaline to the brain

TYROSINE HYDROXYLASE
The rate-limiting enzyme in the biosynthesis of noradrenaline, dopamine and other catecholamines.

have been identified in many genes expressed in the nervous system, including those encoding neuropeptides, neurotransmitter synthetic enzymes, signalling proteins and other transcription factors. CREB binds to CRE sites as a dimer and activates transcription only when both subunits are phosphorylated on their Ser 133 residue. This is because only phosphorylated CREB can interact with the adaptor protein CBP (CREB-binding protein), which in turn stimulates the basal transcription complex. As CREB can be phosphorylated on Ser 133 by protein kinase A (PKA), Ca^{2+}/calmodulin-dependent protein kinase IV, or protein kinases regulated by growth factor–RAS pathways, it represents a point of convergence at which several intracellular messenger pathways can regulate the expression of CRE-containing genes[12,13].

CREB was first implicated in drug addiction[14] because its activation was a predictable consequence of upregulation of the cAMP pathway, one of the best-established adaptations to drugs of abuse. Upregulation of the cAMP pathway, which was first observed in cultured neuroblastoma × glioma cells[15], has since been shown in several regions of the central and peripheral nervous systems in response to chronic opiate administration[8,11,16–20]. As opiates acutely inhibit adenylyl cyclase via G_i-coupled receptors, upregulation of the cAMP pathway is seen as a compensatory homeostatic response of cells to persistent opiate inhibition of the pathway. Upregulation of the cAMP pathway has been implicated in several aspects of opiate addiction, depending on the region of the nervous system involved (TABLE 2).

The LOCUS COERULEUS has been a useful model system in which to study some of the molecular mechanisms that lead to upregulation of the cAMP pathway[8,11]. Upregulation of the cAMP pathway in this brain region is one mechanism underlying physical opiate dependence and withdrawal, and CREB seems to have a central role[21]. Chronic opiate administration increases expression of CREB in the locus coeruleus[22]. This effect seems to be mediated by a homeostatic autoregulatory mechanism: opiate inhibition of the cAMP pathway leads to decreased activity of PKA and lower levels of phosphorylated (activated) CREB, which results in increased transcription of CREB via a CRE site present within the *CREB* gene[23]. This induction of CREB then increases the expression of adenylyl cyclase type VIII and of TYROSINE HYDROXYLASE, via a CRE site present in the genes for these enzymes[21,24]. The precise mechanism by which induction of CREB leads to increased transcription of these genes during the development of opiate dependence remains unknown. So, whereas levels of CREB are increasing in the neurons, the activity of the cAMP pathway remains inhibited by continued opiate activation of opioid receptors. One possibility is that higher levels of CREB, even in its dephosphorylated form, might mediate some transcriptional activation. Another possibility, not excluded by the first, is that the higher levels of CREB are phosphorylated by PKA as the kinase accumulates owing to post-transcriptional mechanisms that are discussed below.

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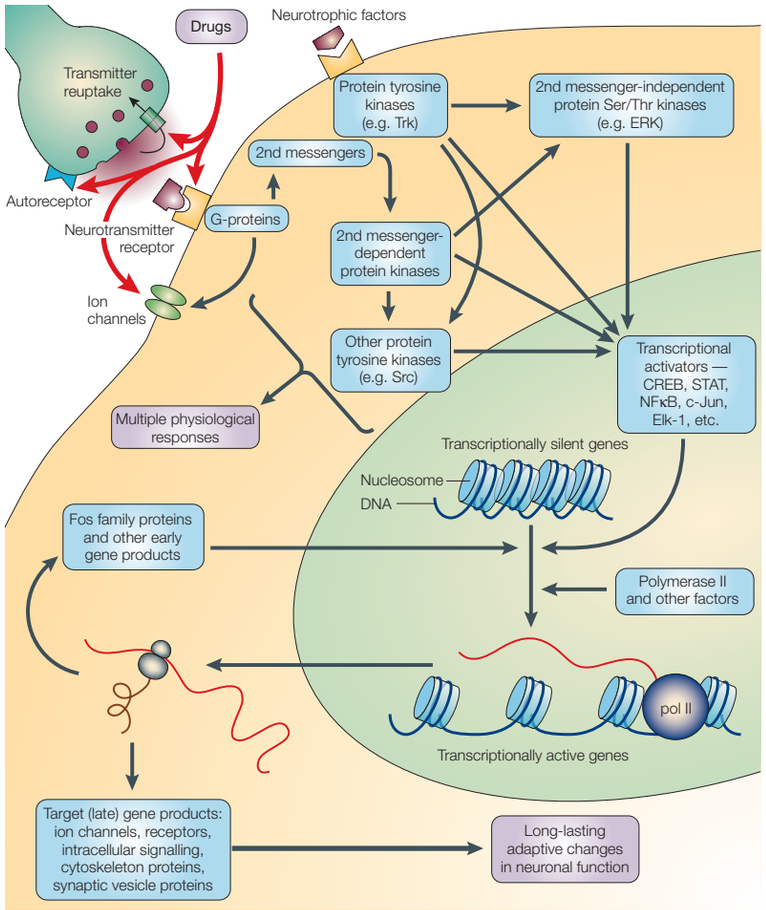
Box 1 | **Regulation of gene expression by drugs of abuse**



The rate of expression of a particular gene is controlled by its location within nucleosomes and by the activity of the transcriptional machinery[10]. A nucleosome is a tightly wound span of DNA that is bound to histones and other nuclear proteins. Transcription requires the unwinding of a nucleosome, which makes the gene accessible to a basal transcription complex. This complex consists of RNA polymerase (pol II, which transcribes the new RNA strand) and numerous regulatory proteins (some of which unwind nucleosomes through histone acetylation). Transcription factors bind to specific sites (response elements; also called promoter or enhancer elements) that are present within the regulatory regions of certain genes, and thereby increase or decrease the rate at which they are transcribed. Transcription factors act by enhancing or inhibiting the activity of the basal transcription complex, in some cases by altering nucleosomal structure through changes in the histone acetylation of the complex.

Regulation of transcription factors is the best-understood mechanism by which changes in gene expression occur in the adult brain[8,9,11]. Most transcription factors are regulated by phosphorylation. Accordingly, by causing repeated perturbation of synaptic transmission and hence of protein kinases or protein phosphatases, repeated exposure to a drug of abuse would lead eventually to changes in the phosphorylation state of particular transcription factors such as CREB that are expressed under basal conditions. This would lead to the altered expression of their target genes. Among such target genes are those for additional transcriptional factors (such as c-Fos), which — through alterations in their levels — would alter the expression of additional target genes. Drugs of abuse could conceivably produce stable changes in gene expression through regulation of many other types of nuclear proteins, but such actions have not yet been shown.

Mice with mutations in the *CREB* gene show decreased development of opiate physical dependence, as indicated by an attenuated WITHDRAWAL SYNDROME after administration of an opioid receptor antagonist[25]. These findings provide strong support for a role of CREB in opiate dependence, but their interpretation is complicated for two main reasons. First, the CREB protein is expressed ubiquitously. So these mice do not reveal those particular brain regions where the physiological functions of CREB mediate opiate action. Second, the mutants do not lack all products of the *CREB* gene and there is evidence that certain CREB isoforms might be upregulated secondary to the mutation. Mice with inducible mutations in CREB (for example, REF. 26), in which the mutation can be targeted to specific brain regions, constitute the next generation of mutant mice that are needed to more fully explore CREB function in opiate physical dependence.

An analogous role for CREB in emotional and motivational aspects of drug dependence would be expected on the basis of the observed upregulation of the cAMP pathway in the NAc in response to chronic opiates, cocaine or alcohol[8,11,17]. Accordingly, chronic morphine and cocaine treatments have been shown to enhance CREB function in this and related striatal regions[27–29]. Overexpression of CREB in the NAc decreases the rewarding effects of opiates and of cocaine, whereas overexpression of a DOMINANT-NEGATIVE mutant form of CREB (which lacks Ser 133) has the opposite effect[30,31]. Similarly, infusion of PKA activators into the NAc

Table 2 | **Upregulation of the cAMP pathway and opiate addiction**

Site of upregulation	Functional consequence
Locus coeruleus*	Physical dependence and withdrawal
Ventral tegmental area‡	Dysphoria during early withdrawal periods
Periaqueductal grey‡	Dysphoria during early withdrawal periods, and physical dependence and withdrawal
Nucleus accumbens	Dysphoria during early withdrawal periods
Amygdala	Conditioned aspects of addiction?
Dorsal horn of spinal cord	Tolerance to opiate-induced analgesia
Myenteric plexus of gut	Tolerance to opiate-induced reductions in intestinal motility and increases in motility during withdrawal

*The cAMP pathway is upregulated within the principal noradrenaline neurons located in this region. ‡Indirect evidence indicates that the cAMP pathway may be upregulated within GABA (γ-aminobutyric acid) neurons that innervate the dopamine and serotonin cells located in the ventral tegmental area and periaqueductal grey, respectively. During withdrawal, the upregulated cAMP pathway would become fully functional and could contribute to a state of dysphoria by increasing the activity of the GABA neurons, which would then inhibit the dopamine and serotonin neurons[18,19].

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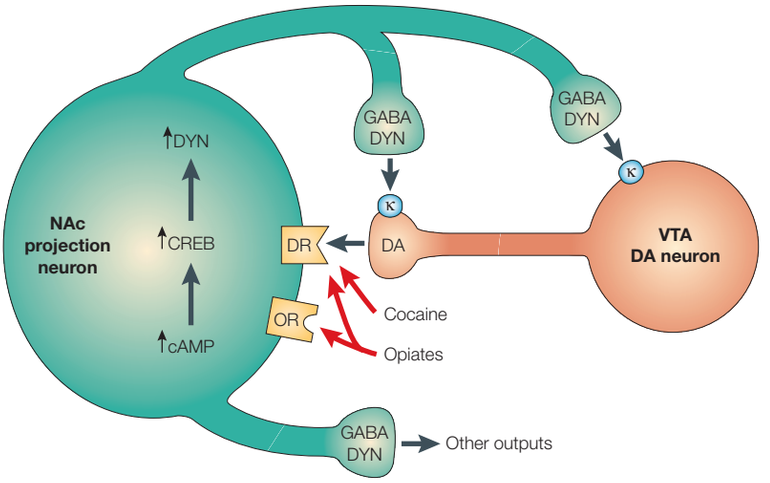

Figure 2 | **Regulation of CREB by drugs of abuse.** The figure shows a dopamine (DA) neuron of the ventral tegmental area (VTA) innervating a class of GABA (γ-aminobutyric acid) projection neuron from the nucleus accumbens (NAc) that expresses dynorphin (DYN). Dynorphin constitutes a negative feedback mechanism in this circuit: dynorphin, released from terminals of the NAc neurons, acts on κ-opioid receptors (κ) located on nerve terminals and cell bodies of the DA neurons to inhibit their functioning. Chronic exposure to cocaine or opiates upregulates the activity of this negative feedback loop through upregulation of the cAMP pathway, activation of CREB and induction of dynorphin.

diminishes cocaine reward, whereas inhibitors of the enzyme increase it[32]. These data indicate that upregulation of the cAMP pathway and CREB in the NAc might mediate a homeostatic adaptation that diminishes further drug responsiveness, as has been proposed to occur in the locus coeruleus. In the NAc, such actions would be expected to attenuate the activity of the reward circuitry, which could mediate some of the dysphoria seen during early phases of withdrawal. However, it must be emphasized that in the case of CREB, this hypothesis is based on the use of place-conditioning assays as the sole measure of drug reward. This assay is often used in initial studies as it is amenable to relatively high throughput. Nevertheless, the field would benefit substantially from the increased use of more complicated behavioural assays (for example, self-administration, conditioned reinforcement and relapse models), which provide a more complete indication of the effect of a molecular perturbation on the complex behaviour of addiction[32,33].

The effects of upregulation of the cAMP pathway and CREB in the NAc are mediated partly by the opioid peptide dynorphin, which is expressed in a subset of NAc medium spiny neurons (FIG. 2). Dynorphin causes dysphoria by decreasing dopamine release within the NAc through an action on κ-opioid receptors that are located on presynaptic dopamine-containing nerve terminals in this region[34–36]. Dynorphin expression is induced in the NAc and related striatal regions after drug exposure[37,38], and this effect seems to be mediated by CREB[27,30]. Moreover, the dysphoria caused by CREB overexpression in the NAc is blocked by a κ-opioid antagonist[30]. CREB seems therefore to increase the gain on this dynorphin-mediated negative feedback circuit and thereby contributes to the generation of aversive states during withdrawal. An important goal of current research is to identify other CREB-regulated genes in the NAc and to relate them to specific features of drug dependence.

Regulation of the cAMP pathway, CREB and dynorphin seems to be relatively short-lived in that the system reverts to normal within a few days or a week after drug withdrawal. As a result, such changes could contribute to the negative emotional state during early phases of withdrawal, but they are not likely to mediate directly the more stable behavioural abnormalities associated with addiction.

ΔFosB. ΔFosB is a member of the Fos family of transcription factors, which dimerize with a member of the Jun family to form activator protein-1 (AP-1) transcription factor complexes. AP-1 complexes then bind to AP-1 sites (consensus sequence, TGAC/GTCA) present in the regulatory regions of many genes[39].

Acute administration of several types of drugs of abuse causes the rapid (1–4 hour) induction of several Fos family members (for example, c-Fos, FosB, Fra-1, Fra-2) in the NAc and dorsal striatum[40–42] (FIG. 3). This induction is also highly transient; it resolves within 4–12 hours of drug administration owing to the instability of these proteins and their mRNAs. By contrast, biochemically modified isoforms of ΔFosB are induced only slightly by acute drug exposure. However, these ΔFosB isoforms begin to accumulate with repeated drug administration owing to their high stability and eventually become the predominant Fos-like protein in these neurons (FIG. 3). This extraordinary stability

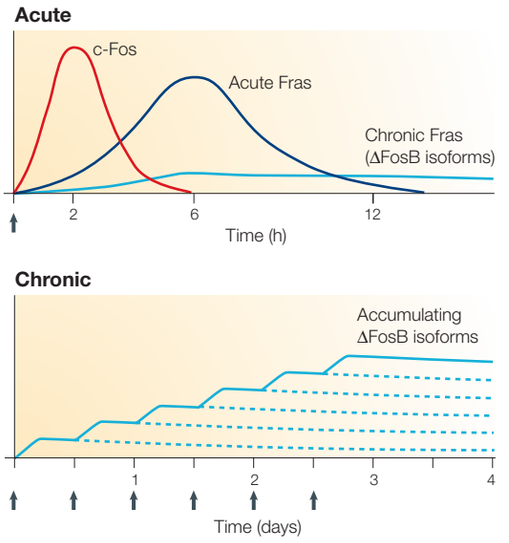

Figure 3 | **Regulation of ΔFosB by drugs of abuse.** The top graph shows the several waves of induction of Fos family proteins in the NAc after a single exposure (black arrow) to a drug of abuse. These proteins include c-Fos and several Fras (fos-related antigens; for example, FosB, Fra-1, Fra-2). All of these proteins of the Fos family are unstable. By contrast, isoforms of ΔFosB are highly stable and therefore persist in the brain long after drug exposure. Because of this stability, ΔFosB accumulates with repeated drug exposures, as shown in the bottom graph.

WITHDRAWAL SYNDROME
A collection of signs and symptoms that appear after sudden cessation of drug intake. Depending on the drug, they can include mild shakiness, sweating, anxiety and even hallucinations.

DOMINANT-NEGATIVE
A mutant molecule that forms heteromeric complexes with the wild type to yield a non-functional complex.

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resides in the ΔFosB protein *per se* and not in its mRNA, which is relatively unstable, like that of other Fos family members[42]. As a result, ΔFosB persists in the brain for relatively long periods of time. This phenomenon is a common response to many classes of addictive drugs. Chronic, but not acute, administration of cocaine, amphetamine, opiates, nicotine, phencyclidine or alcohol has been shown to induce ΔFosB in the NAc and dorsal striatum. This induction seems to be specific to the dynorphin-containing class of medium spiny neurons and this cellular pattern of induction is specific for addictive drugs[41,42]. For example, chronic exposure to antipsychotic drugs also induces ΔFosB in NAc and dorsal striatum, but this induction occurs in the other main subpopulation of medium spiny neurons in these regions[42–44]. ΔFosB is therefore interesting because it provides a molecular mechanism based on the stability of the protein by which drug-induced changes in gene expression can persist long after drug intake stops.

Recent work in which ΔFosB was selectively expressed within the dynorphin-containing class of medium spiny neurons in adult mice provides direct evidence that induction of ΔFosB mediates sensitized behavioural responses to drugs of abuse[45]. Inducible expression of ΔFosB causes increased locomotor and rewarding responses to cocaine and to morphine. In addition, it causes increased cocaine self-administration and increased cocaine-seeking behaviour in an animal model of relapse[46]. Conversely, a transgenic mouse in which a dominant-negative mutant form of c-Jun (which antagonizes the transcriptional effects of ΔFosB) is expressed in NAc and dorsal striatum shows reduced cocaine reward[47]. Together, these findings indicate that ΔFosB might be both necessary and sufficient for the relatively long-lived sensitization to cocaine and perhaps to other drugs of abuse. As such, ΔFosB could function as a sustained molecular switch — presumably one of many — that contributes to relapse after prolonged periods of abstinence[42].

Studies of *fosB* knockout mice further substantiate the involvement of products of this gene in drug action[48]. Some of the abnormalities seen in the *fosB* mutants (such as enhanced behavioural responses to initial cocaine exposure) are discrepant with findings in the inducible systems mentioned above; other abnormalities (for example, lack of sensitization in the mutants to repeated cocaine administration) are consistent with these findings. Indeed, interpretation of the data obtained in the *fosB* knockout mouse is complicated for several reasons. For example, the mice lack both products of the *fosB* gene — ΔFosB and the full-length FosB, which is known to be induced by acute cocaine in NAc and dorsal striatum. As a result, it is not possible to ascribe any particular abnormality to ΔFosB *per se*. In addition, the loss of ΔFosB and FosB is ubiquitous and occurs at the earliest stages of development. These considerations highlight, as I mentioned earlier in the context of CREB, the importance of inducible, cell type-specific mutations, particularly in studies of neural plasticity in the adult brain.

One target gene through which ΔFosB exerts its effects on behaviour seems to be the AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) glutamate receptor subunit *GluR2* (REF. 45). ΔFosB induces GluR2 in the NAc. This effect would be expected to reduce the electrical excitability of NAc neurons, as GluR2-containing AMPA channels show reduced overall conductance and reduced Ca^{2+} permeability. In fact, reduced excitability of NAc neurons has been directly observed in the neurons as a consequence of chronic drug exposure[49]. This reduction could then mediate enhanced reward mechanisms by, for example, making the neurons more sensitive to inhibition by subsequent drug exposure. However, as mentioned above, an understanding of the neural circuitry through which this is achieved is not yet available.

ΔFosB expression is the longest-lasting known molecular change in the brain seen in the context of drug exposure, and perhaps to any other perturbation of the adult brain. Nevertheless, ΔFosB undergoes proteolysis at a finite rate and dissipates to normal levels within a month or two of drug withdrawal. This means that ΔFosB *per se* cannot mediate the extremely long-lived changes in the brain and behaviour associated with addiction. One possibility discussed below is that ΔFosB causes other changes in the brain, which themselves are more permanent.

Other transcriptional mechanisms. It is likely that many transcription factors in addition to CREB and ΔFosB contribute to drug-induced adaptations in the brain. For example, Egr1–3 and Nac-1 are ZINC-FINGER-containing transcription factors that are induced in NAc and dorsal striatum after acute cocaine administration[50,51].

GLUCOCORTICOID receptors are also zinc-finger-containing transcription factors implicated in drug responsiveness[52]. However, it has not yet been possible to show alterations in these proteins that are unique to chronic drug exposure as observed for CREB or ΔFosB.

During development, permanent changes in gene expression, such as those related to organogenesis and cellular differentiation, are thought to occur through stable changes in the structure of nucleosomes, which make sets of genes accessible in some cell types but not in others. Perhaps analogous types of change in nucleosomal structure occur in adult neurons as a consequence of chronic exposure to drugs of abuse. Such a scheme is highly speculative, but it is now amenable to direct investigation given our increased knowledge of chromatin structure and function[10].

Post-transcriptional mechanisms

Changes in gene transcription represent just one of several possible mechanisms by which protein levels can change in a cell. Other mechanisms include alterations in mRNA translation and protein degradation, as well as alterations in the targeting of a protein to its active site within a neuron (FIG. 4). Such mechanisms are much less extensively investigated in models of addiction compared with gene transcription, but two systems illustrate the likely importance of post-transcriptional mechanisms.

ZINC FINGER
Protein module in which cysteine or cysteine–histidine residues coordinate a zinc ion. Zinc fingers are often used in DNA recognition and also in protein–protein interactions.

GLUCOCORTICOIDS
Hormones produced by the adrenal cortex, which are involved in carbohydrate and protein metabolism, but also affect brain function. Cortisol (human) and corticosterone (rodent) are prime examples.

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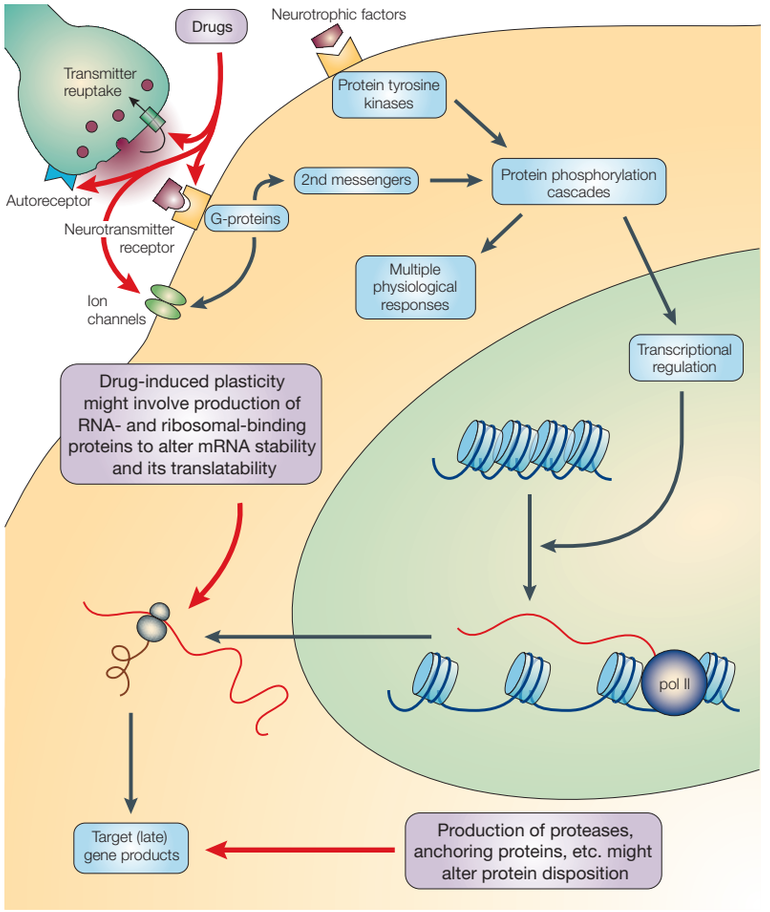

Figure 4 | **Regulation of post-transcriptional mechanisms by drugs of abuse.** The figure shows hypothetical mechanisms by which drug-induced changes in neurotransmission lead to changes in intracellular signalling pathways (for example, protein kinases and protein phosphatases) and to changes in mRNAs or proteins.

As mentioned earlier, upregulation of the cAMP pathway within neurons is an important mechanism of tolerance and dependence. A key part of this upregulation is the increased amount of PKA within certain types of neuron, which is due to the induction of particular catalytic and regulatory subunits of the kinase. Several lines of evidence indicate that induction of PKA subunits might not be achieved at the transcriptional level. For instance, upregulation of PKA immunoreactivity is not associated with detectable changes in subunit mRNA levels in certain brain areas. Similarly, alterations in CREB and ΔFosB do not lead to changes in PKA subunit expression, and the promoters of the PKA subunit genes do not contain identifiable response elements for these or other regulated transcription factors[21,53]. Instead, work in cell culture indicates that induction of PKA might occur through reduced degradation of the subunits[54]. According to this scheme, inhibition of adenylyl cyclase by opiates, for example, causes reduced levels of cAMP. As a result, more PKA molecules exist in the inactive holoenzyme form, which is less vulnerable to degradation within PROTEASOMES. Consequently, PKA subunits accumulate until a new equilibrium is achieved. After removal of the opiate and disinhibition of adenylyl cyclase, cellular cAMP levels rise, which leads to activation of the excess PKA within the cell. Work is now needed to test whether this scheme occurs within neurons *in vivo* in the context of addiction.

Regulation of receptor sensitivity is another example of a post-transcriptional mechanism that contributes to addiction, particularly to tolerance. The precise mechanisms of tolerance remain incompletely understood, but one mechanism involves phosphorylation of receptors followed by their sequestration and internalization. The details of this mechanism are best established for the β-adrenergic receptor, but similar mechanisms seem to operate for G-protein-coupled receptors that are targets for drugs of abuse, such as opioid, dopamine, and cannabinoid receptors[55–58]. Ligand binding to the receptor leads to phosphorylation by any of several G-protein-receptor kinases (GRKs). The phosphorylated receptor can then associate with an ARRESTIN and undergo endocytic internalization through a DYNAMIN-dependent process. The receptor can remain internalized for an extended period of time. Eventually, it can be dephosphorylated and returned to the plasma membrane or, alternatively, it can be degraded by proteases. The function of a receptor can therefore be regulated markedly in the absence of any changes in its transcription or translation. The GRK–arrestin system has been directly implicated in opiate tolerance[56,59,60]. Although such alterations in receptor availability are thought to be readily reversible after agonist removal, adaptations in this system (for example, altered levels of GRKs or arrestins (for example, REF. 61)) could contribute to the more stable behavioural aspects of addiction.

Regulation of synaptic structure

Over the past few years, several groups have documented that repeated exposure to a drug of abuse causes structural changes in specific neuronal cell types. For example, repeated opiate exposure decreases the size and calibre of dendrites and soma of VTA dopamine neurons[62]. The functional consequences of these changes are unknown, but they could reflect a down-regulation of dopamine activity and contribute to the dysphoria of drug withdrawal states. By contrast, repeated cocaine or amphetamine exposure increases the number of dendritic branch points and spines both of medium spiny neurons in the NAc and of pyramidal neurons in the medial prefrontal cortex (both of which receive dopamine inputs)[63,64]. Importantly, these changes have been shown to persist for at least one month after the last drug exposure and are hypothesized to represent the neural substrate for the near-permanent sensitization in drug responsiveness seen in certain animal models of addiction (FIG. 5). However, a link between such dendritic changes and sensitized behavioural responses remains conjectural.

Chronic exposure to opiates also reduces the birth of new neurons in the adult hippocampus[65]. Although the functional significance of such neurogenesis remains a subject of controversy[66], newly born neurons and their integration within existing hippocampal circuits might

PROTEASOME
Protein complex responsible for degrading intracellular proteins that have been tagged for destruction by the addition of ubiquitin.

ARRESTINS
Inhibitory proteins that bind to phosphorylated receptors, blocking their interaction with G proteins and terminating signalling. For example, β-arrestin binds to phosphorylated β-adrenergic receptors and inhibits their ability to activate G_s.

DYNAMIN
Protein involved in the formation of microtubule bundles and in membrane transport.

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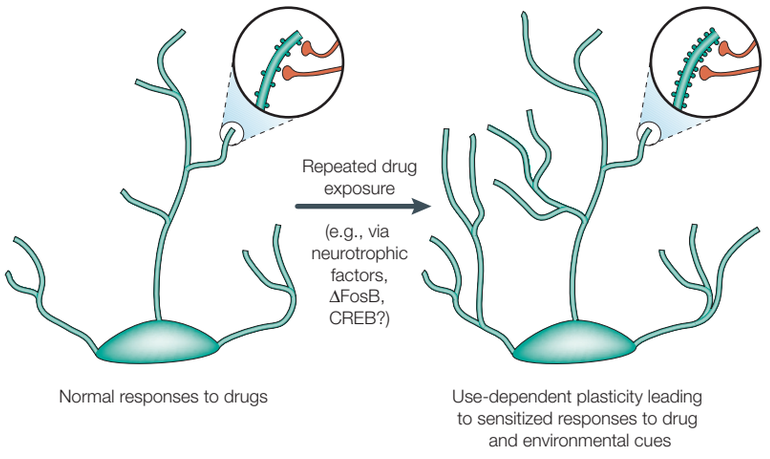

Figure 5 | **Regulation of dendritic structure by drugs of abuse.** The figure shows the expansion of a dendritic tree after chronic exposure to a drug of abuse, as has been observed in the nucleus accumbens and in the prefrontal cortex. The areas of magnification show an increase in dendritic spines, which is postulated to occur in conjunction with activated nerve terminals. Such alterations in dendritic structure, which are similar to those observed in other examples of synaptic plasticity such as long-term potentiation, could mediate long-lived sensitized responses to drugs of abuse or environmental cues.

participate in certain forms of learning and memory[67]. If this were the case, drug-induced regulation of neurogenesis could cause relatively stable changes in hippocampal function and, in consequence, some of the longer-lasting cognitive aspects of addiction.

Clearly, these observations raise the question of the underlying molecular and cellular mechanisms that mediate such alterations in neural structure and neurogenesis. Studies in the VTA–NAc pathway indicate that neurotrophic factors might be involved. For example, infusion of brain-derived neurotrophic factor (BDNF) into the VTA promotes the behavioural actions of drugs of abuse[68,69], whereas infusion of glial-cell-derived neurotrophic factor (GDNF) exerts the opposite effect[70]. Moreover, chronic administration of opiates or cocaine causes alterations in the intracellular signalling cascades for both of these neurotrophic factors[70–72], as well as changes in other neurotrophic factor systems[73]. The results imply a scheme whereby drug exposure perturbs neurotrophic factor function, which then disrupts the homeostatic role normally subserved by these factors in maintaining neuronal function. Drugs of abuse, which act initially on G-protein-coupled receptors or ligand-gated channels (SEE TABLE 1) presumably perturb neurotrophic factor function through the extensive crosstalk that exists between traditional second messenger cascades and protein tyrosine phosphorylation cascades that mediate neurotrophic factor signalling (SEE BOX 1). Given the role of neurotrophic factors in inducing permanent changes during development (including the changes in chromatin structure mentioned earlier), these factors could be central in mediating the stable changes related to addiction.

Although the molecular mechanisms underlying cocaine-induced changes in dendritic structure in the NAc and prefrontal cortex are not known, recent work has suggested one possible scheme. Chronic cocaine administration has been found to increase levels of CYCLIN-DEPENDENT KINASE 5 (Cdk5) within the NAc and related striatal regions[74]. Infusion of a Cdk5 inhibitor into the NAc prevents the cocaine-induced increase of dendritic spine density in this brain region (S. Norrholm, J. Bibb, J. Taylor, E. N., C. Ouimet, and P. Greengard, unpublished observations). Interestingly, the drug-induced upregulation of Cdk5 seems to be mediated through ΔFosB. So Cdk5 is induced upon overexpression of ΔFosB in inducible transgenic mice, and ΔFosB activates the promoter of the *Cdk5* gene in cell culture through a single AP-1 site present within the promoter[74,75]. The implication of these findings is that structural changes caused by repeated cocaine administration might be mediated by induction of ΔFosB and might persist long after the ΔFosB signal itself dissipates.

Relationship to other forms of stable plasticity

In reviewing mechanisms of drug addiction, many remarkable parallels with the learning and memory field become apparent. From a behavioural perspective, certain cardinal features of addiction have been described as forms of memory[3,5,7,9]. They include the conditioned aspects of addiction mentioned earlier, such as the ability of drug-associated cues to induce relapse.

Similarly, from a mechanistic perspective, several of the molecular and cellular adaptations involved in addiction are also implicated in models of learning and memory. Activation of the cAMP pathway and of CREB-mediated transcription in the hippocampus has been related to learning, as well as to LONG-TERM POTENTIATION (LTP)[76–79]. Roles for neurotrophic factors[80,81] and for changes in dendritic spine density[82] have also been implicated in LTP and long-term depression (LTD) in the hippocampus. A further parallel between the two fields is that LTP and LTD have been observed at glutamate synapses in both the VTA and NAc, and that drugs of abuse can modify these forms of plasticity[83–85]. These observations raise the possibility that the molecular and cellular mechanisms implicated in LTP and LTD in the hippocampus (for example, the insertion of AMPA glutamate receptors into subsynaptic regions of stimulated dendritic spines[82,86,87]) might also be relevant in addiction models. Indeed, alterations in glutamate receptor levels and in glutamate-mediated transmission have been reported in the VTA and the NAc after repeated exposure to a drug of abuse, and have been shown to modify drug responsiveness (for example, REFS 45,49,88–91).

However, as is the case for adaptations observed in the addiction field, no molecular or cellular change associated so far with models of learning and memory can account for the existence of essentially permanent memories. Arguing against a reductionist approach, some investigators have viewed learning and memory as processes mediated by use-dependent changes in the activity of particular neural circuits in the brain. However, ultimately such changes must be driven by changes at the molecular and cellular levels at some crucial neurons and synapses in these circuits. Therefore, the key challenges in the addiction and learning and memory fields are equivalent. What stable

CYCLIN-DEPENDENT KINASE 5
A member of a family of cyclin-dependent kinases, Cdk5 is enriched in brain, requires another protein termed p35 for its activation and is implicated in the regulation of neural growth and survival.

LONG-TERM POTENTIATION
A long-lasting increase in the efficacy of synaptic transmission commonly elicited by high-frequency neuron stimulation.

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molecular and cellular changes underlie near-permanent behavioural adaptations? What is the cascade of molecular and cellular events that first establishes and then maintains these long-lasting adaptations? In what way are neural circuits altered by these molecular and cellular adaptations that lead ultimately to a change in complex behaviour? Only through an integrated approach that establishes causal links between the molecular, cellular, circuit and behavioural levels will it be possible to understand the basis of permanent neural and behavioural plasticity.

Links

DATABASE LINKS CREB | ΔFosB | CBP | PKA | Ca^{2+}/calmodulin-dependent protein kinase IV | c-Fos | FosB | Fra-1 | Fra-2 | GluR2 | Egr1–3 | GDNF | Cdk5

ENCYCLOPEDIA OF LIFE SCIENCES Drugs and the synapse | Cocaine and amphetamines

1. Koob, G. F. & Nestler, E. J. Neurobiology of drug addiction. *J. Neuropsychiat. Clin. Neurosci.* **9**, 482–497 (1997).
2. Wise, R. A. Drug-activation of brain reward pathways. *Drug Alcohol Dependence* **51**, 13–22 (1998).
3. Robinson, T. E. & Berridge, K. C. The psychology and neurobiology of addiction: an incentive-sensitization view. *Addiction* **95**, S91–S117 (2000).
4. Koob, G. F., Sanna, P. P. & Bloom, F. E. Neuroscience of addiction. *Neuron* **21**, 467–476 (1998).
5. Robbins, T. W. & Everitt, B. J. Neurobehavioural mechanisms of reward and motivation. *Curr. Opin. Neurobiol.* **6**, 228–236 (1996).
6. Self, D. W. & Nestler, E. J. Relapse to drug seeking: neural and molecular mechanisms. *Drug Alcohol Dependence* **51**, 49–60 (1998).
7. Shaham, Y., Erb, S. & Stewart, J. Stress-induced relapse to heroin and cocaine seeking in rats: a review. *Brain Res. Rev.* **33**, 13–33 (2000).
   Relapse to drug use after some period of abstinence is one of the cardinal features of addiction. An important goal of current drug-abuse research is to understand the neurobiological mechanisms of relapse and to develop improved treatments. Shaham and colleagues provide an outstanding review of this field, and highlight the considerable advances that have been made in developing better animal models of relapse and in using these models to study the underlying neurobiology.
8. Nestler, E. J., Hope, B. T. & Widnell, K. L. Drug addiction: a model for the molecular basis of neural plasticity. *Neuron* **11**, 995–1006 (1993).
9. Berke J. D. & Hyman, S. E. Addiction, dopamine, and the molecular mechanisms of memory. *Neuron* **25**, 515–532 (2000).
10. Carey, M. & Smale, S. T. *Transcriptional Regulation in Eukaryotes* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2000).
11. Nestler, E. J. & Aghajanian, G. K. Molecular and cellular basis of addiction. *Science* **278**, 58–63 (1997).
12. Shaywitz, A. J. & Greenberg, M. E. CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu. Rev. Biochem.* **68**, 821–861 (1999).
13. De Cesare, D. & Sassone-Corsi, P. Transcriptional regulation by cyclic AMP-responsive factors. *Prog. Nucl. Acid Res. Mol. Biol.* **64**, 343–369 (2000).
14. Guitart, X. *et al.* Regulation of CREB phosphorylation by acute and chronic morphine in the rat locus coeruleus. *J. Neurochem.* **5**, 1168–1171 (1992).
15. Sharma, S. K., Klee, W. A. & Nirenberg, M. Dual regulation of adenylate cyclase accounts for narcotic dependence and tolerance. *Proc. Natl Acad. Sci. USA* **72**, 3092–3096 (1975).
16. Terwilliger, R. Z., Beitner-Johnson, D., Sevarino, K. A., Crain, S. M. & Nestler, E. J. A general role for adaptations in G-proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function. *Brain Res.* **548**, 100–110 (1991).
17. Unterwald, E. M., Cox, B. M., Kreek, M. J., Cote, T. E. & Izenwasser, S. Chronic repeated cocaine administration alters basal and opioid-regulated adenylyl cyclase activity. *Synapse* **15**, 33–38 (1993).
18. Bonci, A. & Williams, J. T. Increased probability of GABA release during withdrawal from morphine. *J. Neurosci.* **17**, 796–803 (1997).
19. Jolas, T., Nestler, E. J. & Aghajanian, G. K. Chronic morphine increases GABA tone on serotonergic neurons of the dorsal raphe nucleus: association with an upregulation of the cyclic AMP pathway. *Neuroscience* **95**, 433–443 (2000).
20. Chakrabarti, S., Rivera, M., Yan, S. Z., Tang, W. J. & Gintzler, A. R. Chronic morphine augments Gβγ/Gsα stimulation of adenylyl cyclase: relevance to opioid tolerance. *Mol. Pharmacol.* **54**, 655–662 (1998).
21. Lane-Ladd, S. B. *et al.* CREB (cAMP response element binding protein) in the locus coeruleus: biochemical, physiological, and behavioral evidence for a role in opiate dependence. *J. Neurosci.* **17**, 7890–7901 (1997).
22. Widnell, K. L., Russell, D. & Nestler, E. J. Regulation of cAMP response element binding protein in the locus coeruleus *in vivo* and in a locus coeruleus-like (CATH.a) cell line *in vitro*. *Proc. Natl Acad. Sci. USA* **91**, 10947–10951 (1994).
23. Coven, E. *et al.* Cell-type specific regulation of CREB gene expression: mutational analysis of CREB promoter activity. *J. Neurochem.* **71**, 1865–1874 (1998).
24. Chao, J. R., Ni, Y. G., Chen, J. S., Rahman, Z., & Nestler, E. J. Characterization of the mouse adenylyl cyclase type VIII gene promoter: activiation by cAMP. *Soc. Neurosci. Abstr.* **26**, 125 (2000).
25. Maldonado, R. *et al.* Reduction of morphine abstinence in mice with a mutation in the gene encoding CREB. *Science* **273**, 657–659 (1996).
26. Chen, J. S. *et al.* Transgenic animals with inducible, targeted gene expression in brain. *Mol. Pharmacol.* **54**, 495–503 (1998).
27. Cole, R. L., Konradi, C., Douglass, J. & Hyman, S. E. Neuronal adaptation to amphetamine and dopamine: molecular mechanisms of prodynorphin gene regulation in rat striatum. *Neuron* **14**, 813–823 (1995).
28. Turgeon, S. M., Pollack, A. E. & Fink, J. S. Enhanced CREB phosphorylation and changes in c-Fos and FRA expression in striatum accompany amphetamine sensitization. *Brain Res.* **749**, 120–126 (1997).
29. Shaw, T. Z. *et al.* Upregulation of CRE-mediated transcription during naltrexone-precipitated opiate withdrawal. *Soc. Neurosci. Abstr.* **25**, 422 (1999).
30. Carlezon, W. A. Jr *et al.* Regulation of cocaine reward by CREB. *Science* **282**, 2272–2275 (1998).
31. Barrot, M., Olivier, J. D. A., Zachariou, V., Neve, R. L. & Nestler, E. J. Influence of CREB in the nucleus accumbens shell on the sensitivity to aversive and nociceptive stimuli. *Soc. Neurosci. Abstr.* **26**, 485 (2000).
32. Self, D. W. *et al.* Involvement of cAMP-dependent protein kinase in the nucleus accumbens in cocaine self-administration and relapse of cocaine-seeking behavior. *J. Neurosci.* **18**, 1848–1859 (1998).
33. Nestler, E. J. Genes and addiction. *Nature Genet.* **26**, 277–281 (2000).
34. Hyman, S. E. Addiction to cocaine and amphetamine. *Neuron* **16**, 901–904 (1996).
35. Kreek, M. J. Opiate and cocaine addictions: challenge for pharmacotherapies. *Pharmacol. Biochem. Behav.* **57**, 551–569 (1997).
36. Shippenberg, T. S. & Rea, W. Sensitization to the behavioral effects of cocaine: modulation by dynorphin and kappa-opioid receptor agonists. *Pharmacol. Biochem. Behav.* **57**, 449–455 (1997).
37. Daunais, J. B. & McGinty, J. F. Cocaine binges differentially alter striatal preprodynorphin and zif/268 mRNAs. *Mol. Brain Res.* **29**, 201–210 (1995).
38. Spangler, R. *et al.* Regulation of kappa opioid receptor mRNA in the rat brain by 'binge' pattern cocaine administration and correlation with preprodynorphin mRNA. *Mol. Brain Res.* **38**, 71–76 (1996).
39. Morgan, J. I. & Curran, T. Immediate-early genes: ten years on. *Trends Neurosci.* **18**, 66–67 (1995).
40. Hope, B. T. *et al.* Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron* **13**, 1235–1244 (1994).
41. Moratalla, R., Elibol, B., Vallejo, M. & Graybiel, A. M. Network-level changes in expression of inducible Fos-Jun proteins in the striatum during chronic cocaine treatment and withdrawal. *Neuron* **17**, 147–156 (1996).
42. Kelz, M. B. & Nestler, E. J. ΔFosB: A molecular switch underlying long-term neural plasticity. *Curr. Opin. Neurol.* **13**, 715–720 (2000).
43. Hiroi, N. & Graybiel, A. M. Atypical and typical neuroleptic treatments induce distinct programs of transcription factor expression in the striatum. *J. Comp. Neurol.* **374**, 70–83 (1996).
44. Atkins, J. *et al.* Region-specific induction of ΔFosB by repeated administration of typical versus atypical antipsychotic drugs. *Synapse* **33**, 118–128 (1999).
45. Kelz, M. B. *et al.* Expression of the transcription factor ΔFosB in the brain controls sensitivity to cocaine. *Nature* **401**, 272–276 (1999).
   The authors used the tetracycline-driven gene expression system to generate bitransgenic mice that overexpress ΔFosB selectively within the nucleus accumbens and dorsal striatum in an inducible manner. These mice overcome limitations of conventional transgenics owing to the inducibility and tissue selectivity of transgene expression. The authors show that induction of ΔFosB in these brain regions of adult animals markedly increases the sensitivity to the locomotor-activating and rewarding properties of cocaine.
46. Whisler, K., Kelz, M. B., Chen, J. S., Nestler, E. J. & Self, D. W. Effects of conditional overexpression of ΔFosB in nucleus accumbens on cocaine self-administration and relapse to cocaine-seeking behavior. *Soc. Neurosci. Abstr.* **25**, 811 (1999).
47. Peakman, M.-C. *et al.* Inducible brain-region specific expression of Δc-Jun in transgenic mice decreases sensitivity to cocaine. *Soc. Neurosci. Abstr.* **26**, 124 (2000).
48. Hiroi, N. *et al.* FosB mutant mice: loss of chronic cocaine induction of Fos-related proteins and heightened sensitivity to cocaine's psychomotor and rewarding effects. *Proc. Natl Acad. Sci. USA* **94**, 10397–10402 (1997).
49. White, F. J., Hu, X.-T., Zhang, X.-F. & Wolf, M. E. Repeated administration of cocaine or amphetamine alters neuronal responses to glutamate in the mesoaccumbens dopamine system. *J. Pharmacol. Exp. Ther.* **273**, 445–454 (1995).
50. O'Donovan, K. J., Tourtellotte, W. G., Millbrandt, J. & Baraban, J. M. The EGR family of transcription-regulatory factors: progress at the interface of molecular and systems neuroscience. *Trends Neurosci.* **22**, 167–173 (1999).
51. Mackler, S. A. *et al.* NAC-1 is a brain POZ/BTB protein that can prevent cocaine-induced sensitization in the rat. *J. Neurosci.* **20**, 6210–6217 (2000).
   The authors show that overexpression of Nac-1, a protein regulated by cocaine administration in the nucleus accumbens, antagonizes locomotor sensitization induced by repeated cocaine administration. Thus, cocaine induction of Nac-1, like cocaine activation of CREB, would seem to represent a homeostatic adaptation that serves to diminish responsiveness to subsequent drug exposures.
52. Piazza, P. V. & Le Moal, M. Glucocorticoids as a biological substrate of reward: physiological and pathophysiological implications. *Brain Res. Rev.* **25**, 359–372 (1997).
53. Tasken, K. *et al.* Structure, function, and regulation of human cAMP-dependent protein kinases. *Adv. Second Messenger Phosphoprotein Res.* **31**, 191–204 (1997).
54. Boundy, V. A., Chen, J. & Nestler, E. J. Regulation of cAMP-dependent protein kinase subunit expression in CATH.a and SH-SY5Y cells. *J. Pharmacol. Exp. Ther.* **286**, 1058–1065 (1998).
55. Lefkowitz, R. J. G protein-coupled receptors. III. New roles for receptor kinases and beta-arrestins in receptor signaling and desensitization. *J. Biol. Chem.* **273**, 18677–18680 (1998).
56. Whistler, J. L., Chuang, H. H., Chu, P., Jan, L. Y. & Von Zastrow, M. Functional dissociation of mu opioid receptor signaling and endocytosis: implications for the biology of opiate tolerance and addiction. *Neuron* **23**, 737–746 (1999).
   Tolerance to the analgesic effects of opiates is an impediment in the treatment of chronic pain syndromes but the molecular basis of tolerance has remained obscure. By using tagged opioid receptors

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in cell culture, this study shows that opioid-receptor tolerance probably involves internalization of the receptors and provides evidence for the role of dynamin-dependent endocytosis in this process. The study also establishes clear differences between endogenous opioid peptides versus opiate drugs, both of which are receptor agonists, in eliciting receptor internalization.

57. Ferguson, S. S., Zhang, J., Barak, L. S. & Caron, M. G. Molecular mechanisms of G protein-coupled receptor desensitization and resensitization. *Life Sci.* **62**, 1561–1565 (1998).
58. Zaki, P. A., Keith, D. E. Jr, Brine, G. A., Carroll, F. I. & Evans, C. J. Ligand-induced changes in surface mu-opioid receptor number: relationship to G protein activation? *J. Pharmacol. Exp. Ther.* **292**, 1127–1134 (2000).
59. Bohn, L. M. *et al.* Enhanced morphine analgesia in mice lacking β-arrestin-2. *Science* **286**, 2495–2498 (1999).
60. Bohn, L. M. *et al.* μ-Opioid receptor desensitization by β-arrestin-2 determines morphine tolerance but not dependence. *Nature* **408**, 720–723 (2000).
Mice lacking β-arrestin-2 show reduced tolerance to the analgesic effects of opiates after repeated drug administration, but no difference in the degree of physical dependence as compared to controls. This is the best evidence so far that the G-protein-receptor kinase–arrestin system, which is known to mediate desensitization of many types of G-protein-coupled receptors, is also crucial in opioid-receptor tolerance. In addition, the results further clarify the partly distinct mechanisms that underlie tolerance (G-protein-receptor kinase–arrestin system) versus physical dependence (upregulation of the cAMP pathway).
61. Terwilliger, R. Z., Ortiz, J., Guitart, X. & Nestler, E. J. Chronic morphine administration increases β-adrenergic receptor kinase (βARK) levels in the rat locus coeruleus. *J. Neurochem.* **63**, 1983–1986 (1994).
62. Sklair-Tavron, L. *et al.* Chronic morphine induces visible changes in the morphology of mesolimbic dopamine neurons. *Proc. Natl Acad. Sci. USA* **93**, 11202–11207 (1996).
63. Robinson, T. E. & Kolb, B. Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. *J. Neurosci.* **17**, 8491–8497 (1997).
64. Robinson, T. E. & Kolb, B. Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *Eur. J. Neurosci.* **11**, 1598–1604 (1999).
This study and reference 63 were the first to show that chronic administration of cocaine or the related psychostimulant amphetamine causes alterations in dendritic morphology in specific brain regions. The increases in dendritic length, branch points, and spine density observed in medium spiny neurons of the nucleus accumbens and in pyramidal cells of the prefrontal cortex persist for at least one month after the last drug exposure. Whereas the functional consequences of these changes remain unknown, they could mediate the long-lived sensitization that is observed in behavioural responses to these drugs.
65. Eisch, A. J., Barrot, M., Schad, C. A., Self, D. W. & Nestler, E. J. Opiates inhibit neurogenesis in the adult rat hippocampus. *Proc. Natl Acad. Sci. USA* **97**, 7579–7584 (2000).
66. Gage, F. H. Mammalian neural stem cells. *Science* **287**, 1433–1438 (2000).
67. Fuchs, E. & Gould, E. Mini-review: *in vivo* neurogenesis in the adult brain: regulation and functional implications. *Eur. J. Neurosci.* **12**, 2211–2214 (2000).
68. Horger, B. A. *et al.* Enhancement of locomotor activity and conditioned reward to cocaine by brain-derived neurotrophic factor. *J. Neurosci.* **19**, 4110–4122 (1999).
69. Pierce, R. C., Pierce-Bancroft, A. F. & Prasad, B. M. Neurotrophin-3 contributes to the initiation of behavioral sensitization to cocaine by activating the Ras/Mitogen-activated protein kinase signal transduction cascade. *J. Neurosci.* **19**, 8685–8695 (1999).
70. Messer, C. J. *et al.* Role of GDNF in biochemical and behavioral adaptations to drugs of abuse. *Neuron* **26**, 247–257 (2000).
The authors provide evidence for a role of glial-cell-derived neurotrophic factor (GDNF) in mediating the adaptations to chronic opiate and cocaine administration. GDNF attenuates several biochemical adaptations that are normally induced in the ventral tegmental area (VTA) and the nucleus accumbens (NAc) after repeated opiate or cocaine exposure, as well as certain behavioural responses to the drugs. By contrast, biochemical and behavioural responses to cocaine are enhanced both by an antibody that neutralizes the activity of endogenous GDNF, and by knockout of the *GDNF* gene. Moreover, chronic opiate or cocaine administration reduces the activity of Ret, the tyrosine kinase that mediates the effects of GDNF. These data imply a feedback loop, whereby chronic drug exposure attenuates GDNF signalling, which disrupts the normal influence that GDNF exerts on the VTA–NAc pathway leading to sensitized responses to the drugs.
71. Wolf, D. H., Numan, S., Nestler, E. J. & Russell, D. S. Regulation of phospholipase Cγ in the mesolimbic dopamine system by chronic morphine administration. *J. Neurochem.* **73**, 1520–1528 (1999).
72. Berhow, M. T., Hiroi, N. & Nestler, E. J. Regulation of ERK (extracellular signal regulated kinase), part of the neurotrophin signal transduction cascade, in the rat mesolimbic dopamine system by chronic exposure to morphine or cocaine. *J. Neurosci.* **16**, 4707–4715 (1996).
73. Flores, C., Rodaros, D. & Stewart, J. Long-lasting induction of astrocytic basic fibroblast growth factor by repeated injections of amphetamine: blockade by concurrent treatment with a glutamate antagonist. *J. Neurosci.* **18**, 9547–9555 (1998).
74. Bibb, J. A. *et al.* Cdk5 regulates action of chronic cocaine. *Nature* (in the press).
75. Chen, J. S. *et al.* Induction of cyclin-dependent kinase 5 in hippocampus by chronic electroconvulsive seizures: Role of ΔFosB. *J. Neurosci.* **20**, 8965–8971 (2000).
76. Martin, K. C. & Kandel, E. R. Cell adhesion molecules, CREB, and the formation of new synaptic connections. *Neuron* **17**, 567–570 (1996).
77. Silva, A. J. & Murphy, G. G. cAMP and memory: a seminal lesson from *Drosophila* and *Aplysia*. *Brain Res. Bull.* **50**, 441–442 (1999).
78. Deisseroth, K., Bito, H., Schulman, H. & Tsien, R. W. Synaptic plasticity: A molecular mechanism for metaplasticity. *Curr. Biol.* **5**, 1334–1338 (1995).
79. Yin, J. C. & Tully, T. CREB and the formation of long-term memory. *Curr. Opin. Neurobiol.* **6**, 264–268 (1996).
80. Schuman, E. M. Neurotrophin regulation of synaptic transmission. *Curr. Opin. Neurobiol.* **9**, 105–109 (1999).
81. Korte, M., Staiger, V., Griesbeck, O., Thoenen, H. & Bonhoeffer, T. The involvement of brain-derived neurotrophic factor in hippocampal long-term potentiation revealed by gene targeting experiments. *J. Physiol. (Paris)* **90**, 157–164 (1996).
82. Luscher, C., Nicoll, R. A., Malenka, R. C., & Muller, D. Synaptic plasticity and dynamic modulation of the postsynaptic membrane. *Nature Neurosci.* **3**, 545–550 (2000).
83. Nicola, S. M., Surmeier, J. & Malenka, R. C. Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annu. Rev. Neurosci.* **23**, 185–215 (2000).
A timely review of the physiological effects of dopamine on medium spiny neurons of the nucleus accumbens and dorsal striatum. It discusses dopamine modulation of ion channels through its actions on several subtypes of dopamine receptors. It also covers recent literature on the occurrence of long-term potentiation and long-term depression in these brain regions. Given the central role of dopamine-mediated transmission in drug reinforcement, this review provides a template within which the complex actions of drugs of abuse on the nucleus accumbens can be understood.
84. Jones, S., Kornblum, J. L. & Kauer, J. A. Amphetamine blocks long-term synaptic depression in the ventral tegmental area. *J. Neurosci.* **20**, 5575–5580 (2000).
Shows the development of long-term depression (LTD) at glutamate synapses in the ventral tegmental area (VTA). The authors show that amphetamine, by potentiating the actions of dopamine on D_2-like receptors, completely abolishes LTD in the VTA. This effect could contribute to the demonstrated ability of amphetamine (and perhaps other drugs of abuse) to potentiate glutamate responses in the VTA (for example, see references 49,88) and possibly to cause sensitized behavioural responses as well.
85. Thomas, M. J., Malenka, R. C. & Bonci, A. Modulation of long-term depression by dopamine in the mesolimbic system. *J. Neurosci.* **20**, 5581–5586 (2000).
This study, like reference 84, shows that long-term depression (LTD) occurs at glutamate synapses in the ventral tegmental area (VTA), and that LTD is inhibited by dopamine acting at D_2-like receptors. The authors also show LTD at glutamatergic synapses on medium spiny neurons of the nucleus accumbens, although no effect of dopamine on LTD was seen in this region.
86. Malinow, R., Mainen, Z. F., & Hayashi, Y. LTP mechanisms: from silence to four-lane traffic. *Curr. Opin. Neurobiol.* **10**, 352–357 (2000).
87. Scannevin, R. H. & Huganir, R. L. Postsynaptic organization and regulation of excitatory synapses. *Nature Rev. Neurosci.* **1**, 133–141 (2000).
88. Carlezon, W. A. Jr *et al.* Sensitization to morphine induced by viral-mediated gene transfer. *Science* **277**, 812–814 (1997).
89. Wolf, M. E. The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. *Prog. Neurobiol.* **54**, 679–720 (1998).
90. Bell, K., Duffy, P. & Kalivas, P. W. Context-specific enhancement of glutamate transmission by cocaine. *Neuropsychopharmacology* **23**, 335–344 (2000).
91. Li, Y. *et al.* Both glutamate receptor antagonists and prefrontal cortex lesions prevent induction of cocaine sensitization and associated neuroadaptations. *Synapse* **34**, 169–180 (1999).

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