



Historical review: Molecular and cellular mechanisms of opiate and cocaine addiction

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The National Institute on Drug Abuse was founded in 1974, and since that time there have been significant advances in understanding the processes by which drugs of abuse cause addiction. The initial protein targets for almost all drugs of abuse are now known. Animal models that replicate key features of addiction are available, and these models have made it possible to characterize the brain regions that are important for addiction and other drug effects, such as physical dependence. A large number of drug-induced changes at the molecular and cellular levels have been identified in these brain areas and rapid progress is being made in relating individual changes to specific behavioral abnormalities in animal models of addiction. The current challenges are to translate this increasingly impressive knowledge of the basic neurobiology of addiction to human addicts, and to identify the specific genes that make some individuals either particularly vulnerable or resistant to addiction. In this article, I present a historical review of basic research on opiate and cocaine addiction.

Turn back the clock 30 years. We knew of a range of drugs that caused addiction in some people. A good deal of information was available concerning the behavioral actions of these drugs in animal models and, in some cases, we knew which neurotransmitter system was affected initially by the drugs. It was presumed that, after repeated administration, the drugs induce long-lasting changes in the brain that caused addiction, but the nature of these changes, and the brain areas in which they occur, were almost completely obscure. Addiction was being recognized increasingly as a leading cause of death, morbidity and lost productivity in the USA. The urgent clinical and social need to do something about addiction, along with the maturation of the neurosciences as a field of biomedical research, led, in 1974, to the creation of the National Institute on Drug Abuse (NIDA) as part of the Alcohol, Drug Abuse, and Mental Health Administration (ADAMHA). It wasn't until 1992 that NIDA and the other ADAMHA Institutes (National Institute of Mental Health, and National Institute of Alcohol Abuse and Alcoholism) joined NIH. Although we still lack fundamental knowledge of the causes of addiction, and certainly lack definitive treatments and cures for many patients, there

is no question that the last three decades have marked extraordinary progress in our efforts to tackle drug addiction.

Initial targets of drugs of abuse

By 1974 we knew the basic framework by which many drugs of abuse produce their immediate effects on the nervous system. It was presumed that opiates acted on endogenous opioid receptors, although such 'receptors' had not yet been defined biochemically, let alone cloned molecularly. Psychostimulants such as cocaine and amphetamine were thought to regulate either monoamine reuptake or release. However, the precise molecular target of the drugs was not known, nor was it appreciated which monoamine was the most important for the addicting actions of the drugs. Nicotine was believed to activate nicotinic acetylcholine (nACh) receptors, but, at the time, these receptors had been characterized in *Torpedo* electroplax and related preparations, and little was known about nACh receptors in brain. Alcohol was known to affect numerous neurochemical processes in the brain, but the proximal mechanisms of its effects were obscure. The major hallucinogens had been characterized for their effects on 5-hydroxytryptamine systems in brain, but there was little insight into their initial protein target. And virtually nothing was known about the actions of other commonly abused drugs, such as cannabinoids, phencyclidine and inhalants.

One of the most dramatic advances in drug-abuse research over the past three decades has been the definitive identification of the molecular target for almost every major drug of abuse (Table 1). This advance occurred with the advent of radioligand-binding techniques and the biochemical characterization of drug-binding sites and, ultimately, with the application of molecular biology to clone and isolate these targets. In retrospect, given that drugs of abuse differ greatly in their chemical structure, it is not surprising that each was found to act on its own unique protein target. It is also striking that all drug-abuse targets identified to date are proteins that are involved in synaptic transmission, although different drugs affect different neurotransmitter systems.

The process of identifying initial drug targets has been reviewed recently [1,2] and is not discussed further in this article. Nevertheless, it is important to appreciate that, in addition to improving our understanding of drug action, these discoveries had a major impact on neuroscience in

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Table 1. Initial targets of drugs of abuse

Drug	Properties
Opiates	Agonist at mu, delta and kappa opioid peptide receptors ^a
Cocaine	Indirect agonist at dopamine receptors by inhibiting dopamine transporters ^b
Amphetamine and related stimulants ^c	Indirect agonist at dopamine receptors by stimulating dopamine release ^b
Ethanol	Facilitates GABA _A and inhibits NMDA glutamate receptor function ^d
Nicotine	Agonist at nicotinic acetylcholine receptors
Cannabinoids	Agonist at cannabinoid CB ₁ and CB ₂ receptors ^e
Phencyclidine	Antagonist at NMDA glutamate receptors
Hallucinogens	Partial agonist at 5-HT _{2A} receptors
Inhalants	Unknown

^aActivity at mu and, possibly, delta opioid peptide receptors mediates the addictive effects of opiates. Kappa opioid peptide receptors mediate aversive actions.

^bCocaine and amphetamine exert analogous actions on 5-hydroxytryptamine and noradrenaline systems, which might also contribute to the addictive effects of these drugs.

^cFor example, methamphetamine and methylphenidate.

^dEthanol affects several other ligand-gated channels and, at higher concentrations, voltage-gated channels. In addition, ethanol is reported to influence many other neurotransmitter systems.

^eActivity at cannabinoid CB₁ receptors mediates the addictive effects of cannabinoids; CB₂ receptors are expressed only in the periphery. Proposed endogenous ligands for the CB₁ receptor include the arachidonic acid metabolites anandamide and 2-arachidonylglycerol.

general. Specifically, by defining several novel neurotransmitter and receptor systems that influence brain function and behavior, they increased our understanding of chemical transmission in the brain. Accordingly, these discoveries assisted the development of new medications for numerous psychiatric and neurological disorders and continue to drive CNS drug discovery today.

One early disappointment in the field was the difficulty in accounting for several key features of addiction on the basis of drug regulation of their initial targets. For example, it was widely theorized in the mid 1970s that drug tolerance (diminished drug effect with repeated use) and drug dependence (an altered physiological state that results in a withdrawal syndrome when the drug is withdrawn) could be explained by a relatively simple upregulation or downregulation of the drug target. However, a vast amount of early research failed to find consistent changes in opioid receptors, monoamine transporters and other targets that could account for drug tolerance and dependence. Ironically, more recent work has identified important adaptations in some of these targets that appear to be crucial for addiction (for example [3,4]). Nevertheless, the early failures stimulated the field to look beyond the receptor to possible drug-induced changes in post-receptor signal transduction pathways that were being discovered in the brain at that time.

The cAMP pathway in opiate tolerance and dependence

The roles of cAMP and cAMP-dependent protein kinase (PKA) in mediating the effects of β -adrenoceptors on glycogen metabolism in liver and skeletal muscle were described in the 1950s and 1960s. It was only in the late 1960s and early 1970s that cAMP and PKA were implicated in neural-specific phenomena, in particular, the regulation of synaptic transmission [5]. Although controversial at the time, such a role for cAMP and PKA became well established during the ensuing decade and was expanded to include numerous families of second messengers and protein kinases.

In 1975, this evolving understanding of the molecular basis of synaptic transmission was applied to drug addiction. Sharma, Klee and Nirenberg [6] added morphine to neuroblastoma \times glioma cells in culture and demonstrated that morphine initially decreases cellular levels of cAMP. However, with continued exposure, cAMP levels recover to

normal and, on addition of an opioid receptor antagonist, cAMP levels increase far above control values. These observations, which are summarized in Figure 1, indicate that tolerance and dependence-like phenomena can be seen at the single-cell level, and caused the authors to hypothesize that adaptations in the cAMP pathway contribute to opiate tolerance and dependence. Collier and colleagues independently provided similar lines of evidence for the involvement of the cAMP pathway in opiate dependence [7].

The next major advance was the application of this hypothesis to the brain, in particular, to neurons of the locus coeruleus (LC), which is the major noradrenaline-containing nucleus in the brain. These neurons were shown to develop opiate tolerance and dependence at the cellular level: acutely, opiates decrease the firing rate of LC neurons; the

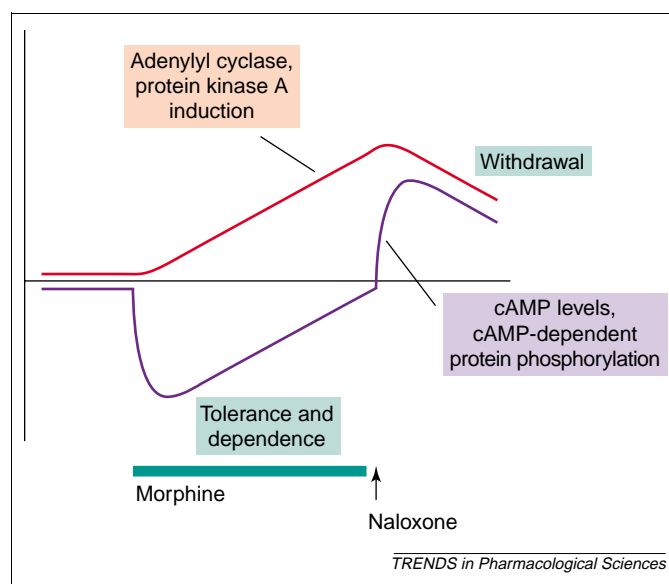


Figure 1. Upregulation of the cAMP pathway as a mechanism of opiate tolerance and dependence. Opiates acutely inhibit the functional activity of the cAMP pathway (indicated by cellular levels of cAMP and cAMP-dependent protein phosphorylation). With continued opiate exposure, functional activity of the cAMP pathway gradually recovers, and increases far above control levels following removal of the opiate (e.g. by administration of the opioid receptor antagonist naloxone). These changes in the functional state of the cAMP pathway are mediated via the induction of adenylyl cyclase and protein kinase A (PKA) in response to chronic administration of opiates. Induction of these enzymes accounts for the gradual recovery in the functional activity of the cAMP pathway that occurs during chronic opiate exposure (tolerance and dependence) and activation of the cAMP pathway observed on removal of opiate (withdrawal).

firing rate recovers toward normal with continued opiate exposure; and the firing increases several-fold above normal levels on administration of an opioid receptor antagonist [8]. In addition, a role of cAMP in partly mediating the acute electrophysiological actions of opiates on these neurons was suggested [9]. This information led our group to investigate opiate actions on the cAMP pathway in the LC in greater detail. We demonstrated the same general changes in this pathway in the LC *in vivo* as reported earlier for cells in culture: opiates acutely inhibit adenylyl cyclase and cAMP-dependent protein phosphorylation in the LC; this inhibition recovers with chronic opiate administration (tolerance); and increases far above normal upon administration of an opioid receptor antagonist (dependence and withdrawal) [10–12]. Work on the LC also provided possible molecular mechanisms by which these changes might occur. Thus, during chronic opiate administration, several isoforms of adenylyl cyclase and PKA subunits are induced in LC neurons. This can account for the changes in the functional activity of the cAMP pathway that are observed during the development of tolerance and dependence and during withdrawal (Figures 1 and 2) [10–14].

It has been known for many years that the LC is an important neural substrate for physical dependence on opiates [15–17]. By contrast, other noradrenaline-containing nuclei in brain have been implicated recently in the motivational aspects of addiction [18]. Over the years, it has been possible to directly relate upregulation of the cAMP pathway in the LC to the electrophysiological changes that occur in these neurons following chronic opiate administration and to physical dependence and withdrawal [15–23]. Although some authors question the role of the LC and the cAMP pathway in the LC in opiate withdrawal [24], such a role has been confirmed by several groups. In addition, knowledge of opiate action in the LC led to the introduction of clonidine, an α_2 -adrenoceptor agonist, as the first non-opiate treatment of physical opiate-withdrawal syndromes [25]. Moreover, the contribution of the cAMP pathway to opiate dependence in the LC has largely guided studies of addiction of opiates and other drugs in many other brain regions.

Thus, subsequent to the discoveries in the LC, several laboratories provided evidence that similar upregulation of the cAMP pathway occurs in several regions of the CNS and PNS and, thereby, accounts for the diverse aspects of opiate action that are mediated by these neural circuits (Table 2) [26–30]. For example, although upregulation of

the cAMP pathway in the LC contributes to physical opiate dependence, similar biochemical adaptations in reward regions of the brain [e.g. nucleus accumbens (NAc)] contribute to changes in drug reward and addiction (discussed later).

Molecular basis of opiate dependence in the LC

A current challenge in the field is to better understand the mechanism by which chronic administration of opiates upregulates adenylyl cyclase and PKA. Several lines of evidence indicate a role for the cAMP-response-element-binding protein (CREB) transcription factor. Phosphorylation of CREB is inhibited acutely by opiates in the LC, recovers during chronic opiate treatment, and increases above normal during withdrawal [31]. These functional changes might be mediated, in part, by the induction of CREB expression by morphine in this brain region, similar to the scheme shown in Figure 1 [32]. Accordingly, CREB-mediated transcription is increased in the LC and elsewhere by chronic administration of opiates, and is increased even further during withdrawal [33]. Moreover, knockdown of CREB levels in the LC using antisense oligonucleotides blocks the ability of chronic opiates to upregulate some, but not all, components of the cAMP pathway (Figure 2) [14]. Such knockdown also blocks some of the effects of morphine on the electrophysiological state of LC neurons, and attenuates the development of physical dependence on opiates and withdrawal. Similar results have been obtained more recently by either increasing or decreasing CREB levels in the LC using viral-mediated gene transfer [34]. These findings are consistent with the observation that mice that are globally deficient in CREB develop lower levels of physical dependence on opiates and withdrawal [35]. A remaining mystery, however, is the mechanism by which opiate exposure switches from acute inhibition of the cAMP pathway and CREB to chronic upregulation.

Neural circuitry of reinforcement and addiction

Our conceptualization of drug addiction was confused for decades by the fact that key drugs of abuse, such as opiates and alcohol, cause physical dependence as well as addiction. For this reason, the clinical definition of addiction became intertwined with definitions of dependence. Remnants of this confusion persist to the present day and the *Diagnostic and Statistical Manual* (American Psychiatric Association) [36] continues to categorize addiction as ‘drug dependence’.

Table 2. Upregulation of the cAMP pathway in opiate addiction

Site of upregulation	Functional consequence
Locus coeruleus ^a	Physical dependence and withdrawal
Ventral tegmental area ^b	Dysphoria during early withdrawal periods
Periaqueductal gray ^b	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 reduction in intestinal motility and increase in motility during withdrawal

^aThe cAMP pathway is upregulated in the principal noradrenaline-containing neurons located in this region.

^bIndirect evidence indicates that the cAMP pathway is upregulated in GABA-containing neurons that innervate dopamine- and 5-hydroxytryptamine (5-HT)-containing neurons in the ventral tegmental area and periaqueductal gray, respectively. During withdrawal, this upregulated cAMP pathway should become fully functional and might contribute to a state of dysphoria by increasing the release of GABA, which would inhibit the dopamine-containing and 5-HT-containing neurons [27,30].

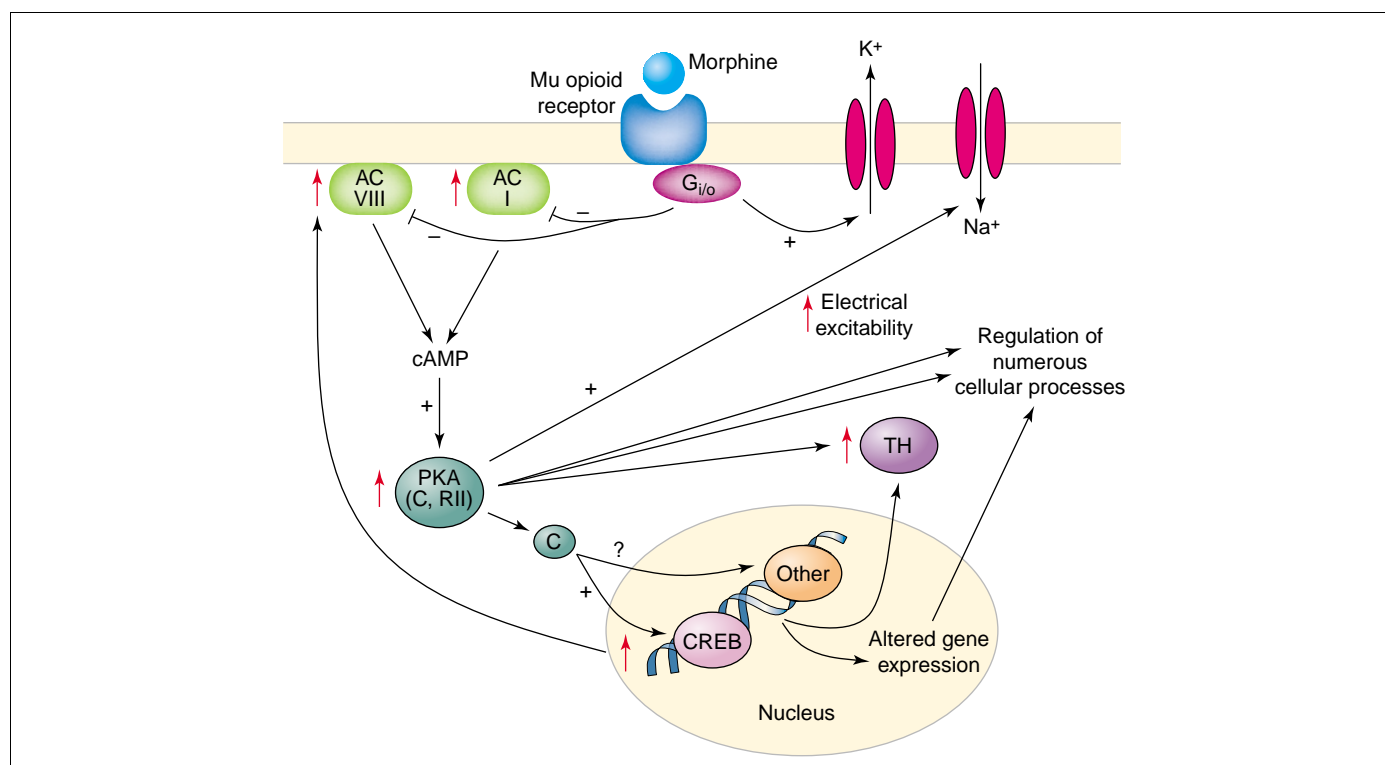


Figure 2. Opiate actions in the locus coeruleus (LC). Opiates acutely inhibit LC neurons by increasing the conductance of an inwardly rectifying K^+ channel via coupling with subtypes of $G_{i/o}$ and, possibly, by decreasing an Na^+ -dependent inward current via coupling with $G_{i/o}$ and the consequent inhibition of adenylyl cyclase (AC), reduced levels of protein kinase A (PKA) activity and reduced phosphorylation of the channel or pump responsible. Inhibition of the cAMP pathway also decreases the phosphorylation of many other proteins and, thereby, affects numerous other neuronal processes. For example, it reduces the phosphorylation state of cAMP response element-binding protein (CREB), which might initiate some of the longer-term changes in LC function. Chronic administration of morphine increases the levels of AC I, AC VIII, PKA catalytic (C) and regulatory (RII) subunits, and several phosphoproteins, including CREB and tyrosine hydroxylase [TH (the rate-limiting enzyme in noradrenaline biosynthesis)] (indicated by red arrows). These changes contribute to the altered phenotype of the drug-addicted state. For example, the intrinsic excitability of LC neurons is increased by enhanced activity of the cAMP pathway and Na^+ -dependent inward current, which contributes to the tolerance, dependence and withdrawal exhibited by these neurons. Upregulation of AC VIII and TH is mediated via CREB, whereas upregulation of AC I and of the PKA subunits appears to occur via an unidentified, CREB-independent mechanism. Reproduced, with permission, from [28]. ©1997. American Association for the Advancement of Science.

However, during the 1970s and 1980s it became increasingly clear that physical dependence is a largely separable phenomenon from addiction. Some drugs that are very addictive (e.g. cocaine) do not produce prominent physical dependence. Moreover, Olds and colleagues [37] demonstrated that rodents work to electrically stimulate relatively discrete areas of brain, which demonstrates the existence of, so-called, brain-reward regions. Subsequently, several groups found that rodents also work to self-administer drugs of abuse (but not other drugs) and that this self-administration behavior is disrupted by lesioning these brain-reward regions [38–42]. By contrast, physical dependence and withdrawal to opiates and >alcohol are mediated largely by distinct CNS regions. We now know that the most important brain-reward circuit involves dopamine-containing neurons in the ventral tegmental area (VTA) of the midbrain and their target areas in the limbic forebrain, in particular, the NAc and frontal regions of cerebral cortex (Figure 3). In fact, the VTA–NAc pathway seems to be a site where virtually all drugs of abuse converge to produce their acute reward signals. Two major mechanisms are involved: First, all drugs of abuse increase dopamine-mediated transmission in the NAc, although by very different mechanisms; second, some drugs also act directly on NAc neurons by dopamine-independent mechanisms [38–42].

This evolving knowledge of drug action on the VTA–NAc pathway represented a major advance in the field. It provided insight into mechanisms of drug addiction and the neurobiology of motivation in general [42,43]. The discovery that dopamine-containing neurons regulate behavioral responses to food [44] and drugs of abuse [45] as far back in evolution as worms and flies underscored the primal power of this reward circuit. It also helped focus investigators' attention on possible neural substrates of compulsive behavior to non-drug rewards, in conditions such as pathological overeating, pathological gambling and sex addictions. Abnormalities in these brain-reward regions have also been implicated recently in motivational defects in depression [46].

Despite these advances, it is also true that the addiction field became overly focused (one might say addicted) on this single circuit. NIDA support went overwhelmingly to researchers focused on the VTA–NAc pathway, despite the fact that it is just one part of a series of parallel, distributed circuits that are known to control reward and motivation (Figure 3). In a similar vein, the field overly invested in use of the self-administration paradigm to study drug reward. Innumerable studies examined the ability of dopamine-related and other pharmacological agents to regulate drug self-administration behavior. This focus persisted, despite the clinical knowledge that drug addiction is not, in

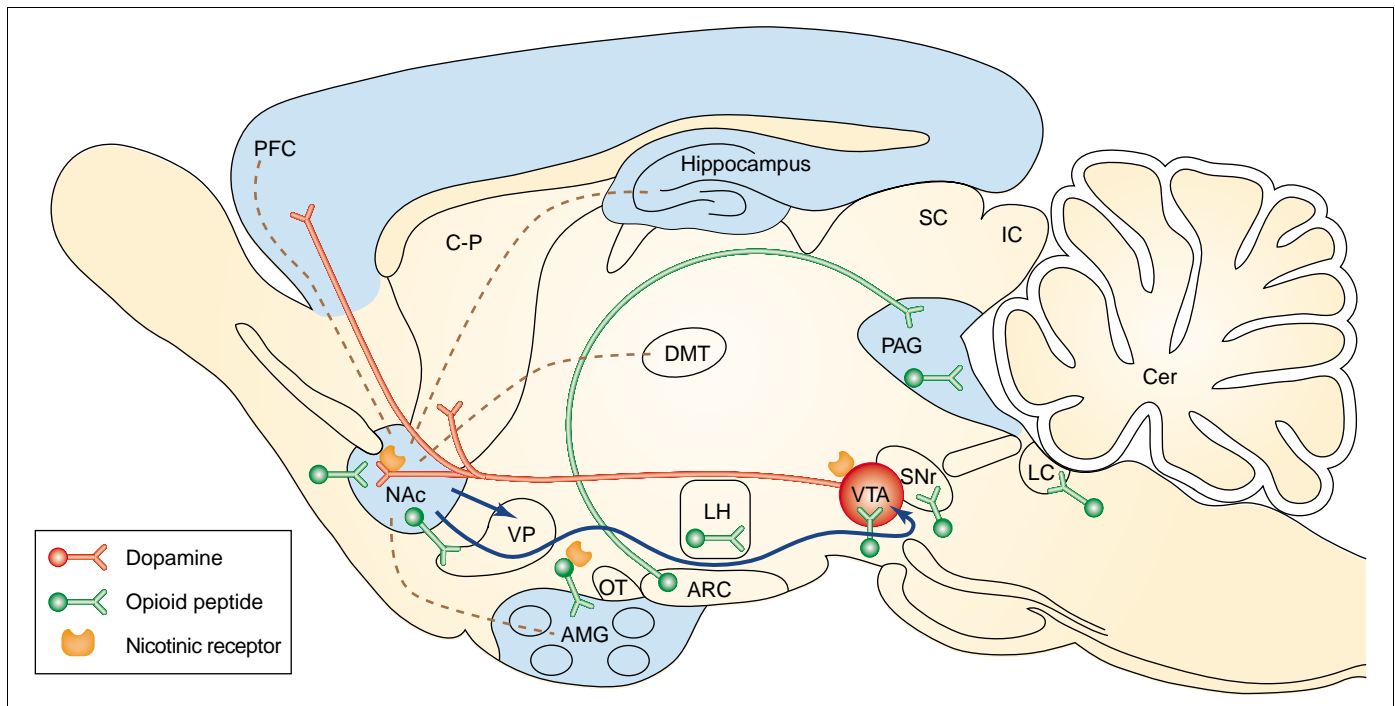


Figure 3. Key neural circuits of addiction. Limbic afferents to the nucleus accumbens (NAc) are shown as brown dashed lines. Blue arrows represent efferents from the NAc that are thought to be involved in drug reward. Projections of the mesolimbic dopamine system that are thought to be a crucial substrate for drug reward are shown in red: this system originates in the ventral tegmental area (VTA) and projects to the NAc and other limbic structures, including the prefrontal cortex (PFC), ventral domains of the caudate-putamen (C-P), the olfactory tubercle (OT) and the amygdala (AMG) (projections to the OT and AMG are not depicted). Neurons that contain opioid peptides and are involved in opiate, ethanol and, possibly, nicotine reward are shown in green. These opioid peptide systems include the local enkephalinergic circuits (short segments) and the hypothalamic midbrain β -endorphin circuit (long segment). The approximate distribution of GABA_A receptor complexes, which might contribute to ethanol reward, is shown as light blue areas. Nicotinic acetylcholine receptors, which are thought to be located on neurons that contain either dopamine or opioid peptides, are also shown. Abbreviations: ARC, arcuate nucleus; Cer, cerebellum; DMT, dorsomedial thalamus; IC, inferior colliculus; LC, locus coeruleus; LH, lateral hypothalamus; PAG, periaqueductal gray; SC, superior colliculus; SNr, substantia nigra pars reticulata; VP, ventral pallidum. Based on [16]. Reproduced, with permission, from Nature Reviews Neuroscience (<http://www.nature.com/reviews>) [30]. ©2001. Macmillan Magazines Ltd.

essence, a disorder of acute drug reward. Rather, the core feature of addiction is the compulsive seeking and taking of the drug, and the persistent risk of relapse even after years of abstinence. Although investigators started to model drug craving and relapse in animals as early as 1981 [47], it wasn't until the mid-1990s that such approaches were used to investigate the neural circuits and neurotransmitter systems that are responsible for craving and relapse [47–50]. This work confirmed the importance of the VTA–NAc pathway as a major rheostat of reward, and further emphasized the key roles played by related limbic circuits (e.g. amygdala, hippocampus and frontal cortex) in controlling the particularly long-lived features of addiction that are central to relapse [50–54]. Now, these new, behavioral approaches are providing powerful models to develop fundamentally new medications for drug addiction.

Molecular and cellular mechanisms of addiction in the VTA–NAc pathway

At the start of the 1990s, the identification of brain-reward regions and the development of increasingly sophisticated animal models of addiction made it possible to search for drug-induced changes in these regions that account for the complex behavioral abnormalities that underlie an addicted state. The vast majority of the work to date has focused, as outlined above, on the VTA–NAc, but crucial efforts directed toward other key regions are starting.

Role of the cAMP pathway and CREB

One of the earliest findings was the discovery that, like opiates, chronic administration of either cocaine or alcohol upregulates the cAMP pathway in the NAc [26,55]. As would be expected, the drugs also activate CREB in this brain region [33,56–58]. There is now compelling evidence that upregulation of the cAMP pathway and CREB in the NAc represents a mechanism of 'motivational tolerance and dependence': these molecular adaptations decrease an individual's sensitivity to the rewarding effects of subsequent drug exposures (tolerance) and impair the reward pathway (dependence) so that after removal of drug the individual is left in an amotivational, depressed-like state [59–64]. The importance of motivational tolerance and dependence for human addiction is described elsewhere [39].

The ability to relate something as reductionistic as a transcription factor to a complex behavioral endpoint required the development of new tools to manipulate gene expression in localized brain areas of adult animals. Tools such as viral-mediated gene-transfer and inducible, cell-targeted mutations in mice dramatically accelerated the establishment of causal relationships between molecules, such as CREB, and particular behavioral abnormalities that are associated with addiction.

By identifying target genes in the NAc through which the cAMP pathway and CREB produce behavioral abnormalities [65], it might be possible to develop new treatments that reverse these changes. An illustration of

this point is the opioid peptide, dynorphin, which normally suppresses activity of the VTA–NAc reward circuit by stimulating kappa opioid peptide (KOP) receptors and so inhibiting dopamine release [66]. Increasing evidence indicates that CREB induction of dynorphin is an important mediator of the CREB behavioral phenotype in this circuit (Figure 4) [57,59,61,64]. These findings have driven research to investigate the use of KOP receptor antagonists to treat the negative emotional symptoms that characterize early drug withdrawal [67].

Common molecular underpinnings of addiction

At about the same time that several drugs of abuse were shown to upregulate the cAMP pathway in the NAc, many groups reported numerous, additional adaptations in the VTA–NAc pathway, some of which are also common to several drugs of abuse. These include alterations in levels of G-protein subunits, tyrosine hydroxylase (the rate limiting enzyme in dopamine biosynthesis), neurofilament proteins, glutamate receptors and neuropeptide systems [55,68–72]. A corollary of these findings is that the cAMP pathway is just one of several intracellular signaling pathways that are altered by drug exposure. Although the precise functional consequences of most of these adaptations remain to be seen, this work has led to the notion that drugs of abuse converge, in part at least, on common neural and molecular substrates to produce acute reward and chronic changes in reward mechanisms that contribute to addiction [68].

Role of Δ FosB

One such common, chronic action of drugs of abuse is induction of another transcription factor, Δ FosB, in the NAc. In 1991, two groups found that acute administration of cocaine induced c-Fos and several other proteins of the Fos family in the NAc and dorsal striatum [73,74]. These

proteins and their mRNAs are unstable, so that levels of the proteins return to normal within 8–12 h of drug exposure. In 1992, we studied the effect of chronic administration of cocaine on this transcription factor family. Based on the ability of cocaine to induce sensitization to many of its behavioral effects, we predicted that repeated exposure to cocaine would cause progressively greater induction of Fos proteins. The opposite was observed: chronic administration of cocaine dramatically reduced the ability of subsequent exposure to induce c-Fos and other Fos proteins. Instead, it caused the accumulation of high levels of activator protein 1 (AP-1) complexes (these are transcriptionally active dimers of Fos and related, Jun-family proteins) [75]. This finding indicated that chronic administration of cocaine induced a novel Fos protein that was responsible for the persistent AP-1 activity. Indeed, over the next several years, we demonstrated that the Fos proteins responsible for this long-lived AP-1 complex are modified isoforms of Δ FosB, a truncated splice variant of the *FosB* gene [76–79]. Moreover, the longevity of this complex is caused by the extraordinary stability of Δ FosB isoforms, which is in stark contrast to other Fos family members. The molecular basis of this unique stability is a subject of current investigation.

In more recent years, we and other groups have found that virtually all drugs of abuse induce Δ FosB in the NAc (and in some cases the dorsal striatum) after chronic administration [76,80–83]. In addition, repeated consumption of non-drug rewards, for example, excessive wheel-running behavior and sucrose drinking, also induces Δ FosB in these regions [84,85]. Insight into the role played by the induction of Δ FosB in drug and other rewarding behaviors came from studies of inducible transgenic mice in which either Δ FosB or a dominant-negative antagonist of Δ FosB and other Fos proteins are overexpressed in adult animals with some selectivity in the NAc and dorsal striatum [86,87]. Overexpression of Δ FosB increases sensitivity to the locomotor-activating and rewarding effects of cocaine and morphine, increases cocaine self-administration, and increases incentive drive for cocaine in progressive ratio tests [86,88–90]. Overexpression also increases wheel-running and sucrose intake [84,90]. Conversely, expression of the dominant-negative Δ FosB decreases sensitivity to cocaine and morphine [87,89] and studies of the dominant-negative Δ FosB in other behavioral assays are ongoing. Together, these findings support the view that Δ FosB is both necessary and sufficient for sensitizing animals to drug and non-drug rewards and might even increase drive for such rewards. Moreover, because Δ FosB is stable, it can drive such behavioral changes for weeks and months after the last drug exposure. In this way, Δ FosB could be a sustained molecular switch that helps to initiate and maintain a state of addiction [85]. A recent DNA microarray study confirms the dominant role of Δ FosB in mediating the long-term effects of drugs on gene expression (Figure 5) [65].

Mechanisms of 'permanent' plasticity in addiction

However, even the induction of Δ FosB is not as long-lived as some of the behavioral abnormalities that are associated with addiction, which can last a lifetime. Consequently, a key

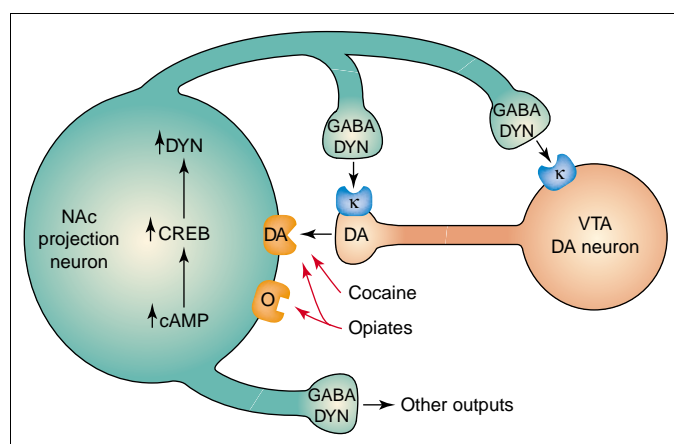


Figure 4. Regulation of cAMP response element-binding protein (CREB) by drugs of abuse. The figure shows a dopamine (DA)-containing neuron from the ventral tegmental area (VTA) that innervates a class of GABA-containing projection neurons in the nucleus accumbens (NAc) that express dynorphin (DYN). DYN acts as a negative feedback mechanism in this circuit. DYN released from the terminals of neurons from the NAc acts on kappa opioid peptide (κ) receptors on nerve terminals and cell bodies of DA-containing neurons and inhibits their activity. Chronic exposure to either cocaine or opiates and, possibly, other drugs of abuse upregulates this negative feedback loop by upregulating the cAMP pathway, which activates CREB and induces the formation of DYN. Abbreviation: O, opioid receptor. Reproduced, with permission, from Nature Reviews Neuroscience (<http://www.nature.com/reviews>) [30]. ©2001. Macmillan Magazines Ltd.

challenge in the addiction field is to understand how repeated drug administration causes such extraordinarily stable changes in the brain. One hypothesis is that chronic drug administration causes structural changes in neurons of the reward pathway. Chronic morphine causes a reduction in the size of dopamine-containing neurons in the VTA [91] and reduces the density of dendritic spines of medium spiny neurons in the NAc [92]. By contrast, chronic administration of cocaine causes an expansion in the dendritic arborization of NAc neurons that can persist for months after the last drug exposure [93]. What molecular mechanisms are responsible for these drug-induced changes in neuronal morphology? One possibility is that some molecular adaptations, even though they are transient, can initiate structural adaptations that last longer. There is evidence, for example, that Δ FosB-mediated induction of cyclin-dependent kinase 5 might partly mediate the increase in density of dendritic spines on neurons in the NAc following chronic cocaine administration [94,95]. Identification of other target genes for Δ FosB could provide further clues about the mechanisms of long-lived changes in the brain [65].

Although heuristically important, there is no direct evidence that the structural changes observed in VTA–NAc neurons underlie the long-lived, behavioral plasticity induced by drug exposure. This challenge, to understand the molecular and cellular basis of near-permanent behavioral changes that accompany addiction, is analogous to the learning and memory field, where, despite elegant molecular and cellular models, there is little insight into the neurobiological basis of behavioral memory. This common challenge is interesting because, over the past decade, the molecular and cellular pathways of drug addiction on the one hand, and of learning and memory on the other, have converged [96–98]. Learning and memory and drug addiction are modulated by the same neurotrophic factors (e.g. brain-derived neurotrophic factor), share several intracellular signaling cascades and depend on activation of CREB. They are associated with similar adaptations in neuronal morphology (e.g. changes

in dendritic spine density), and both are accompanied by alterations in synaptic plasticity (e.g. long-term potentiation and long-term depression) at particular glutamatergic synapses in the brain [50,99–101]. There has also been recent convergence in the brain regions that are considered important for the molecular and cellular plasticity that underlies addiction and memory. Thus, whereas the addiction field has focused largely on the VTA–NAc pathway, and the learning and memory field on the hippocampus, amygdala and cortex, we now know that all of these regions contribute to inter-related circuits that are crucial in addiction, learning and memory. These molecular connections between addiction and memory are consistent with work, now decades old, that demonstrated the involvement of associative and operational learning in addiction phenomena.

Future directions

The pace of identifying drug-induced molecular changes in the VTA–NAc and in the many other brain regions important for addiction has increased dramatically over the past 5 years with the advent of genomic and proteomic tools. These studies have validated the notion that drugs of abuse produce some common, chronic actions in reward regions in the brain and, as might be expected, have highlighted that each drug also exerts its own specific effects. For example, impressive advances have been made in appreciating how repeated administration of opiates alters opioid receptor trafficking, and how such alterations contribute to addiction (not reviewed here) [3,4]. A major challenge for the next phase of drug-addiction research is to figure out how to handle the vast amounts of data that are generated by genomics and proteomics. Ultimately, new clues to the molecular underpinnings of addiction might have to come from considering coordinated changes in multiple proteins in multiple brain regions, something that is not feasible with today's technologies.

Another major gap in the field is the difficulty in understanding precisely how molecular and cellular changes in neurons in the reward circuitry in the brain actually mediate the behavioral phenomena of reward and addiction. We still do not know, for example, how decreased excitability of NAc neurons causes 'reward,' and how altered activity of these cells contributes to craving and relapse. Such understanding requires appreciation of how the NAc, and its inter-related brain-reward structures, function as complex neural circuits, which is not yet available.

We also need to understand what makes some individuals particularly vulnerable to addiction and others relatively resistant. Epidemiological investigations indicate that ~50% of the risk of drug addiction (including addiction to opiates, cocaine, nicotine and alcohol) is genetic, but the specific genes involved have not yet been identified. Only with the identification of these genes will it be possible to understand how genetic and non-genetic factors interact to determine an individual's risk for an addictive disorder.

Finally, we need to increasingly translate the impressive body of information about the basic neurobiology of addiction from animal studies to helping human addicts. Impressive strides have been made in clinical investigations of

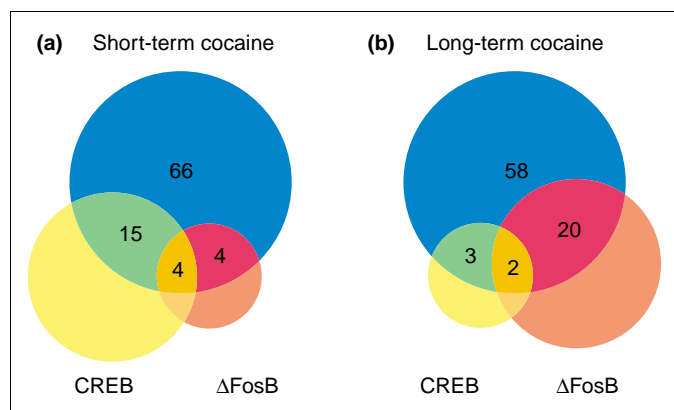


Figure 5. The roles of cAMP response element-binding protein (CREB) and Δ FosB in the regulation of gene expression by cocaine. Mice were treated with cocaine for either (a) 5 days or (b) 4 weeks, and then RNA, isolated from the nucleus accumbens, was subjected to DNA microarray analysis. The Venn diagrams show the number of genes whose regulation by shorter- or longer-term cocaine administration (blue circles) is accounted for by Δ FosB (red) and CREB (yellow). CREB has an important role in earlier phases of cocaine exposure, whereas Δ FosB has an increasingly dominant role during more prolonged periods of drug administration. Reproduced, with permission, from Nature (<http://www.nature.com>) [65].

addiction, in particular, in the areas of brain imaging and genetics, and several medication and non-medication treatments of addiction that have been developed over the past 30 years. Nevertheless, available treatments are inadequate for many addicts. Our hope and expectation is that improved understanding of addiction at the molecular and cellular levels will lead to definitive treatments for addiction and, eventually, to cures and preventive measures. A lot of work remains to be done.

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