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MOLECULAR AND CELLULAR BIOLOGY OF ADDICTION

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Addiction to alcohol, tobacco, and illegal drugs represents a substantial burden to societies worldwide. In terms of health-related outcomes, addiction results in enormous direct medical costs, premature mortality (tobacco alone may be responsible for 450,000 deaths yearly in the United States), and disability. In terms of broader social costs, addiction results in crime, negative impacts on families, derailed lives, and personal suffering. The major categories of drugs most likely to produce addiction are psychostimulants (including cocaine and amphetamines), opiates, ethanol, nicotine, marijuana, and phencyclidine-like drugs. Understanding the molecular and cellular actions of addictive drugs is obligatory if we are to better understand pathophysiology and develop potent pharmacotherapies to treat addiction. Of course, the molecular and cellular information presented in this chapter cannot be applied directly to the behavioral expression of addiction without putting it into the context of systems level neuroscience described in other chapters.

Acutely, addictive drugs are both rewarding (i.e., interpreted by the brain as intrinsically positive) and reinforcing (i.e., behaviors associated with drug use tend to be repeated). With repeated use, however, addictive drugs produce molecular changes that, within a vulnerable brain, promote continued drug-taking behavior in a manner that becomes increasingly difficult to control. The central feature of addiction is compulsive drug use—the loss of control over the apparently voluntary acts of drug seeking and drug taking. Once it has taken hold, addiction tends to follow a chronic course with periods of abstinence (that may or may not follow treatment), followed by relapse to active drug use. Even after extended periods of drug abstinence, the risk of relapse remains high. From the point of view of developing treatments, a central problem in addiction research includes understanding the molecular processes that lead to compulsive use and the long-term risk of relapse.

Addiction (defined as compulsive use) is not the only long-term effect of addictive drugs. Both addictive and many nonaddictive drugs may produce tolerance and dependence. Tolerance refers to the diminishing effect of a drug after repeated administration at the same dose, or to the need for an increase in dose to produce the same effect. Dependence represents an adaptive state that develops as a homeostatic response to repeated drug administration. Dependence is typically unmasked when drug taking stops, leading to withdrawal symptoms. Withdrawal symptoms may even emerge during active drug use as a result of tolerance, helping to drive increasing dosages or shorter intervals between doses.

Among the addictive drugs, ethanol and opiates produce dependence that has a somatic component, manifested by somatic symptoms during withdrawal, such as hypertension, tremor or seizures for ethanol, and hypertension, lacrimation, and abdominal cramps for opiates. All addictive drugs, including the psychostimulants, can produce an emotional–motivational component of dependence, manifested by symptoms such as dysphoria, anhedonia, and drug craving.

Tolerance and dependence may be prominent features accompanying addiction, but are not required. Indeed, when produced by addictive drugs, tolerance and withdrawal symptoms tend to resolve within days to weeks and therefore cannot account for the persistence of drug addiction (as manifest by the tendency to relapse) for many years. Indeed, both tolerance and dependence can occur with nonaddictive drugs as well. For example, B-adrenergic agonists inhaled for asthma, many antihypertensive drugs, and shorter-acting serotonin selective reuptake inhibitors may produce dependence and withdrawal symptoms on cessation, but do not produce compulsive drug seeking and drug taking. Based on these considerations, the molecular mechanisms underlying tolerance and dependence, and those responsible for addiction may overlap, but cannot be identical.

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One other long-term effect of addictive drugs, best documented for psychostimulants, is sensitization, in which repeated administration of a drug elicits escalating effects of a given dose. Sensitization can be operationally defined as a leftward shift in the drug's dose–response curve (1). Because behavioral sensitization to drugs in animal models can be quite long-lived, it has been considered by some to be a model for long-lasting aspects of human drug addiction.

Not every individual who experiments with drugs becomes addicted. Indeed, the likelihood that a person will experiment with drugs, use them repetitively, and progress to addiction, appear to be the product of complex gene–gene and gene–environment interactions, acting together with contextual variables, such as drug availability. Factors related to vulnerability are discussed elsewhere in this volume. This chapter has a dual focus. First, it discusses the initial molecular targets of addictive drugs in the brain; then the molecular and cellular changes induced by drugs in the brain that might be responsible for such clinically significant aspects of drug abuse syndromes as tolerance, dependence, sensitization, and addiction. An enormous number of drug-induced molecular and cellular changes in brain function are already known, not all of which turn out to have clinical relevance. Thus, the chapter does not attempt to produce an exhaustive list of the known molecular effects of addictive drugs, but focuses on a subset of those that illustrate important principles and that can be related to the long-term effects of addictive drugs in humans.

With exceptions from a small number of human postmortem studies, most of what we know about the molecular and cellular actions of addictive drugs comes from animal models. The integration of such information about drug action in the brain with information about human risk factors is in its early stages and will benefit enormously from the eventual discovery of risk-producing alleles from human genetic studies. The discovery of alleles that confer vulnerability to drug use or addiction will help focus molecular and cellular studies of pathophysiology, as well as suggest biochemical pathways that can be exploited for treatment.

MOLECULAR TARGETS OF ADDICTIVE DRUGS

The overall effect of each of the addictive drugs depends on the particular neurons and circuits that express their molecular targets, and the nature of those targets. Thus, for example, morphine-like opiates are analgesic and sedating, whereas cocaine is a psychomotor stimulant; these different properties are based on differences in localization and functional properties of the proteins with which they interact, the μ-opioid receptor for morphine and the dopamine reuptake transporter (DAT) for cocaine. However, as described in other chapters in this section, addictive drugs share the ability to activate mesocorticolimbic dopamine projections that are critical substrates for both rewarding and reinforcing effects of natural stimuli. Mesocorticolimbic dopamine projections originate in the ventral tegmental area (VTA) of the ventral midbrain and project to structures that include the nucleus accumbens (NAc) (a complex structure within the ventral striatum that is the best-established substrate for reinforcement), and the prefrontal cerebral cortex. *In vivo* microdialysis studies have indicated that most if not all addictive drugs, including cocaine, amphetamines, opiates, nicotine, and ethanol, cause selective elevation of extracellular dopamine levels in the NAc, and blockade of dopamine neurotransmission in this region attenuates most measurable reinforcing and rewarding effects of addictive drugs (2,3). The powerful control over behavior exerted by addictive drugs is thought to result from the brain's inability to distinguish between the activation of reward circuitry by drugs and natural activation of the same circuitry by useful behaviors (e.g., behavioral related to eating or reproduction). Any activity, whether related to drug taking or survival, that activates this circuitry tends to be repeated; however, activation of reward circuitry by addictive drugs can be much more reliable and powerful than activation triggered by natural reinforcers, facilitating repetitive drug use, and with it, the initiation of molecular mechanisms that may produce tolerance, dependence, sensitization, and compulsive use. Although the mesocorticolimbic dopamine system is a site of convergence for the rewarding effects of virtually all major classes of addictive drugs, these drugs act by very different mechanisms.

Psychostimulants

The best-characterized and most widely abused psychostimulants are cocaine and the amphetamines. The details of their mechanisms of actions differ, but both result in increases of extracellular dopamine and other monoamines and produce similar effects on behavior. In humans, psychostimulants increase alertness and produce a sense of well being. In animal studies, psychostimulants produce a dose-dependent increase in locomotor activity at low doses and stereotypies at high doses. If cocaine or amphetamine is used repeatedly, some acute drug effects may diminish (tolerance), whereas others are enhanced (sensitization).

Cocaine and amphetamines produce their effects by potentiating monoaminergic transmission through actions on dopamine, serotonin, and norepinephrine reuptake transporters (4). These proteins normally transport previously released neurotransmitter back into the presynaptic nerve terminal, and thereby terminate transmitter action. Cocaine binds to these transporters and competitively inhibits their functioning, thereby increasing the duration of action of neurotransmitter released into the synaptic cleft. Amphetamines and related drugs increase dopamine, serotonin, and norepinephrine neurotransmission by acting as a substrate for their transporters. Amphetamines are transported into

the presynaptic terminal where they cause neurotransmitter release by reversing the usual direction of transport (i.e., causing transmitter to move into the synapse).

Whereas psychostimulants affect all three transporters, it is their actions at the DAT that are most directly related to the reinforcing effects of psychostimulant drugs. Lesions of the dopamine system or administration of dopamine receptor antagonists, but not similar manipulations of serotonin or noradrenergic systems, markedly attenuate cocaine self-administration. The central role of the DAT in psychostimulant action is highlighted in studies using mice in which the DAT has been genetically inactivated. In the absence of the DAT, animals are insensitive to the locomotor stimulatory effects of cocaine and amphetamine (5). By contrast, animals lacking serotonin (5-HTT$^{-/-}$) or norepinephrine (NET$^{-/-}$) transporters exhibit normal locomotor responses to psychostimulants (6,7). Interestingly DAT$^{-/-}$ animals still self-administer cocaine and amphetamine to some degree (8), but the interpretation of this result is complex because DAT$^{-/-}$ animals have very high levels of extracellular dopamine at baseline (lacking a DAT to remove synaptic dopamine), which might magnify a psychostimulant-mediated effect on norepinephrine or serotonin. Much evidence demonstrates that the dopamine system is obligatory for psychostimulant-induced reinforcement, but the serotonin and noradrenergic systems, whose transporters are also inhibited by psychostimulants, may play a role as well.

Opiates

The opiates and their synthetic analogues are the most effective analgesic agents known, and at the same time can produce tolerance, dependence (including somatic dependence), and addiction. Physical dependence on opiates can contribute to addiction, but can also occur independently of it. For example, patients with cancer pain may become physically dependent on these drugs but do not compulsively abuse them.

Opiate drugs bind to receptors for three subtypes of receptors that normally bind endogenous opioid peptides. The three subtypes, denoted μ, κ, and δ, are members of the G protein-coupled receptor family, and all interact with G proteins of the G_i/G_o types. This coupling results in inhibition of adenylyl cyclase, activation of inwardly rectifying K^+ channels, and inhibition of voltage-gated Ca^{2+} channels. Opiate receptors thus typically mediate inhibitory responses that reduce membrane excitability and reduce likelihood of cell firing and neurotransmitter release; however, the different opiate receptor subtypes are expressed on different cells, resulting in different biological effects when stimulated. Morphine-like opiates, including heroin, are both analgesic and addictive, and interact with highest affinity with the μ-receptor.

Morphine-like opiates suppress afferent nociceptive information by acting on opiate receptors contained within a descending pathway extending from the periaqueductal gray matter of the midbrain, to the rostroventral medulla, and then to the dorsal horn of the spinal cord. These same drugs appear to produce both reward and reinforcement by means of at least two mechanisms: (a) activation of the VTA, which results in dopamine release in the NAc; and (b) direct binding to opiate receptors in the NAc, an action that is independent of dopamine. Activation of VTA dopamine neurons by opiates results from disinhibition: morphine-like opiates inhibit GABAergic interneurons in the VTA that tonically inhibit the dopamine projection neurons (9). Increased activity of these dopamine neurons produces increases in extracellular dopamine levels in the NAc. Consistent with this arrangement, the reinforcing effects of intravenous heroin can be partly attenuated by administration of an opioid receptor antagonist directly into the VTA or lesioning the dopaminergic neurons of the VTA. Opiates also produce reinforcement through direct dopamine-independent action on μ, and perhaps δ, receptors expressed by NAc neurons. Consistent with this mechanism, morphine is self-administered even in the presence of dopamine receptor blockade or following a 6-hydroxydopamine lesion that destroys dopamine neurons (10,11). Moreover, lesions of the NAc or pharmacologic μ-receptor antagonists applied to the NAc dose dependently reduce the reinforcing effects of heroin and morphine (12,13). Thus, opiates and dopamine work through different postsynaptic receptors within the NAc to produce reinforcement.

M and δ-opioid receptor subtypes, both of which are present in the VTA and NAc, may both play a role in opiate reinforcement. In contrast, κ-opioid receptor activation is not reinforcing. Activation of κ receptors can decrease dopamine release in the NAc by both presynaptic mechanisms-there are κ receptors on a subset of dopamine terminals. As a result stimulation of κ opiate receptors may produce aversive responses in both animals and humans. As will be described in the following, κ opiate receptors may play a role in the emotional–motivational aspects of withdrawal from psychostimulants.

Ethanol

Ethanol is a central nervous system depressant that produces behavioral disinhibition, euphoria, reduced anxiety, decreased motor coordination, and sedation. The major mechanisms underlying behavioral effects, including reinforcement, are thought to be facilitation of $GABA_A$ receptors and inhibition of NMDA glutamate receptors (14). At higher doses, ethanol also inhibits the functioning of most voltage-gated ion channels. The molecular mechanisms by which ethanol affects these receptors and channels are not yet certain; two types of mechanisms have been hypothesized. One possible mechanism attributes the effects of ethanol on receptors and channels to its generalized effects on cell membranes, in which it is highly soluble. Certain

ligand-gated and voltage-gated channels may be preferentially affected by ethanol because, as complex multimeric proteins, they may be particularly vulnerable to ethanol-mediated changes in their lipid environment. The alternative hypothesis is that ethanol interacts with specific hydrophobic regions of these proteins to produce allosteric changes in structure, but the convincing demonstration of such interactions is still lacking.

Whether it acts via its general effects on membranes or, more specifically, in interaction with particular regions of proteins, ethanol has been shown to allosterically regulate the $GABA_A$ receptor to enhance GABA-activated Cl^- flux. The anxiolytic and sedative effects of ethanol, like those of barbiturates and benzodiazepines, are believed to result from facilitation of the $GABA_A$ receptor, although the precise mechanism differs for each drug. For example, distinct binding sites on the receptor have been identified for barbiturates and benzodiazepines. The convergence of actions of ethanol, barbiturates, and benzodiazepines on a single receptor result in more than additive effects, which can be responsible for lethal overdoses. In addition, these agents all produce cross-tolerance, thus permitting the use of benzodiazepines in ethanol detoxification protocols.

Not all $GABA_A$ receptors are ethanol sensitive. $GABA_A$ receptor complexes are heteropentamers comprised of combinations of the various members of five distinct subunit families. The subunit combinations vary in different cell types, leading to differences in the sensitivity of $GABA_A$ receptors to ethanol in different brain regions.

Ethanol also acts as an NMDA glutamate receptor antagonist, and allosterically inhibits the passage of glutamate-activated Na^+ and Ca^{2+} currents through the NMDA receptor. Other actions of ethanol that are possibly relevant to its psychotropic effects include potentiation of the action of serotonin at $5\text{-}HT_3$ receptors, which, like NMDA receptors, are excitatory, cation-selective ion channels.

The mechanisms by which ethanol produces reinforcement are not yet known in their entirety. The reinforcing effects of ethanol are partly explained by its ability to activate mesocorticolimbic dopamine circuitry (15), with enhanced release of dopamine in the NAc. It is not known whether this effect is mediated by disinhibition of dopamine neurons at the level of the VTA or whether it occurs at the level of the NAc, nor is it known whether it is caused primarily by facilitation of $GABA_A$ receptors or inhibition of NMDA receptors. Finally, it is not know to what degree opioid, serotonin, and other systems play a role in ethanol-mediated reinforcement. Thus, for example, not only $GABA_A$ receptor antagonists but also opiate antagonists, decrease ethanol self-administration and ethanol-related behavioral effects in rats (16–18). The opiate antagonist naltrexone reduces ethanol self-administration in animals; moreover, naltrexone and other opioid receptor antagonists reduce ethanol consumption, relapse to active drinking, and craving clinically (19,20).

Serotonin also appears to be involved in ethanol consumption and reinforcement; ethanol consumption is generally curbed by experimental manipulations that increase serotonergic function, and experiments with rats selectively bred for ethanol preference suggest that strong ethanol preference is associated with reduced serotonergic function. $5\text{-}HT_3$ antagonists such as ondansetron can block both ethanol-induced dopamine release in the NAc and ethanol consumption in rats. Mice lacking $5\text{-}HT_{1B}$ serotonin receptors consume higher levels of ethanol yet demonstrate less ataxia (21).

Nicotine

Nicotine is the main psychoactive ingredient of tobacco and is responsible for the stimulant effects, reinforcement, and dependence that result from tobacco use. Cigarette smoking rapidly delivers nicotine into the bloodstream. Nicotine differs from cocaine and opiates in that it is powerfully reinforcing in the absence of subjective euphoria.

The effects of nicotine are caused by its activation of nicotinic acetylcholine receptors (nAChRs). Nicotinic AChRs are ligand-gated cation channels located both presynaptically and postsynaptically. Presynaptic nAChRs facilitate neurotransmitter release. The reinforcing effects of nicotine depend on an intact mesolimbic dopamine system; nicotine-induced increases in locomotor behavior are also blocked by destruction of mesolimbic dopamine nerve terminals or cell bodies (22). Moreover, nicotine increases dopamine neurotransmission and energy metabolism in the nucleus accumbens (23).

Nicotinic AChRs containing $\alpha 6$ and B2 subunits are highly expressed in VTA dopamine neurons, and seem to be involved in both nicotine-induced dopamine release and reinforcement and in nicotine-induced locomotor activation (24,25). Systemic nicotine self-administration is disrupted when nicotinic receptor antagonists are administered directly into the VTA but not when they are administered into the NAc. Nicotine may also have some ability to stimulate dopamine release in the NAc, however, mediated by presynaptic nAChRs located on dopamine terminals within the NAc. Nicotinic AChRs on VTA dopamine neurons are normally activated by cholinergic innervation from the laterodorsal tegmental nucleus or the pedunculopontine nucleus.

Nicotine may also affect reinforcement via the opioid peptide system. Not only dopamine antagonists, but also opiate antagonists, block nicotine-induced behaviors and self-administration (26,27). These findings suggest a role for endogenous opioid systems in the reinforcing effects of nicotine, and raise the possibility that such antagonists may be of use in the treatment of nicotine addiction.

Cannabinoids

Δ-9-Tetrahydrocannabinol (THC) is the major psychoactive compound contained in marijuana. THC produces ef-

fects in humans that range from mild relaxation, euphoria, analgesia, and hunger to panic attacks. Reinforcing effects of cannabinoids comparable to those of other addictive drugs have not been demonstrated in animals, but cannabinoids have been shown to decrease reward thresholds and promote conditioned place preference in rats (28,29). THC increases mesolimbic dopamine transmission in the NAc shell, probably via a μ-opioid receptor-mediated mechanism because μ-receptor antagonists prevent the THC-induced dopamine increases in the brain mesolimbic area (30). Cannabinoids have also been reported to inhibit excitatory glutamatergic neurotransmission in the substantia nigra pars reticulata (31).

THC binds to two cannabinoid receptors denoted CB_1 and CB_2. Of the two, only the CB_1 receptor is expressed in the central nervous system, with high levels in the basal ganglia and limbic system (32). The endogenous ligand for the CB1 receptor appears to be an arachidonic acid derivative, anandamide; however, the nature of anandamide's function in the brain remains speculative. Evidence indicates that other endogenous ligands also may bind at this receptor.

Despite ongoing debates about the addictiveness of cannabinoids in humans, there appear to be many compulsive marijuana users. Withdrawal symptoms typically are not reported with termination of long-term marijuana use, but withdrawal symptoms have been demonstrated in a laboratory setting after four days of marijuana smoking (33). Cannabinoid dependence can be demonstrated experimentally with the use of cannabinoid receptor antagonists, which precipitate profound withdrawal symptoms that are somatic and emotional–motivational. In animals chronically treated with THC, a selective cannabinoid receptor antagonist produced withdrawal symptoms that included head shakes, facial tremors, tongue rolling, biting, wet dog shakes, and ptosis (34). Neurobiologically, withdrawal effects include increases in c-fos expression in the basal ganglia systems and CRF release in the amygdala (35).

Phencyclidine-Like Drugs

Phencyclidine (PCP or angel dust) and ketamine are related drugs classified as dissociative anesthetics. These drugs exhibit psychotomimetic properties, but are distinguished from hallucinogens by their distinct pharmacologic effects, including their reinforcing properties and risks related to compulsive abuse.

The reinforcing properties of PCP and ketamine are mediated by the binding to specific sites in the channel of the NMDA glutamate receptor, where they act as noncompetitive NMDA antagonists. PCP is self-administered directly into the NAc, where its reinforcing effects are believed to result from the blockade of excitatory glutamatergic input to the same medium spiny NAc neurons inhibited by opioids, and also by increases in extracellular dopamine. In contrast, hallucinogens, such as LSD, act at $5\text{-}HT_2$ serotonin receptors.

MOLECULAR AND CELLULAR MECHANISMS OF LONG-LIVED DRUG EFFECTS

Homeostasis Versus Associative Learning

Diverse behaviors, symptoms, and signs of substance use disorders coexist clinically, but depending on the drug and on the stage of the disorder, these may involve multiple molecular mechanisms occurring in diverse neural circuits. Heuristically, the types of molecular mechanisms involved in the long-lived effects of addictive drugs may be divided into two major classes: homeostatic adaptations and associative learning. Homeostatic adaptations can be understood as compensatory responses of cells or circuits to excessive bombardment by a drug or to excessive drug-induced neurotransmitter stimulation (e.g., excessive dopamine stimulation). These adaptations tend to dampen drug effects, thus playing a critical role in tolerance and dependence. The adapted state of neurons or neural systems may be unmasked on drug cessation, leading to the production of withdrawal symptoms, as illustrated in the following. Homeostatic adaptations typically occur within reversible bounds, and with removal of the drug, tend to dissipate over days to weeks.

Although clinically significant, homeostatic mechanisms cannot account for the persistent tendency of addicted individuals to relapse, even years after any withdrawal symptoms have subsided. Relapse often occurs on re-exposure to cues associated with drug use, consistent with an important role for associative learning (36). Although homeostatic mechanisms are thought to represent reversible global alterations in the sensitivity of neurons or circuits to neurotransmitters or drugs, associative learning is thought to represent long-lived or permanent alterations in patterns of synaptic connectivity that encode specific information (37). The clear separation between homeostasis and associative learning that has been implied, however, is an oversimplification. For example, there is recent evidence that associative learning mechanisms and compensatory adaptations may interact. Thus, for example, associative learning mechanisms have recently been shown to play a role tolerance to opiate analgesia, that is, the expression of tolerance may be context-dependent (38). Moreover, molecular adaptations that occur as a homeostatic response to drug bombardment may alter the threshold for associative learning involving affected cells.

Recruitment of Different Molecular Mechanisms Over Time

During the earliest periods of drug experimentation, mesocorticolimbic reward circuits are activated via different

mechanisms by different classes of drugs. As noted, a shared property of addictive drugs is to promote dopamine release in multiple forebrain regions, including the NAc, but also including the dorsal striatum, amygdala, and hippocampus, in which dopamine release can act as a reinforcement signal, thus controlling learning processes (39,40). As drug use continues, tolerance may occur, leading to dosage escalation. Depending on the drug, somatic dependence and/or emotional–motivational dependence my sustain drug seeking and drug use in attempts to avoid the aversive state of withdrawal. The emotional–motivational aspects of tolerance and dependence may largely occur within the mesocorticolimbic circuitry itself, but molecular adaptations occur in other circuits as well in a drug-specific manner reflecting the location of the target molecules for the given drug. Sensitization to some drug effects may occur, a phenomenon that is especially well documented for psychostimulants. Sensitization may act, *inter alia,* to increase the incentive salience of the drug, and thereby contribute to compulsive drug use (41). At the same time, multiple memory systems are affected by drugs of abuse (42) and, undoubtedly contribute to sustaining active drug use and late relapses (37). What follows are examples of different molecular processes that contribute to different aspects and stages of substance use disorders. These illustrations have been chosen based on the depth of available information, and likely relevance to the clinical situation in humans.

Adaptations That Produce Tolerance and Somatic Dependence to Opiates

Opiates and ethanol produce somatic dependence and withdrawal because their targets are expressed on cells and circuits that regulate bodily functions such as autonomic activity. Tolerance and dependence are generally thought to represent homeostatic adaptations that compensate for overstimulation by a drug or neurotransmitter. During withdrawal, the overcompensated system is suddenly unopposed by the drug it had adapted to counteract. Consequently, withdrawal symptoms appear that generally are opposite to the immediate effects produced by the drug. The molecular adaptations probably responsible for some aspects of tolerance and somatic dependence are best understood for opiates (43).

With repeat administration of mu agonist opiates such as morphine or heroin, both tolerance and dependence emerge. There is a significant somatic component to heroin dependence as manifest by the classic heroin somatic withdrawal syndrome. It had initially been hypothesized that opiate dependence would correlate with significant changes in expression of endogenous opioid peptides or opioid receptors or changes in opioid receptor affinity. This has not turned out to be the case; rather opiate tolerance and dependence appear to be caused by adaptation in postreceptor signaling mechanisms in opiate receptor-bearing cells (Fig. 96.1).

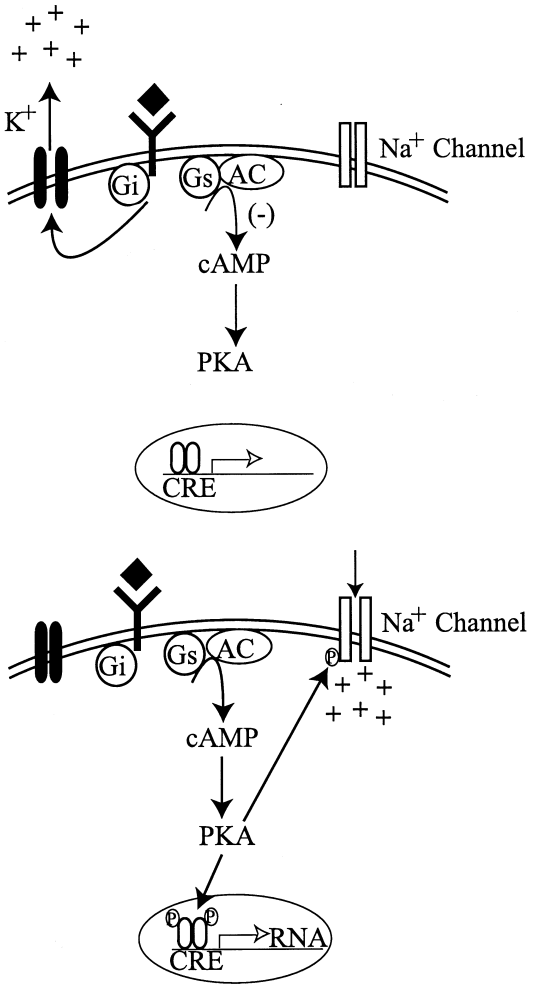

FIGURE 96.1. Mechanism of opiate tolerance and dependence in the locus ceruleus: Acute administration of opiates increases outward K^+ current, thereby hyperpolarizing locus ceruleus cells *(top)*. With chronic opiate use the cAMP signaling system is up-regulated, leading to PKA-dependent phosphorylation of the Na^+ channel. In this state, the channel is more active, allowing Na^+ ions to flow into the cell, increasingly the intrinsic excitability of the cell. Up-regulation of the cAMP system also increases CREB Ser^{133} phosphorylation and CRE-dependent gene transcription. Alterations in CRE-driven genes may contribute to the increased LC neuron excitability as well *(bottom)*.

The locus ceruleus (LC), located in the dorsal pons, is the major noradrenergic nucleus of the brain and regulates arousal, attention, and vigilance. It is involved in responses to stress, and together with other noradrenergic cell groups plays a role in regulation of the autonomic nervous system. Morphine-like opiates acutely inhibit the firing of LC neurons, but tolerance and dependence occur within the LC with continued administration. Thus, despite continued opiate exposure, LC firing rates gradually return to their basal levels. At this point, administration of an opioid receptor

antagonist, such as naloxone or naltrexone, causes a dramatic increase in LC firing rates. In animals, the period of rapid LC firing correlates with the somatic withdrawal syndrome, and drugs, such as the α_2-adrenergic receptor agonist clonidine, which inhibit LC firing, attenuate withdrawal symptoms.

Many of the adaptations that produce LC-mediated tolerance and dependence depend on the cyclic AMP (cAMP) pathway. In the LC, as in most other cell types, μ-opioid receptor activation inhibits the cAMP pathway via Gi activation and stimulates an inwardly rectifying K^+ current by means of direct interactions of the G protein $\beta\gamma$ subunits with the channel. M-opiate receptors also inhibit a Na^+ current that is dependent on the cAMP-dependent protein kinase (protein kinase A or PKA) for its activation, and is thus dependent on an active cAMP system. Taken together, the actions of μ agonist opiates on these K+ and Na+ channels expressed by LC neurons decrease the excitability and inhibit the firing of the LC. With long-term opiate administration, however, a homeostatic compensatory response occurs: key components of the cAMP pathway become up-regulated in LC neurons; thus, for example there are increased concentrations of adenylyl cyclase and protein kinase A. This up-regulation increases the intrinsic excitability of LC neurons, by activating the cAMP-dependent Na^+ current. The activation of this current may explain why LC firing rates return to normal despite the continued presence of an opiate (an example of tolerance). These observations may also explain the dramatic increase in LC firing that occurs if an opiate antagonist such as naloxone is administered to precipitate withdrawal (illustrating dependence). The up-regulation of the cAMP pathway has increased the intrinsic excitability of the LC neuron, but as long as μ-opiate agonists continue to be administered, this excitability is counteracted.

Activation of LC neurons during opiate withdrawal owes not only to changes in intrinsic excitability, but also partly to glutamatergic projections to the LC from the nucleus paragigantocellularis of the medulla. Lesions of the paragigantocellularis, or glutamate receptor antagonists administered locally in the LC, attenuate withdrawal-induced increases in LC firing rates by approximately 50%. An up-regulated cAMP pathway also may mediate this effect, as long-term use of opiates causes up-regulation of the cAMP pathway in the paragigantocellularis and its major afferents.

The mechanisms by which the cAMP pathway becomes up-regulated are complex, and may involve both transcriptional and translational mechanisms; however, the importance of transcription factor CREB is supported by experiments in mice with a partial knockout (hypomorphic allele) of CREB. In these animals two of the major CREB isoforms, α and Δ, were disrupted. After an opiate administration paradigm that would be expected to produce opiate dependence and naloxone-precipitated withdrawal, these mice exhibited markedly reduced signs of withdrawal including complete absence of sniffing and ptosis (44,45).

This is not the whole story, however. Opiate-induced up-regulation of PKA does not involve CREB and may be mediated posttranslationally. The inactive PKA holoenzyme is a heterotetramer composed of two regulatory and two catalytic subunits. When the regulatory subunits are bound by cAMP, the catalytic subunits are free to phosphor late substrate proteins. However, free catalytic subunits of PKA are highly vulnerable to proteolysis, whereas inactive subunits bound to regulatory subunits are proteolysis-resistant. It is currently speculated that PKA subunits accumulate in the LC during long-term opiate treatment because the enzyme is inhibited by the persistent presence of an opiate, keeping it in its inactive holoenzyme form in which subunits would be degradation-resistant. As the number of enzyme molecules increases, the kinase activity can be more readily activated by the low levels of cAMP.

Adaptations That May Produce Tolerance and Somatic Dependence on Ethanol

Like opiates, ethanol produces somatic dependence and withdrawal, although the clinical syndrome is quite distinct, and potentially more dangerous. The molecular mechanisms are less well understood than those underlying opiate tolerance and dependence, but the comparison is instructive. There is some evidence that homeostatic adaptations occur in response to ethanol that decrease $GABA_A$ receptor expression and increase NMDA receptor expression on some neurons. The decrease in receptors for the major inhibitory neurotransmitter and the increase in excitatory receptors would make neurons intrinsically more excitable. With removal of ethanol, a drugs that facilitates $GABA_A$ receptor-mediated Cl− currents and inhibits NMDA receptors, a state of increased neural excitability would be unmasked (Fig. 96.2) leading to withdrawal symptoms such as agitation, tremor, hypertension, and seizures. The decrease in $GABA_A$ receptor function is possibly due in part to decreased $GABA_A$-1 subunit expression in the striatum, cortex, and hippocampus (46). Conversely, chronic ethanol appears to increase the number and function of NMDA receptors (47). During withdrawal, glutamate release is increased for up to 36 hours in the NAc, hippocampus, and striatum (48). Overall, there is increasing evidence to suggest that that the neuronal hyperexcitability evident during ethanol withdrawal result from the combination of reduced $GABA_A$ receptor-mediated inhibition and increased glutamatergic excitation (Fig. 96.2).

Adaptations That Produce Emotional and Motivational Aspects of Dependence

An emotional and motivational component of dependence has been hypothesized to reflect neural adaptations to exces-

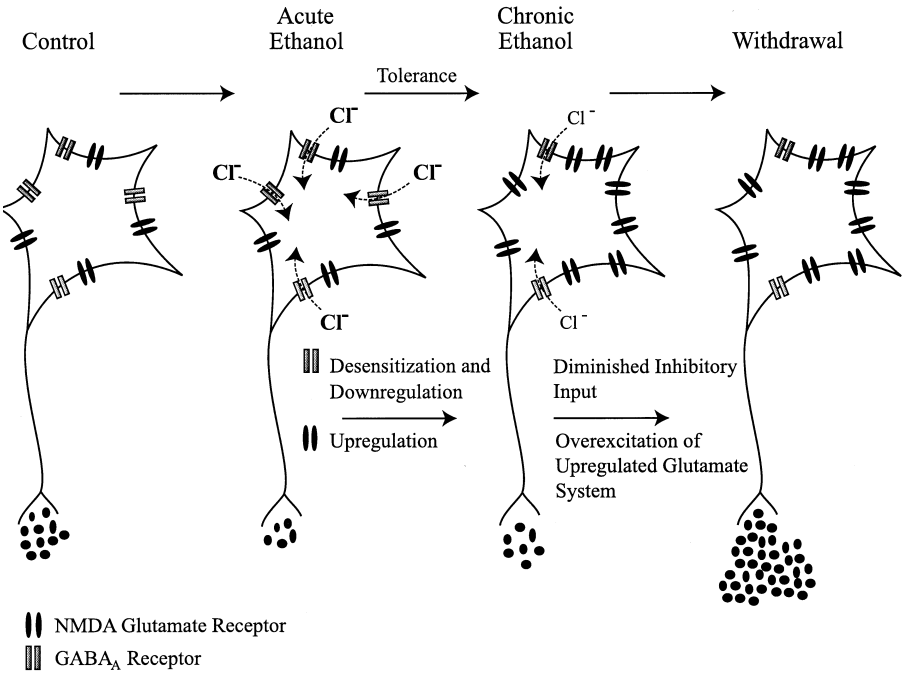

FIGURE 96.2. Hypothetical scheme to explain increased neuronal excitability with ethanol withdrawal: Acute ethanol exposure increases chloride conductance via the GABA$_A$ receptor and inhibits NMDA glutamate receptors, thereby reducing neuronal excitability and glutamate release. With chronic ethanol exposure, there is putative down-regulation of GABA$_A$ receptor subunits and possibly up-regulation of NMDA receptors. On ethanol withdrawal, inhibitory input from ethanol is removed; therefore, excitatory influences are relatively unopposed. The neurons release increased quantities of glutamate, which may act on up-regulated receptors. The unopposed cellular hyperexcitability can promote seizure activity as groups of neurons become overexcited.

sive dopamine release within brain reward pathways. Emotional and motivational aspects of dependence are inferred in humans from the dysphoria, anhedonia, and, anxiety that may accompany withdrawal from psychostimulants (49) and other addictive drugs. One animal model for emotional–motivational aspects of dependence is elevation of brain stimulation reward thresholds. Direct electrical stimulation of brain reward pathways is both rewarding and reinforcing. Acute administration of addictive drugs decreases the level of stimulation necessary to achieve rewarding levels of stimulation. However, if drugs are repeatedly administered and then withdrawn, there is a marked increase in the threshold for achieving reward, as if the brain reward pathways are in a state of decreased responsiveness (50). Based on this and other models, it has been hypothesized that reduced mesolimbic dopaminergic activity is associated with the emotional–motivational aspects of dependence and withdrawal; however, brain reward circuitry is complex, and it is to be expected that many adaptive processes occur that contribute to dependence. For example, relevant adaptations likely occur both in NAc and in VTA neurons. Indeed there is evidence of up-regulation of the cAMP pathway with chronic administration of addictive drugs both in NAc neurons and in the GABAergic interneurons that innervate dopaminergic neurons of the VTA. Up-regulation of the cAMP pathway in VTA interneurons during withdrawal could lead to increased GABA release and consequently to reduced firing of the dopaminergic cells on which they synapse. Such activity might partially account for the reductions in dopaminergic neurotransmission from the VTA to the NAc observed during early phases of withdrawal and that are believed to contribute to withdrawal symptoms.

One mechanism that could contribute to aversive states that occur with psychostimulant withdrawal is up-regulation of the neuropeptide dynorphin. In the dorsal and ventral striatum, levels of prodynorphin mRNA and dynorphin peptides increase significantly following repeated administration of psychostimulants (51,52). A significant increase in prodynorphin mRNA is observed after rats self-administer cocaine (53) and, in postmortem studies of cocaine-dependent human drug abusers, there is a marked induction of prodynorphin, but not other peptide mRNAs in the striatum (54).

Dynorphin peptides are relatively selective for the κ opiate receptor, and exert inhibitory actions in the nervous system via the G protein, Gi. Stimulation of κ opiate receptors on dopamine terminals within the dorsal and ventral striatum appears to decrease dopamine release. Consistent with this, activation of κ receptors is associated behaviorally

with an aversive dysphoric syndrome both in humans (55) and rats (56). Thus, increases in dynorphin peptides produced by chronic cocaine or amphetamine administration may inhibit dopamine release and contribute to emotional–motivational aspects of psychostimulant withdrawal.

Regulation of prodynorphin gene expression by psychostimulants has been shown to be dependent on D1 dopamine receptor stimulation (57) because selective D1 receptor agonists inhibit it. Moreover, the prodynorphin gene is expressed in the striatum in D1 receptor bearing cells (58). D1 receptors are coupled to G_s/G_{olf}, and thus stimulate adenylyl cyclase to produce cAMP, which in turn activates PKA. PKA can then phosphorylate numerous substrates including CREB, a transcription factor that binds cAMP response elements (CREs) in numerous genes. Indeed, cocaine and amphetamine (59) have been shown to induce phosphorylation of CREB in striatal neurons via a D1 receptor-mediated mechanism, and the prodynorphin gene has been shown to be CREB regulated in these same cells (60). Thus, at the same time that D1 receptor stimulation acutely contributes to the acute rewarding effects of cocaine and amphetamine, it also initiating a cascade of homeostatic events that eventually yield compensatory adaptations to excess dopamine stimulation. One of these adaptations is induction of dynorphin peptides (Fig. 96.3).

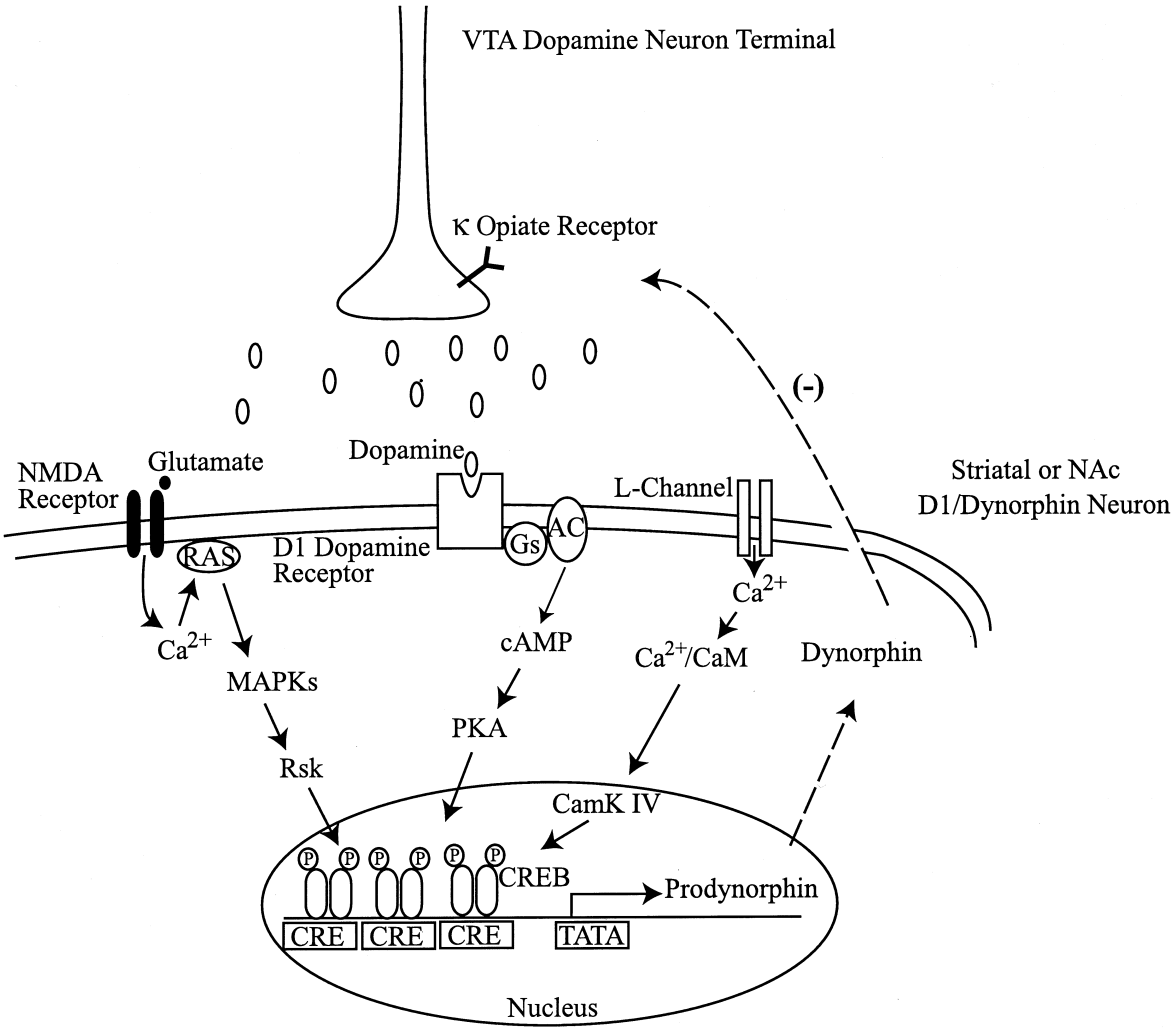

FIGURE 96.3. Dynorphin gene regulation by psychostimulants: implications for central motive states. Cocaine and amphetamine increase levels of dopamine in the nucleus accumbens and dorsal striatum. D1 receptor stimulation by dopamine leads to activation of the cAMP pathway, phosphorylation of CREB and ultimately the transcription of CRE-regulated genes such as c-fos and prodynorphin. Glutamate, via the NMDA receptor, as well as L-type calcium channels similarly contribute to CREB phosphorylation and CRE-driven gene expression in these dopaminoceptive neurons. Release of dynorphin inhibits dopamine release by binding to its presynaptic target, the κ opiate receptor, on DA nerve terminals. The dynorphin negative-feedback mechanism for controlling dopamine levels also contributes to aversive feelings and dysphoria due to its actions at the κ opiate receptor.

Corticotropin Releasing Factor May Also Contribute to Emotional–Motivational Aspects of Withdrawal

One type of adaptation that occurs outside the mesocorticolimbic dopamine system that may contribute to aversive states, and thus drug seeking, is up-regulation of corticotropin releasing factor (CRF). This neuropeptide is expressed in the hypothalamus, central nucleus of the amygdala, and other brain regions. In the hypothalamus CRF has been shown to be critical in the initiation of stress hormone cascades that culminate in the release of cortisol from the adrenal cortex. CRF released in the central nucleus of the amygdala has been implicated in anxiety states. Several studies have implicated CRF systems in the mediation of many of the angiogenic and aversive aspects of drug withdrawal. Increased release of CRF, particularly in the central nucleus of the amygdala, occurs during withdrawal from ethanol, opiates, cocaine, and cannabinoids. CRF antagonists have reversed at least some of the aversive effects of cocaine, ethanol, and opiate withdrawal in laboratory animals.

Alterations in Expression of Transcription Factors May Impact Diverse Neuronal Processes

Drug-induced changes in expression of transcription factors may lead to the altered expression of specific target genes, which in turn may affect both homeostatic adaptations and associative learning. In addition to regulating the peptide precursor gene, prodynorphin, D1 receptor-mediated stimulation of CREB induces a large number of immediate early genes (IEGs) including several that encode transcription factors, including c-fos, fras, junB, and zif268 (61–64). One interesting finding is that one CREB regulated transcription factor, ΔFosB, a truncated isoform of Fos B has a long half-life compared to all other members of the Fos family of transcription factors. Unlike other members of the Fos family, ΔFosB is only slightly induced by acute stimulation, but because it is long lived, it begins to accumulate with repeated stimulation, including repeated administration of addictive drugs (65). Thus, long-term, but not short-term, administration of cocaine, amphetamine, opiates, nicotine, or PCP induces ΔFosB in the NAc and dorsal striatum. Of all known molecules that exhibit altered levels following drug stimulation, ΔFosB is the longest lived currently known. Accumulation of ΔFosB represents a molecular mechanism by which drug-induced changes in gene expression can persist for weeks—even after drug use has been discontinued. The biological significance of ΔFosB induction will be better understood following identification of the genes that it regulates. Certain AMPA glutamate receptor subunit-encoding genes are among the candidates. Even though ΔFosB is stable, it is ultimately degraded; thus, by itself it cannot mediate the lifelong changes in behavior that accompany addiction.

Associative Learning

Both humans and animals readily learn to self-administer addictive drugs; behaviors that require the specific recognition of drug-associated cues, and the performance of complex action sequences. Cues associated with drug administration acquire motivational significance as illustrated by the conditioned place preference paradigm; for example, rats will choose to spend more time in a location in which they have passively received an injection of psychostimulants than in another location paired with saline injection (66). Associative learning also appears to play a role in psychostimulant sensitization. If, for example, a rat is taken from its home cage to a novel "test" cage for intermittent amphetamine injections, the sensitized locomotor response to a challenge dose is much greater if the challenge is also given in that test cage than if given in a different environment (67,68). Such context dependence can dominate the behavioral effects with sensitization expressed in the drug-associated location, no sensitization at all in a different environment (69,70). In drug-addicted humans, late relapses appear to involve associative learning, as they often occur after encounters with people, places, or other cues previously associated with drug use (71,72). As described, conditioned responses to drug-associated cues persist far longer than withdrawal symptoms (36), and can occur despite years of abstinence from drugs.

At a systems level, context-dependent sensitization in animal models and cue-conditioned relapse in humans suggests that the brain stores specific patterns of drug-related information. Homeostatic responses that increase or decrease the gain on the overall responsiveness of dopaminergic or other neurotransmitter systems in the brain could not mediate selective responsiveness to specific contexts or cues. Thus, general homeostatic mechanisms are not adequate to explain these phenomena. Elsewhere it has been argued that core features of addiction arise from the inappropriate recruitment of molecular mechanisms normally responsible for associative learning (37). On this view, the persistence of drug addiction reflects the persistence of the memory for this learned experience in the form of altered patterns of synaptic connectivity.

At the molecular level, stimulation of dopamine D1 receptors in multiple brain regions, including striatum, promotes activation of the transcription factor CREB (59,73) and a transient burst of altered gene expression (74). The induction of multiple transcription factors by this mechanism has already been described. Other psychostimulant induced IEG products that have been described in the striatum include *homer-1a, narp, arc,* and many others (62,74, 75). Some of the genes induced by dopamine and psychostimulants in the striatum have been hypothesized to play

a role in hippocampal LTP, making it tempting to speculate that they may ultimately have a role in synaptic remodeling in the striatum (76–79). Indeed, D1 receptors have been shown to be required for normal hippocampal long-term potentiation (LTP), an important model of synaptic plasticity. For LTP in the CA1 region of the hippocampus to persist for more than 2 or 3 hours ("late-phase" LTP; L-LTP) there must be increases in postsynaptic cAMP, phosphorylation of CREB, gene transcription, and protein synthesis (64,80–82). The requirement for activation of gene expression seems to be transient, because blockers of transcription or translation disrupt hippocampal L-LTP if they are given within a few hours of the LTP-inducing stimulus, but not if given later (83). Activators of the cAMP cascade, including D1 agonists, can induce L-LTP (84,85). D1 receptor blockade inhibits hippocampal L-LTP (85–87), and D1-knockout mice do not show L-LTP (88). Therefore, D1 receptor activation in the hippocampus may act to gate synaptic plasticity, helping to determine whether changes in synaptic strength are long lasting or merely transient.

A role for dopamine receptors in the modification of synaptic strength fits well with the idea that increases in extracellular dopamine can act as a reinforcement learning signal in striatum (89). LTP (and also LTD, long-term depression) is found at corticostriatal synapses *in vivo* (90) and *in vitro* (91,92). Some groups have found that striatal LTP can be modified by dopamine receptor stimulation (91,93,94). Moreover, based on genetic manipulations, CREB has been implicated in both invertebrate and vertebrate models of synaptic plasticity and long-term memory (80–82,95). Moreover, changes in striatal synaptic physiology and synaptic structure occur in response to psychostimulant administration (96). At the systems level, dorsal regions of striatum appear to be involved in the learning and execution of complex automatized behavioral sequences, particularly in response to external cues. Ventral striatal areas are involved in acting on the motivational significance of such cues. Thus drug-induced synaptic plasticity in both regions may contribute to drug use through consolidation of drug-taking and -seeking behaviors. Many questions remain, but the central outstanding issue is the identification of genes transiently induced by addictive drugs, the products of which produce stable remodeling of synapses.

CONCLUSION

All of the initial molecular targets of drugs of abuse have been characterized and cloned. However, the molecular biology of processes relevant to tolerance, dependence, sensitization, and most important, compulsive drug use, and late relapse, are in their relatively early stages. Striking progress has been made in identifying large numbers of molecular changes initiated by drugs of abuse, but coherent biological implications of these changes can currently be described for only a few situations, such as somatic dependence on opiates. Even for more difficult problems, however, powerful tools are on the horizon. It is imperative, for example, to investigate the mechanisms by which dopamine excess might produce long-lived pathological associative memories that could underlie compulsive drug use and late relapse. Fortunately, in the very near future, a complete set of mammalian genes will be available in arrays, and similar collections of proteins will follow, albeit with some delay. Given these reagents, we will be limited only by our neurobiological imaginations.

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