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Feature Article

Molecular Mechanisms of Drug Addiction

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Drug addiction has afflicted mankind for centuries, yet the mechanisms by which particular drugs lead to addiction, and the genetic factors that make some individuals particularly vulnerable to addiction, have remained elusive. From a clinical perspective, drug abuse continues to exact enormous human and financial costs on society, yet all currently available treatments for drug addiction are notoriously ineffective. The search for a better understanding of the neurobiological mechanisms underlying the addictive actions of drugs of abuse and of the genetic factors that contribute to addiction should be given a high priority, as this should result in crucial advances in our ability to treat and prevent drug addiction.

From the basic neuroscience perspective, study of the neurobiology of drug addiction offers a novel opportunity to establish the biological basis of a complex and clinically relevant behavioral abnormality. Many prominent aspects of drug addiction in people can be clearly reproduced in laboratory animals, in striking contrast to most other forms of neuropsychiatric illness, such as psychotic and affective disorders, animal models for which are much harder to interpret. Advances made in the study of drug addiction should provide important insights into mechanisms underlying some of these other disorders.

Three terms related to drug abuse are used commonly: *tolerance, dependence,* and *addiction.* Tolerance represents a reduced effect upon repeated exposure to a drug at a constant dose, or the need for an increased dose to maintain the same effect. Dependence is defined as the need for continued exposure to a drug so as to avoid a withdrawal syndrome (physical and/or psychological disturbances) when the drug is withdrawn. Dependence is considered a priori to result from adaptive changes that develop in body tissues in response to repeated drug exposure. The traditional distinction between physical and psychological dependence is somewhat artificial, since both are mediated by neural mechanisms, possibly even similar neural mechanisms, as will be seen below. Addiction is defined as the compulsive use of a drug despite adverse consequences. In the past, physical dependence was part of the definition of addiction. However, the requirement for physical dependence as a necessary or sufficient aspect of drug addiction is no longer considered valid. Many drugs with no abuse potential, for example, β-adrenergic antagonists, clonidine, and tricyclic antidepressants, can produce marked physical symptoms on withdrawal. On the other hand, many unquestionably severe abusers of some drugs have little or no physical withdrawal syndrome upon cessation of drug exposure (e.g., most marijuana or cocaine users). Similarly, not all drugs of abuse produce tolerance to all of their effects.

This article reviews the results of recent research efforts that have begun to characterize the neurobiological basis of compulsive drug use. Its major focus is on opiates and cocaine, since the addictive mechanisms underlying the actions of these drugs are the best understood.

Cellular site of drug addiction

The discovery of endogenous opiate receptors in the 1970s raised the possibility that opiate addiction might be mediated by changes in these receptors. However, a decade of research has failed to identify consistent changes in the number of opiate receptors, or changes in their affinity for opiate ligands, under conditions of opiate addiction (Loh and Smith, 1990). Changes in levels of endogenous opioid peptides also do not appear to explain prominent aspects of opiate tolerance and dependence. The discovery that cocaine and other addictive psychostimulants acutely inhibit the reuptake or stimulate the release of monoamines throughout the brain has focused study of their addictive mechanisms on the regulation of monoamine neurotransmitters and their receptors. These studies too have been disappointing because it has been difficult to demonstrate consistent long-term changes in specific neurotransmitter or receptor systems in brain regions thought to underlie psychostimulant addiction (see Clouet et al., 1988; Liebman and Cooper, 1989; Peris et al., 1990).

The failure to account for important aspects of opiate and psychostimulant addictions in terms of regulation of neurotransmitters and receptors has shifted attention to postreceptor mechanisms. Most types of neurotransmitter receptors present in brain produce most of their physiological responses in target neurons through a complex cascade of intracellular messengers. These intracellular messengers include G-proteins (Simon et al., 1991), which couple the receptors to intracellular effector systems, and the intracellular effector systems themselves, which include second messengers, protein kinases and protein phosphatases, and phosphoproteins (Nestler and Greengard, 1984,

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1989). Regulation of these intracellular messenger pathways mediates the effects of the neurotransmitter–receptor systems on diverse aspects of neuronal function, including gene expression. Given that many important aspects of drug addiction develop gradually and progressively in response to continued drug exposure, and can persist for a long time after drug withdrawal, it is likely that the regulation of neuronal gene expression is of particular relevance to addiction.

In recent years, the increasing knowledge of intracellular messenger pathways has provided an experimental framework for studies of the molecular mechanisms underlying drug addiction. These investigations have demonstrated that changes in the activity of G-proteins and the cAMP second messenger and protein phosphorylation pathway mediate important aspects of opiate, and possibly cocaine, addiction in a number of drug-responsive brain regions.

Molecular mechanisms underlying opiate tolerance, dependence, and withdrawal: studies in the locus coeruleus

The locus coeruleus (LC) of the rat has served for many years as a useful model of opiate action. The LC is the largest noradrenergic nucleus in brain, located bilaterally on the floor of the fourth ventricle in the anterior pons. It is particularly suited for biochemical and molecular investigations, as it is a relatively homogeneous brain region that has been extensively characterized anatomically and electrophysiologically.

Pharmacological and behavioral studies have indicated that modulation of LC neuronal firing rates contributes to physical aspects of opiate addiction, namely, physical dependence and withdrawal, in several mammalian species, including primates (see Redmond and Krystal, 1984; Rasmussen et al., 1990). The importance of the LC in mediating opiate addiction is highlighted by a recent study that examined the effects of local injection of an opiate receptor antagonist into various brain regions of opiate-dependent rats (Maldonado et al., 1992). The most severe opiate withdrawal syndrome was produced by antagonist injections into the LC, which, in fact, elicited a withdrawal syndrome even more severe than that seen following intracerebroventricular administration.

Acute opiate action in the LC

The mechanism of acute opiate action in the LC, based on electrophysiological and biochemical studies, is well established and is shown schematically in Figure 1 (top). Acutely, opiates decrease the firing rate of LC neurons via activation of an inward rectifying K^+ channel (Aghajanian and Wang, 1987; North et al., 1987) and inhibition of a slowly depolarizing, nonspecific cation channel (Aghajanian and Wang, 1987; M. Alreja and G. K. Aghajanian, unpublished observations). Both actions are mediated via pertussis toxin–sensitive G-proteins (i.e., G_i and/or G_o) (Aghajanian and Wang, 1986; North et al., 1987), and inhibition of the nonspecific cation channel is mediated by reduced neuronal levels of cAMP and activated cAMP-dependent protein kinase (Aghajanian and Wang, 1987; Wang and Aghajanian, 1990; Alreja and Aghajanian, 1991). Opiates acutely inhibit adenylate cyclase activity in the LC (Duman et al., 1988; Beitner et al., 1989), as is the case in many other brain regions (see Childers, 1991), and inhibit cAMP-dependent protein phosphorylation (Guitart and Nestler, 1989). Such regulation of protein phosphorylation presumably mediates the effects of opiates on the nonspecific cation channel through the phosphorylation of the channel itself or some associated protein. Opiate regulation of protein phosphorylation also probably mediates the effects of opiates on many other aspects of LC neuronal function, including some of the initial steps underlying longer-term changes associated with addiction.

Chronic opiate action in the LC

Upon chronic opiate treatment, LC neurons develop tolerance to the acute inhibitory actions of opiates, as neuronal firing rates recover toward pretreatment levels (Aghajanian, 1978; Andrade et al., 1983; Christie et al., 1987). The neurons also become dependent on opiates after chronic exposure, in that abrupt cessation of opiate treatment, for example, by administration of an opiate receptor antagonist, leads to an elevation in LC firing rates manyfold above pretreatment levels (Aghajanian, 1978; Rasmussen et al., 1990).

The tolerance and dependence exhibited by LC neurons during chronic opiate exposure occur in the absence of detectable changes in opiate receptors or opiate-regulated ion channels themselves (see Christie et al., 1987; Loh and Smith, 1990).[1] This raises the possibility that intracellular messenger pathways may be involved. Indeed, over the past several years, it has been demonstrated that chronic administration of opiates leads to a dramatic upregulation of the cAMP system at every major step between receptor and physiological response (Fig. 1, bottom). Chronic opiate treatment increases levels of $G_{i\alpha}$ and $G_{o\alpha}$ (the active subunits of the G-proteins G_i and G_o) (Nestler et al., 1989), adenylate cyclase (Duman et al., 1988), cAMP-dependent protein kinase (Nestler and Tallman, 1988), and a number of MARPPs (morphine- and cAMP-regulated phosphoproteins) (Guitart and Nestler, 1989). Among these MARPPs is tyrosine hydroxylase (TH) (Guitart et al., 1990), the rate-limiting enzyme in the biosynthesis of catecholamines. These various intracellular adaptations to chronic opiate treatment are mediated via persistent activation of opiate receptors: the adaptations are blocked by concomitant treatment of rats with naltrexone, an opiate receptor antagonist, and are not produced by a single morphine injection.

Direct evidence for a functional role of an upregulated cAMP system in opiate addiction in the LC

The upregulated or "hypertrophied" cAMP system in the LC can be viewed as a compensatory, homeostatic response of LC neurons to the inhibition devolving from chronic opiate treatment (Fig. 1). According to this view, opiate upregulation of the cAMP system increases the intrinsic excitability of LC neurons and thereby accounts, at least in part, for opiate tolerance, dependence, and withdrawal exhibited by these neurons (Nestler, 1990). In the opiate-tolerant/dependent state, the combined presence of the opiate and the upregulated cAMP system would return LC firing rates toward pretreatment levels, whereas removal of the opiates would leave the upregulated cAMP system unopposed, leading to withdrawal activation of the neurons. This model, which is similar to one proposed previously based

[1] It is important to note that the lack of consistent effects of chronic opiates on opiate receptors in the LC and elsewhere is based exclusively on ligand binding studies, since to date no opiate receptor has been cloned or purified. It may well prove to be true that when a more complete analysis of opiate receptors is possible, changes in the receptors (e.g., altered expression, phosphorylation) associated with drug addiction will be revealed. Similarly, rigorous investigation of opiate regulation of specific ion channels must await molecular characterization of these channels.

on studies of cultured neuroblastoma × glioma cells (Sharma et al., 1975; Collier, 1980), is supported by several lines of evidence.

First, cAMP and agents that elevate cAMP levels excite LC neurons via the activation of cAMP-dependent protein kinase and subsequent activation of the nonspecific cation channel (Wang and Aghajanian, 1990). In fact, the *spontaneous* firing rate of LC neurons requires an active cAMP system and the opening of the nonspecific cation channel (Alreja and Aghajanian, 1991). Second, the time course by which certain components of the upregulated cAMP system revert to normal levels during naltrexone-precipitated opiate withdrawal parallels the rapid, early phase of the time course of recovery of LC neuronal firing rates and of various behavioral signs during withdrawal (Rasmussen et al., 1990). Third, upon bath application of naltrexone, LC neurons in brain slices obtained from morphine-dependent animals exhibit spontaneous firing rates more than twofold higher compared to LC neurons in slices from normal animals (Fig. 2*A*) (Kogan et al., 1992). Earlier studies failed to detect such withdrawal activation of morphine-dependent LC neurons *in vitro,* possibly due to the poor condition of the brain slices used and the small number and nonrandom samples of neurons examined (Andrade et al., 1983; Christie et al., 1987). Since most major afferents to the LC are severed in the brain slice preparation, the results establish that an increased intrinsic excitability caused by chronic opiate exposure contributes to opiate dependence in these cells. Fourth, LC neurons from morphine-dependent animals show a greater maximal responsiveness to cAMP analogs *in vitro* (Fig. 2*B*) (Kogan et al., 1992). Taken together, these results provide strong evidence to support the view that the opiate-induced upregulation of the cAMP system represents one mechanism by which opiates produce addictive changes in LC neurons.

Molecular mechanisms underlying opiate upregulation of the cAMP system in the LC

One of the central questions raised by these studies concerns the molecular mechanisms by which chronic opiate administration leads to upregulation of the cAMP system in LC neurons. Recent evidence indicates that many of the intracellular adaptations are attributable to changes in the levels of specific proteins and their mRNAs (Nestler et al., 1989; Guitart et al., 1990; Nestler, 1990), suggesting that regulation of gene expression may be involved in opiate addiction in this brain region.

Neurotransmitters influence gene expression via second messenger–dependent phosphorylation and/or induction of a class of nuclear proteins referred to as transcription factors—proteins that bind to specific DNA sequences (termed *response elements*) in the promoter regions of genes and thereby increase or decrease the rate at which those genes are transcribed. Two general types of mechanisms appear to be involved. In the first, protein kinases, activated in response to a first and second messenger stimulus, phosphorylate and activate transcription factors that are already present in the cell. CREB (cAMP response element binding) proteins function in this manner. CREB proteins consist of a family of related transcription factors that mediate many of the effects of cAMP (and probably of calcium), and of those neurotransmitters that act through cAMP (or calcium), on gene expression (Goodman, 1990; Montminy et al., 1990; Sheng et al., 1991). In the second mechanism, protein kinases, in some cases via phosphorylation and activation of CREB or a CREB-like protein, stimulate the expression of a family of genes en-

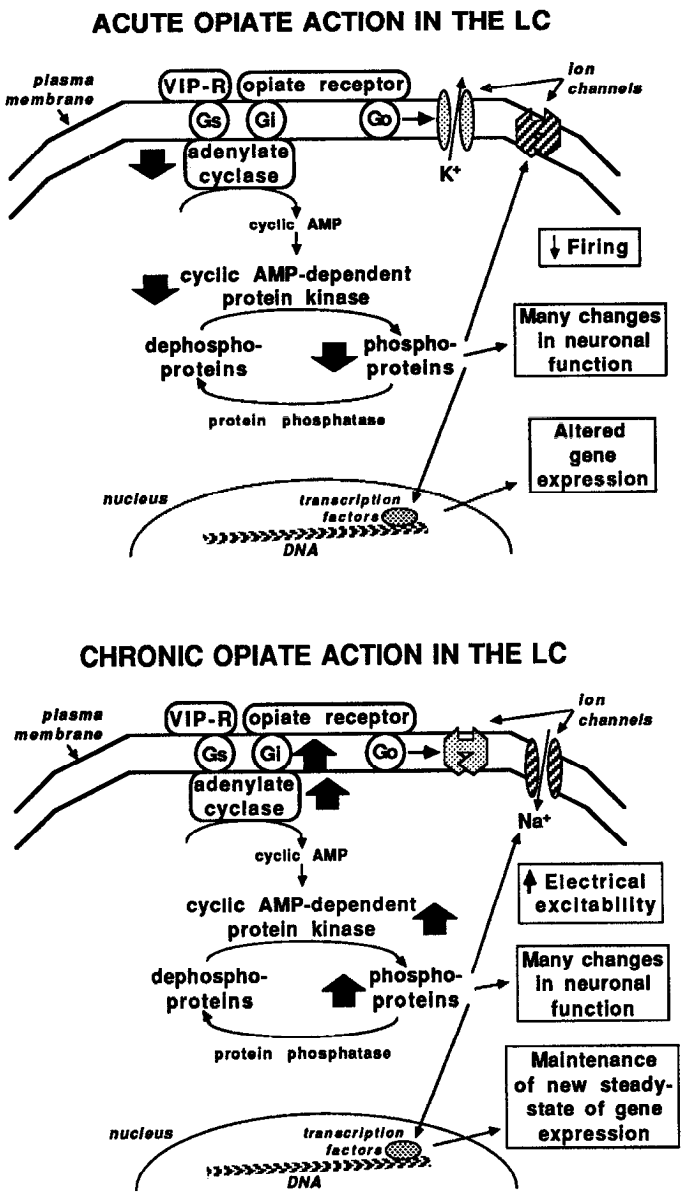

Figure 1. Schematic illustration of the mechanisms of acute and chronic opiate action in the LC. *Top,* Opiates acutely inhibit LC neurons by increasing the conductance of a K^+ channel (*stippled*) via coupling with a pertussis toxin–inhibitable G-protein (perhaps G_o), and by decreasing the conductance of a nonspecific cation channel (*hatched*) via coupling with G_i (the inhibitory G-protein) and the consequent inhibition of the cAMP pathway (*large downward arrows*) and reduced phosphorylation of the channel or a closely associated protein. Inhibition of the cAMP pathway, via decreased phosphorylation of numerous other proteins, would affect many processes in the neuron; in addition to reducing firing rates, for example, it would initiate alterations in gene expression via regulation of transcription factors. *Bottom,* Chronic administration of opiates leads to a compensatory upregulation of the cAMP pathway (*large upward arrows*), which contributes to opiate dependence in the neurons by increasing their intrinsic excitability via increased activation of the nonspecific cation channel. In addition, upregulation of the cAMP pathway presumably would be associated with persistent changes in transcription factors that maintain the chronic morphine-treated state. Chronic opiate administration also leads to a relative decrease in the degree of activation of the K^+ channel due to tolerance, the mechanism of which is unknown. Also shown in the figure are *VIP-R,* vasoactive intestinal polypeptide receptor (VIP is a major activator of the cAMP pathway in the LC), and G_s, the stimulatory G-protein that activates adenylate cyclase. Modified from Nestler (1990).

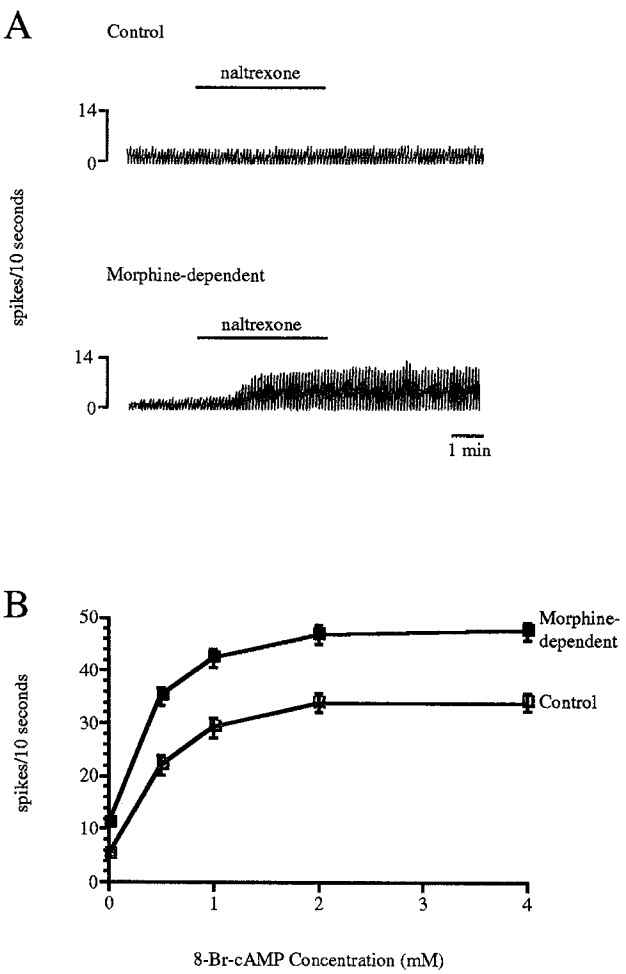

Figure 2. Elevated basal firing rates and enhanced responses to 8-bromo-cAMP in LC neurons in brain slices from morphine-dependent rats. *A,* Extracellular recordings showing the effect of bath application of the opiate receptor antagonist naltrexone (100 μM), on the spontaneous firing rates of LC neurons from a control and morphine-dependent animal recorded 1 hr after brain slice preparation. The figure illustrates that the spontaneous firing rate of LC neurons from dependent animals is more than twofold higher compared to those from control animals. *B,* Dose–response curves of LC neurons from control (*open squares*) and morphine-dependent (*solid squares*) animals to 8-bromo-cAMP illustrating the increased maximal response of the cells from morphine-dependent animals. Data represent mean firing rates ± SEM of an average of 35 neurons tested at every concentration from five rats in each group (control, $N = 176$ cells; morphine-dependent, $N = 178$ cells). Each of the morphine-dependent rates is significantly different from the control rates ($p < 0.01$). From Kogan et al. (1992).

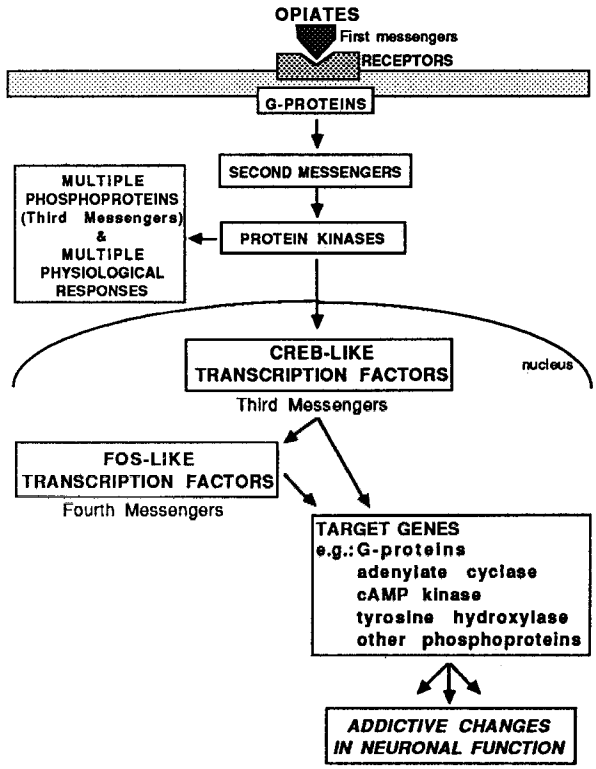

Figure 3. Intracellular messenger pathways through which opiates and other extracellular agents could regulate gene expression in target neurons. *CREB-like* transcription factors refer to those that are expressed constitutively, and regulated by extracellular agents primarily through changes in their degree of phosphorylation. *Fos-like* transcription factors refer to those that are expressed at very low levels under basal conditions, and regulated by extracellular agents primarily through induction of their expression (presumably via CREB-like proteins). Both types of mechanisms could contribute to the addictive actions of opiates and other drugs of abuse. Modified from Hyman and Nestler (1992).

coding transcription factors, referred to as immediate-early genes, for example, c-*fos,* c-*jun,* and zif268. The newly synthesized immediate-early gene products return to the nucleus, where they regulate the expression of other genes (Sheng and Greenberg, 1990; Morgan and Curran, 1991). Figure 3 illustrates the putative mechanisms involving changes in gene expression that mediate the addictive actions of opiates, and other drugs of abuse, in the nervous system.

Based on this scheme, studies have been performed to identify the specific transcription factors through which opiates might regulate the expression of G-proteins and the cAMP system in the LC. It has been shown that acute administration of opiates decreases levels of c-*fos* expression in the LC, and that such decreased expression persists with chronic opiate administration. In contrast, expression of c-*fos* and c-*jun* is increased severalfold during naltrexone-induced opiate withdrawal (Hayward et al., 1990). These results indicate that decreased expression of c-*fos* (and related transcription factors) might play a role in triggering and maintaining some of the intracellular adaptations to chronic morphine exposure, and that increased levels of the transcription factors might be involved in reversing the changes in the intracellular messengers to pretreatment levels during withdrawal.

More recently, it has been possible to study opiate regulation of one particular CREB protein (referred to simply as CREB) in the LC by use of an *in vitro* phosphorylation and immunoprecipitation procedure (Guitart et al., 1992a). It was found that acute morphine administration decreases the extent of phosphorylation of CREB in the LC, an effect that diminishes after chronic exposure to morphine. In contrast, opiate withdrawal increases CREB phosphorylation in this brain region (Guitart et al., 1992a). This regulation of CREB phosphorylation is consistent with the known effects of acute and chronic opiate administration, and opiate withdrawal, on the activity of the cAMP system in the LC.

These studies may well represent only the tip of the iceberg of opiate effects on transcription factors, with many more effects likely to be observed as it becomes possible to study the in-

creasing number of transcription factors implicated in brain signal transduction. Nevertheless, these studies highlight the utility of the LC as a model system for the investigation of transcription factor regulation. In LC neurons, specific candidate target genes have been identified, and changes in their rates of expression have been shown to be physiologically important. Thus, it should be possible to delineate the precise molecular steps by which opiates regulate the expression of these genes and, as a result, understand the cellular basis of tolerance, dependence, and withdrawal.

Extrinsic factors in opiate addiction in the LC

The preceding discussion of opiate addiction focused on factors that are intrinsic to LC neurons. However, extrinsic factors also contribute to the raising of LC neuronal firing rates during opiate withdrawal. Lesions of the nucleus paragigantocellularis (PGi), a region in the rostral medulla that provides a major excitatory input to the LC (Ennis and Aston-Jones, 1988), attenuate by about 50% the severalfold increase in LC neuronal firing rates upon withdrawal *in vivo* (Rasmussen and Aghajanian, 1989). Intracerebroventricular or intracoerulear administration of kynurenic acid or other glutamate receptor antagonists produces a similar effect (Rasmussen and Aghajanian, 1989; Akaoka and Aston-Jones, 1991), consistent with the view that the PGi input to the LC is mediated by an excitatory amino acid, presumably glutamate. In view of the twofold withdrawal activation of LC neurons observed in brain slices *in vitro,* and the approximately 50% reduction in withdrawal activation induced by PGi lesions or kynurenic acid, it would appear that intrinsic and extrinsic factors each contribute about equally to the overall withdrawal activation of LC neurons *in vivo.*

Where do the changes occur that underlie the role of the PGi in withdrawal activation of LC neurons, and what is their nature? These changes might occur in nerve terminals within the LC of axons projected from the PGi, in cell bodies within the PGi, or in any afferents that innervate the PGi (e.g., from spinal regions). Indeed, upregulation of the cAMP system induced by chronic opiate administration, similar to that which occurs in the LC, has been observed in cultures of dorsal root ganglion–spinal cord—a major afferent to the PGi (Makman et al., 1988; Terwilliger et al., 1991a)—and in the PGi itself (D. Beitner-Johnson and E. J. Nestler, unpublished observations). These findings indicate that, as outlined in Figure 4, an upregulated cAMP system may contribute to opiate dependence in several neuronal cell types, which summate to lead to the greatly increased firing rates of LC neurons *in vivo.*

The findings also demonstrate the likely complexity of the types of mechanisms underlying opiate addiction even for such a homogeneous and "simple" brain region as the LC, and the critical importance of considering neural networks when attempting to understand drug addiction. This view is further supported by growing evidence for a role of glutamatergic neurotransmission in opiate tolerance, dependence, and withdrawal. Thus, administration of the NMDA glutamate receptor antagonist MK-801 has been shown to attenuate the development of tolerance and physical dependence (Trujillo and Akil, 1990), and administration of kynurenic acid has been shown to decrease the severity of opiate withdrawal (Rasmussen et al., 1991). It must be borne in mind, of course, that glutamatergic neurotransmission occurs at a large fraction of all synpases in the brain, such that these observations may indicate the requirement for multiple, intact neural networks in the development and expression of opiate addiction and not a *specific* role for glutamate in these phenomena.

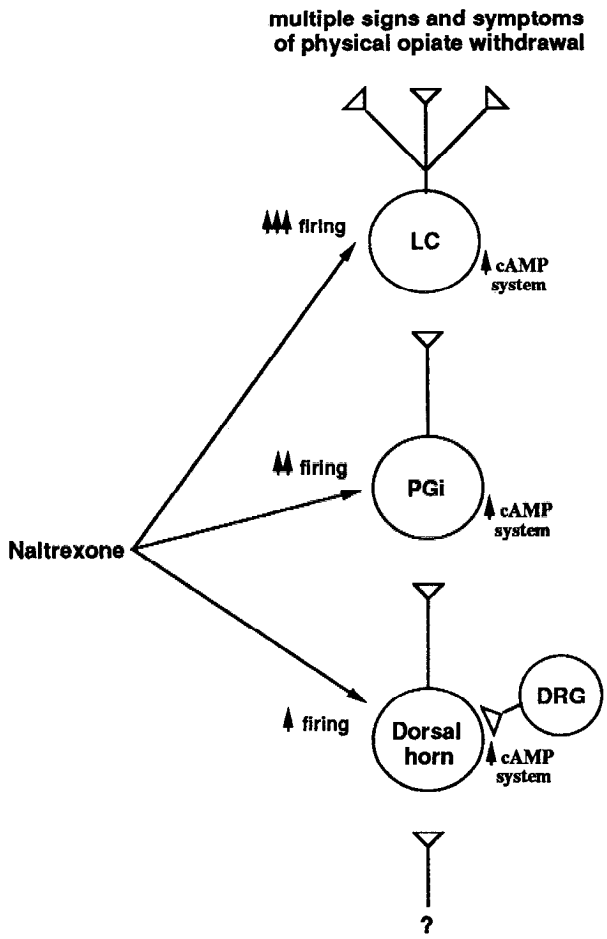

Figure 4. Role of extrinsic and intrinsic factors in withdrawal activation of the LC. An upregulated cAMP system represents part of the intrinsic changes that opiates induce in a number of neuronal cell types [including LC, PGi, and dorsal root ganglion (*DRG*)–spinal cord (*dorsal horn*)] that contribute to tolerance, dependence, and withdrawal. The figure illustrates that sudden opiate withdrawal via systemic administration of an opiate receptor antagonist (e.g., naltrexone) would reveal these intrinsic changes in each of the various neurons in which they occur. The occurrence of such changes at several steps along a particular neural relay pathway—for example, from DRG–spinal cord to PGi to LC—would lead to escalating activation of the neurons in that pathway during withdrawal.

General role for G-proteins and an upregulated cAMP system in opiate addiction

Biochemical studies have thus demonstrated upregulation of the cAMP system in response to chronic opiate exposure in a number of discrete regions of the CNS in addition to the LC, including the dorsal root ganglion–spinal cord, nucleus accumbens (NAc), amygdala, and thalamus (Makman et al., 1988; Crain and Shen, 1990; Terwilliger et al., 1991a), indicating that upregulation of adenylate cyclase and cAMP-dependent protein kinase may represent a common mechanism by which a number of opiate-sensitive neurons adapt to chronic morphine administration.

Among the regions that exhibit an upregulated cAMP system with chronic opiate administration, there are different types of changes in the levels of G-protein subunits: increased levels of

$G_{i\alpha}$ and $G_{o\alpha}$ are observed in the LC and amygdala, but decreased levels of $G_{i\alpha}$ are observed in the dorsal root ganglion–spinal cord and NAc. These differential G-protein responses could account for the differences between *homologous* and *heterologous* desensitization observed in these neuronal tissues in response to chronic opiate treatment (Terwilliger et al., 1991a). Dorsal root ganglion neurons show heterologous desensitization: tolerance develops to the effects of not only opiates, but also of other agents, such as α_2-adrenergic agonists and 5-HT. In contrast, LC neurons show homologous desensitization: tolerance develops to the effects of opiates, but not to those of α_2-adrenergic agonists.

Molecular mechanisms of drug reward: studies in the mesolimbic dopamine system

Drugs that are abused by humans are considered reinforcing or rewarding, in that drug use often leads to further drug use. Virtually all drugs that are rewarding in people are also rewarding in laboratory animals. The rewarding properties of a drug are considered the core cause of its addictiveness. This may seem paradoxical: since addiction is defined as the compulsive use of a drug despite *adverse* consequences, what is the reward that leads to compulsive drug use? One possibility is that the drug is acutely rewarding and that reward occurs with repeated administrations, such that the drive for that reward overshadows the normal concerns of appetite, sleep, lawful behavior, and social taboos. Another possibility, not incompatible with the first, is that repeated drug exposure produces adaptive changes (dependence) in brain regions relevant to reward, such that discontinuation of the drug leads to a psychological withdrawal syndrome that is eased by subsequent drug administration (Koob et al., 1992). According to this view, the psychological withdrawal syndrome would be severe enough to overshadow other life concerns. What is the nature of drug reward and psychological withdrawal? Drug-induced reward could be an intense euphoria or "high" that occurs with drug administration, whereas psychological withdrawal could reflect a dysphoria or "crash" that occurs with cessation of drug exposure.

Drug reward has been studied in animals by use of three major experimental paradigms. *Self-administration* and *intracranial self-stimulation* are operant paradigms where animals learn to perform a task in exchange for administration of a drug or electrical stimulation of a neural pathway, respectively. *Conditioned place preference* is a classical conditioning paradigm where animals learn to associate the experience of a drug with a particular context. These studies have established the mesolimbic dopamine system as one important neural substrate of drug reward. The mesolimbic dopamine system consists of dopaminergic neurons in the ventral tegmental area (VTA) and their various projection regions, notably, the NAc. Animals self-administer opiates directly into one or both of these brain regions and develop conditioned place preference in response to local drug administration. Conversely, lesions of the VTA-NAc pathway block systemic self-administration of opiates as well as the development of conditioned place preference. Similar results have been obtained with self-administration of cocaine and other psychostimulants such as amphetamine (Wise and Bozarth, 1987; Clouet et al., 1988; Koob and Bloom, 1988; Liebman and Cooper, 1989). In addition, the ability of opiates and psychostimulants acutely to increase extracellular levels of dopamine in the NAc is shared with a number of other drugs of abuse, including ethanol, nicotine, and Δ^9-tetrahydrocannabinol (DiChiara and Imperato, 1988; Chen et al., 1990). These findings have led to the view that the VTA and NAc are critical "brain reward regions" that mediate the reinforcing actions (i.e., craving) of many drugs of abuse. It should be emphasized that other components of the mesolimbic dopamine system, such as the medial prefrontal cortex (Goeders and Smith, 1983) and ventral pallidum (Hubner and Koob, 1990), as well as non-dopaminergic pathways (Koob and Bloom, 1988), have also been implicated in drug reward mechanisms.

Common actions of morphine and cocaine in the VTA-NAc pathway

Identification of specific brain regions implicated in drug reinforcement has led to a large number of investigations of possible biochemical changes in these regions associated with addictive phenomena. However, as mentioned above, studies of neurotransmitter and receptor regulation have failed to account for important aspects of opiate and cocaine reinforcement, particularly that seen after chronic drug exposure. This failure has led to the view that adaptations in postreceptor mechanisms may play a critical role in drug action on the mesolimbic dopamine system.

Regulation of G-proteins and the cAMP pathway by opiates and cocaine. The finding of opiate regulation of the G-protein/cAMP system in the NAc raised the possibility that other addictive drugs, such as cocaine, might produce similar intracellular adaptations in the NAc. Indeed, it was found that chronic, but not acute, administration of cocaine decreases levels of $G_{i\alpha}$ and $G_{o\alpha}$ (Nestler et al., 1990), and increases levels of adenylate cyclase and cAMP-dependent protein kinase (Terwilliger et al., 1991a), in the NAc. Morphine and cocaine regulation of these intracellular messenger proteins was not observed in the other major dopaminergic system in the brain, the nigrostriatal system, which consists of dopaminergic neurons in the substantia nigra and their major projection region, the caudate–putamen. Moreover, regulation of G-proteins and the cAMP system was not seen in response to other classes of psychotropic drugs that lack reinforcing properties, including haloperidol (an antipsychotic drug), and imipramine or fluoxetine (antidepressant drugs).

With respect to cocaine, these biochemical actions can be understood within a functional context of known electrophysiological effects of the drug on NAc neurons. Chronic cocaine administration has been shown to produce supersensitivity of NAc neurons to the inhibitory actions of D1-dopaminergic agonists (Henry and White, 1991). This supersensitivity occurs in the absence of consistent changes in levels of D1-receptors (see Clouet et al., 1988; Peris et al., 1990), suggesting the involvement of postreceptor mechanisms. As D1-receptors are generally thought to exert their effects via the G-protein G_s and the subsequent activation of the cAMP pathway, the observed increase in adenylate cyclase and cAMP-dependent protein kinase, together with the observed decrease in G_i (without a change in levels of G_s), could account for D1-receptor supersensitivity observed electrophysiologically. Although the electrophysiological effects of chronic morphine administration on NAc neurons have not yet been studied, effects similar to those observed following chronic cocaine administration would be predicted based on the biochemical observations.

Identification of morphine- and cocaine-regulated phosphoproteins. To study further the putative intracellular targets of chronic opiate and cocaine action in the VTA and NAc, drug

regulation of the next step in the cAMP signal transduction pathway, namely, individual phosphoprotein substrates of cAMP-dependent protein kinase, was investigated. Chronic administration of morphine or cocaine was found to exert similar effects on levels of many of the same phosphoproteins in the mesolimbic dopamine system; these have been termed MCRPPs (morphine- and cocaine-regulated phosphoproteins) (Beitner-Johnson and Nestler, 1991; Beitner-Johnson et al., 1992a). One of the MCRPPs identified to date is TH. In initial studies, morphine and cocaine were found to increase the *in vitro* levels of TH phosphorylation in the VTA, an effect shown subsequently to be due to drug-induced increases in the total amount of the enzyme, without a change in its degree of phosphorylation (Beitner-Johnson and Nestler, 1991). In contrast, chronic administration of morphine or cocaine was shown to decrease the degree of phosphorylation of TH in the NAc without a change in the total amount of the enzyme. Since dephosphorylation of TH decreases its catalytic activity, the morphine- and cocaine-induced decrease in TH phosphorylation in the NAc is probably associated with decreased enzyme activity in this brain region. Indeed, this observed dephosphorylation of TH could account for the reduced levels of *in vivo* dopamine synthesis observed in response to chronic cocaine administration in the NAc (Brock et al., 1990), and for reduced *in vivo* levels of basal and morphine-stimulated dopamine release in the NAc in response to chronic administration of morphine (Acquas et al., 1991). As TH present in the NAc is located within dopaminergic nerve terminals of axons projected from the VTA, the results indicate that this enzyme can be regulated by morphine and cocaine differentially in cell body and nerve terminal regions of the mesolimbic dopamine system. The possible consequences of such differential regulation are discussed in greater detail below.

Four other MCRPPs have also been identified (Beitner-Johnson et al., 1992a). Three correspond to the major constituents of neurofilaments (NFs): NF-H (high M_r), NF-M (medium M_r), and NF-L (low M_r). The fourth corresponds to a novel NF-like protein referred to as α-internexin or, alternatively, as NF-66 kDa. Morphine and cocaine decrease the total amounts of most of these NF proteins, but increase the degree of their phosphorylation, in the VTA.

In the course of these studies, it was also found that NF-H, NF-M, NF-L, and α-internexin are highly enriched within the VTA compared to many other brain regions examined (Beitner-Johnson et al., 1992a,b). The possibility that NFs are abundantly expressed in VTA neurons would suggest that these dopaminergic cells display some specialized function subserved by these proteins that is altered under the morphine- and cocaine-addicted state. Morphine and cocaine regulation of NF proteins is not associated with a general disruption of the neuronal cytoskeleton, as chronic morphine administration has no effect on several other cytoskeletal or cytoskeletal-associated proteins studied, which included α- and β-tubulin, actin, vimentin, synaptophysin, tau, and synapsin 1 (Beitner-Johnson et al., 1992a). Moreover, morphine and cocaine regulation of TH and the NF proteins in the mesolimbic dopamine system, like the changes in the G-protein/cAMP system, showed temporal, regional, and pharmacological specificity.

The similar effects of chronic morphine and cocaine administration on G-proteins, the cAMP pathway, and several target phosphoproteins in the mesolimbic dopamine system are particularly striking since acute administration of the two drugs exerts opposite electrophysiological effects on VTA neurons (Matthews and German, 1984; Henry et al., 1989); yet both drugs are clearly psychologically addicting, indicating that they probably produce some similar functional changes in the mesolimbic system after chronic administration. Indeed, chronic morphine and cocaine treatment have both been shown to increase the spontaneous firing rate of VTA neurons (Henry et al., 1989; M. Jeziorski and F. J. White, personal communication). It is possible, then, that the similar effects of chronic morphine and cocaine administration on intracellular messenger proteins represent part of the biochemical basis of long-term functional changes in the VTA-NAc pathway that modify drug reward mechanisms.

Molecular mechanisms of cocaine action. Attention has been given recently to the possibility that some of the long-term effects of cocaine on the mesolimbic dopamine system are achieved at the level of gene expression. Acute administration of cocaine has been shown to induce the expression of c-*fos*, c-*jun*, zif/268, and a number of related immediate-early genes in the NAc (Graybiel et al., 1990; Young et al., 1991; Hope et al., 1992). Fos- and Jun-related proteins form dimers (called AP-1 complexes) that bind to a specific sequence of DNA, termed the AP-1 site. AP-1 binding activity (a measure of the level of AP-1 complexes) is induced in the NAc by acute cocaine administration, as would be expected from the increased expression of immediate-early genes (Hope et al., 1992). In contrast, chronic administration of cocaine abolishes the ability of a subsequent acute dose to increase the expression of these transcription factors in the NAc, and yet leads to a persistent increase in AP-1 binding activity, with elevated levels observed for at least 3 d after the last chronic dose of cocaine (Hope et al., 1992).

These observations indicate that while chronic administration of cocaine produces a desensitization in the inducibility of certain immediate-early genes, for example, c-*fos* and c-*jun*, it leads to a concomitant accumulation of some as yet unidentified Fos- and/or Jun-like protein(s). Such a change in the composition of the AP-1 complex could alter its transcriptional activity and/or specificity and thereby lead to some of the changes in gene expression seen in response to chronic cocaine administration in the NAc, for example, alterations in G-proteins and the cAMP system described above.

Genetic factors in drug reward

Growing evidence indicates that genetic factors influence the predilection to drug addiction (see Pickens and Svikis, 1988). In humans, such an influence is well established for alcoholism and widely presumed to exist for other drug addictions. Moreover, numerous inbred strains of animals exhibit widely differing degrees of predilection to addiction to various drugs of abuse, and marked individual differences have been observed within single outbred strains. Such genetic factors are likely to influence the predilection to addiction by determining the neurochemical responses the drugs elicit in the brain acutely, and/or the long-term adaptations induced in the brain after chronic drug exposure.

Biochemical differences in the VTA-NAc pathway in inbred rat strains. To study further the relevance of morphine and cocaine regulation of G-proteins, the cAMP pathway, and the various MCRPPs in the mesolimbic dopamine system to drug reward mechanisms, these intracellular messenger proteins were studied in the VTA-NAc pathway of the inbred Lewis and Fischer 344 rat strains. Lewis rats self-administer opiates, cocaine,

and alcohol at much higher rates than Fischer rats (Li and Lumeng, 1984; Suzuki et al., 1988; George and Goldberg, 1989) and also develop greater degrees of conditioned place preference to systemically administered morphine and cocaine (Guitart et al., 1992b). Furthermore, cannabinoids facilitate self-stimulation of the mesolimbic dopamine pathway in Lewis but not Fischer rats (Gardner and Lowinson, 1991). It was found that, in drug-naive rats, the NAc of the Lewis strain contains lower levels of $G_{i\alpha}$, higher levels of adenylate cyclase and cAMP-dependent protein kinase, and lower levels of TH than that of the Fischer strain (Beitner-Johnson et al., 1991; Terwilliger et al., 1991b). In addition, the VTA of the Lewis strain contains higher levels of TH and lower levels of NF proteins than that of the Fischer strain (Beitner-Johnson et al., 1991; Guitart et al., 1992b).

Several pieces of correlative evidence support the speculation that the strain differences in G-proteins, the cAMP system, TH, and NF proteins may underlie the strain differences in drug preference. First, the strain differences in each of these proteins are highly localized to the VTA-NAc pathway. Such differences are not, for example, seen in the nigrostriatal dopamine system, which is structurally related to the mesolimbic dopamine system but generally not implicated in drug reward mechanisms. Second, the difference in levels of TH observed in the mesolimbic dopamine systems of Lewis and Fischer rats presumably reflects different levels of dopaminergic function in the VTA-NAc pathway, which would be expected to alter levels of drug preference. Third, the inherent levels of each of the specific intracellular signaling proteins in the VTA-NAc pathway of the drug-preferring Lewis rat, compared to the relatively non-drug-preferring Fischer rat, resemble morphine- and cocaine-induced changes in the levels of the proteins in outbred Sprague–Dawley rats compared to vehicle-treated animals. These findings raise the possibility that different levels of expression of these intracellular signaling proteins in the VTA-NAc pathway could contribute to individual genetic predilection to drug addiction (Beitner-Johnson et al., 1991).

Model of the biochemical basis of drug reward

Figure 5 summarizes the effects of chronic morphine and cocaine administration on intracellular messenger proteins in the mesolimbic dopamine system, and the different levels of these proteins observed in these brain regions between Lewis and Fischer rats. Based on these data, it can be speculated that a drug-addicted (or genetically drug-preferring) state is associated with higher levels of TH and lower levels of NFs in the VTA, and lower levels of G_i and TH and higher levels of adenylate cyclase and cAMP-dependent protein kinase in the NAc.

What functional changes occur in the mesolimbic dopamine system in the drug-addicted state as a result of the concerted action of these biochemical adaptations? First, as alluded to above, the activity of the dopamine system is regulated differently in dopaminergic cell bodies and dendrites in the VTA and in dopaminergic nerve terminals in the NAc. This is consistent with the view that dopaminergic neurotransmission subserves different functions in these two brain regions. Thus, higher levels of TH (and hence higher levels of dopaminergic function) in the VTA would be expected to increase the autoinhibitory influence of dopamine acting on D2-dopamine receptors on these neurons (the principal action of dopamine within this brain region). This effect could lead to subsensitivity of D2-receptor function and increased spontaneous firing of the neurons, both of which have been observed electrophysiologically (Henry et al., 1989; Jeziorski and and White, personal communication). In contrast, lower levels of active TH and of dopaminergic function in the NAc would be expected to decrease the various pre- and postsynaptic effects of dopamine acting on D1 and D2 (and probably other) dopamine receptor subtypes. This relative dopamine deficiency could be the stimulus that leads to D1-receptor supersensitivity in NAc neurons in response to chronic administration of cocaine.

The mechanism by which TH is regulated differentially in the VTA and in the NAc is unknown, but it might involve NF proteins. Studies have shown that lower levels of NFs are associated with decreased rates of axonal transport and decreased axonal caliber (see Hammerschlag and Brady, 1989). A decrease in levels of NFs in the drug-addicted (or genetically drug-preferring) state could, then, result in decreased transport of TH from cell bodies in the VTA to nerve terminals in the NAc. At a constant rate of TH synthesis, this would tend to lead to a buildup of TH in the VTA, with either no change in enzyme levels in the NAc (as observed in the morphine- and cocaine-treated states) or lower amounts of TH in the NAc (as observed in Lewis rats). In fact, we have found recently that chronic morphine treatment does impair axonal transport in the VTA-NAc pathway (Beitner-Johnson and Nestler, 1992). Taken together, our findings provide strong evidence for the view that drug addiction is associated with structural alterations in mesolimbic dopamine neurons which reduce the ability of these cells to transmit dopaminergic signals to neuronal elements in the NAc. Such structural alterations in response to chronic exposure to psychostimulants have been observed in preliminary observations with neurons cultured *in vitro* (Cubells et al., 1991).

Despite the evidence for a role of the mesolimbic dopamine system in drug reward mechanisms, the precise process by which this system influences drug reinforcement remains unknown. One possibility is that dopamine (and, therefore, the VTA) influences drug reward by modulating the synaptic efficacy of polysynaptic neural pathways from cortical to subcortical structures that travel through the NAc (and related mesolimbic projection areas). According to this scheme, drugs of abuse would produce their acute and chronic effects on reward (i.e., produce craving) by influencing this pathway. As stated earlier, the cocaine-induced changes in levels of G-proteins, adenylate cyclase, and cAMP-dependent protein kinase observed in the NAc could underlie the D1-receptor supersensitivity observed electrophysiologically in this brain region, and a similar supersensitivity of D1-receptor function would be expected for chronic morphine, at least under certain treatment conditions. Such altered activity of G-proteins and the cAMP pathway in the NAc would be expected to change the synaptic responsiveness of NAc neurons not only to dopamine, but also to a host of other incoming neurotransmitter signals that innervate this brain region. Such altered responsiveness of NAc neurons to these other synaptic inputs might represent one of the major changes in the brain that underlie drug addiction and craving.

Future directions

The studies described here support the view that through the investigation of intracellular messenger pathways, it will be possible to understand the biochemical and molecular mechanisms by which drugs of abuse induce changes in brain function that underlie addiction. Studies of neurons of the LC have provided the clearest indication to date of the specific biochemical mechanisms involved in opiate addiction. Adaptations in G-proteins

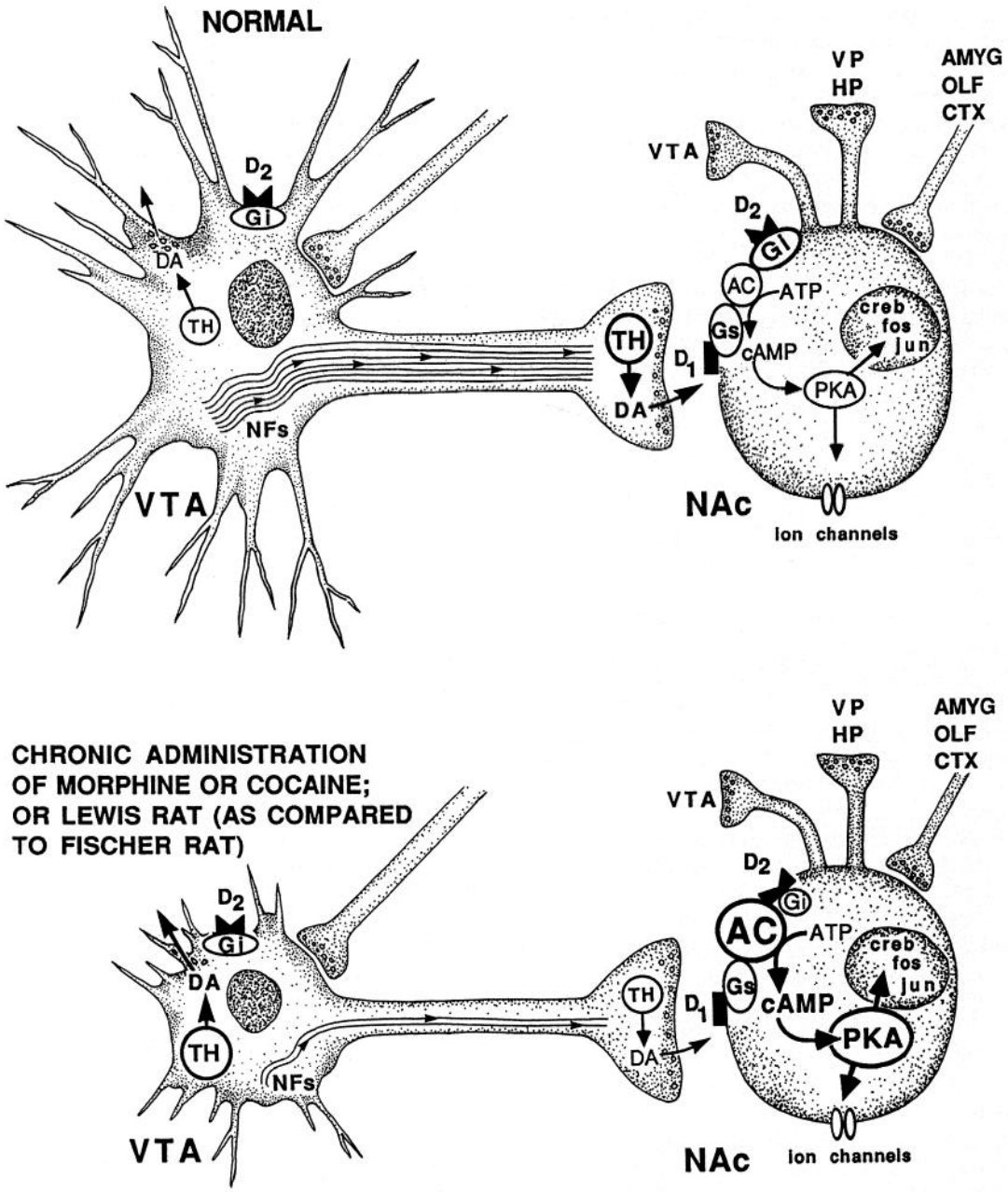

Figure 5. Schematic summary of similar biochemical manifestations of the "drug-addicted" and "genetically drug-preferring" state. The *top panel* depicts a normal VTA neuron projecting to an NAc neuron. Shown in the VTA neuron are TH, dopamine (*DA*), presynaptic dopamine receptors (D_2) coupled to G-proteins (*Gi*), and neurofilaments (*NFs*). Shown in the NAc neuron are dopamine receptors (D_1 and D_2), G-proteins (*Gi* and *Gs*), components of the intracellular cAMP system (*AC,* adenylate cyclase; *PKA,* cAMP-dependent protein kinase; and possible substrates for the kinase–ion channels and the nuclear transcription factors *CREB, fos,* and *jun*), as well as major inputs and outputs of this region (*VP,* ventral pallidum; *HP,* hippocampus; *AMYG,* amygdala; *OLF,* olfactory cortex; *CTX,* other cortical regions). The *bottom panel* depicts a VTA neuron projecting to the NAc after chronic administration of morphine or cocaine, or in an untreated Lewis (genetically drug-preferring) rat as compared to a relatively non-drug-preferring Fischer rat. In the drug-addicted or drug-preferring animal, TH levels are increased in the VTA and decreased in the NAc (due to either decreased phosphorylation as for morphine and cocaine, or decreased enzyme levels as in Lewis vs. Fischer rats). In addition, NF levels are decreased in the VTA in the drug-addicted and drug-preferring animal, as observed recently for chronic morphine (Beitner-Johnson and Nestler, 1992). Such a decrease in NFs may be associated with alterations in neuronal structure, decreases in axonal caliber, and/or decreases in axonal transport rate in these cells. This hypothetical decrease in axonal transport may account for the lack of correspondingly increased levels of TH in dopaminergic terminals in the NAc. Decreased TH levels imply decreased dopamine synthesis, and may result in reduced dopaminergic transmission to the NAc. In the NAc of the drug-addicted or drug-preferring animal, G_i is decreased, and adenylate cyclase and cAMP-dependent protein kinase activities are increased, changes that could account for D1-receptor supersensitivity observed electrophysiologically.

It should be noted that alterations in dopaminergic transmission probably influence many cell types within the NAc, as well as other nerve terminals in the NAc. Similarly, altered local dopaminergic transmission in the VTA would influence other VTA neurons, as well as nerve terminals that innervate this brain region. Thus, biochemical alterations in the mesolimbic dopamine system could potentially lead to altered neuronal function in many other brain regions as well. From Beitner-Johnson et al. (1992b).

and the cAMP second messenger and protein phosphorylation system have been shown to play an important role in mediating aspects of opiate tolerance, dependence, and withdrawal in this cell type. It is likely that many other mechanisms, perhaps involving other intracellular messenger systems, also contribute to opiate addiction. Nevertheless, studies in the LC have provided one of the first cases where it has been possible to understand an aspect of opiate addiction at the molecular, electrophysiological, and behavioral levels of analysis.

Neurons of the LC, which play a role in physical opiate dependence, and neurons of the NAc, which contribute to the psychological reinforcing actions of opiates, show similar biochemical adaptations to chronic administration of opiates. This supports the view that similar biochemical mechanisms mediate both physical and psychological aspects of drug addiction, depending on the neuronal cell type involved. The findings emphasize that the distinction between physical and psychological dependence is arbitrary: both are due to changes in brain function mediated via biochemical adaptations in specific neuronal cell types that lead to alterations in the functional state of these neurons and in the particular behavioral parameters subserved by the neurons.

In the mesolimbic dopamine system, chronic administration of morphine or cocaine exerts similar actions on G-proteins and the cAMP second messenger and protein phosphorylation pathway, whereas other classes of psychotropic drugs that are not reinforcing are without significant effect on this intracellular messenger pathway. These findings support the possibility that common biochemical changes mediate aspects of morphine and cocaine reinforcement and craving. Future studies should reveal whether chronic exposure to other classes of abused drugs elicits similar intracellular adaptations in the mesolimbic dopamine system.

The manner in which adaptations in G-proteins, the cAMP system, TH, and NF proteins might contribute to drug addiction remains unknown. The scheme proposed in Figure 5, while highly conjectural, defines specific hypotheses regarding the functional sequelae of the drug-induced biochemical changes in the mesolimbic dopamine system that can now be tested by direct experimental means. In particular, future studies are needed to establish a direct causal link between the various biochemical adaptations and (1) electrophysiological changes induced in VTA and NAc neurons by chronic opiate and cocaine administration, as well as (2) behavioral measures of drug reward and craving. Future studies will also be needed to determine the precise molecular mechanisms by which opiates and cocaine alter the levels of intracellular messenger proteins in the mesolimbic dopamine system.

Recent studies on inbred rat strains raise the possibility that biochemical mechanisms similar to those underlying opiate and cocaine action may be involved in the genetic predisposition of some individuals to drug addiction. Clearly, our hypothesis that Lewis and Fischer strain differences in the various intracellular signaling proteins are related to strain differences in drug preference must be viewed with caution, as much work is needed to establish such a connection between these two observations. These two rat strains are known to differ genetically in several other ways, including aging and stress and immune responses (see Beitner-Johnson et al., 1991). The biochemical differences presented here could conceivably be associated with some of these other phenotypic differences. On the other hand, it is possible that some of these other processes are, in fact, connected with drug preference. The difference in immune responsiveness between the Lewis and Fischer strains has been associated with a difference in levels of corticotropin-releasing factor (CRF) in specific brain regions (Sternberg et al., 1989a,b). Such a difference in the activity of the central CRF system could conceivably contribute also to the strain difference in drug preference, given the increasing evidence for the involvement of the CRF/glucocorticoid system in drug reward (Calogero et al., 1989; Goeders et al., 1990; Maccari et al., 1991; Piazza et al., 1991).

Studies of the biochemical and molecular basis of drug addiction have several important clinical implications. A better understanding of the neurobiological mechanisms underlying the addictive actions of drugs of abuse and of the genetic factors that contribute to drug addiction is bound to lead to the development of pharmacological agents that prevent or reverse the actions of the drugs on specific target neurons. Such drugs could be used not only to treat physical abstinence syndromes, but also to reduce the craving for drugs of abuse. Their availability would represent a revolutionary step in our battle against drug addiction.

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