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Regulation of drug-taking and -seeking behaviors by neuroadaptations in the mesolimbic dopamine system

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Abstract

Previous studies have identified several neuroadaptations to chronic drug use, but relatively few have been functionally linked to addiction-related changes in drug-taking and -seeking behaviors. This article summarizes our past and present studies on the contribution of drug-induced neuroadaptations in the mesolimbic dopamine system to addiction-related changes in drug self-administration and the propensity for relapse in drug withdrawal. Our studies suggest that drug-induced up-regulation in cyclic AMP (cAMP)–protein kinase A (PKA) signaling in the nucleus accumbens (NAc) contributes to escalating drug intake and a propensity for relapse by differentially altering the sensitivity of D_1 and D_2 dopamine receptors that regulate drug-taking and -seeking behaviors. In addition, our studies suggest that drug-induced neuroplasticity at excitatory synapses in both the ventral tegmental area (VTA) and the NAc also facilitates drug-seeking behavior and the propensity for relapse. Finally, the role of both transient and enduring neuroadaptations in regulating drug-seeking behavior is discussed in view of different learning- and memory-based interactions.
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1. Introduction

The transition from recreational drug use to an addicted state is marked by a dramatic escalation in the amount of drug consumed during self-administration, and by profound increases in drug craving in withdrawal leading to compulsive drug-seeking behavior (American Psychiatric Association, 1994; Self, in press). Recently, these behavioral changes have been modeled in animals using self-administration procedures designed to dissociate "addicted" from "non-addicted" states (Ahmed and Koob, 1998; Deroche et al., 1999; Ahmed et al., 2000; Piazza et al., 2000; Sutton et al., 2000). In these studies, the addicted phenotype can be artificially produced by prolonging daily access to drug self-administration (Ahmed and Koob, 1998; Deroche et al., 1999), or selected from

outbred populations based on individual differences in preferred levels of drug intake (Piazza et al., 2000; Sutton et al., 2000). Importantly, escalating drug intake has been specifically related to a propensity for drug seeking during withdrawal (Sutton et al., 2000), thereby encompassing both addictive traits in a subpopulation of outbred rats. These behavioral differences suggest that differences also exist in the neural substrates that regulate these behaviors. Clarification of the neurobiological changes underlying the transition to addiction, whether induced by chronic drug use, or facilitated by an inherent predisposition, is of paramount importance to ultimately understand the disease.

The pharmacological regulation of drug self-administration behavior is complex, but critical aspects of the addiction process can be studied by dividing self-administration into two temporally distinct phases often referred to as drug seeking and drug-taking (or drug intake). Drug seeking is reflected by behavior aimed at approaching and performing responses that

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deliver drug injections. Most studies measure the level of effort an animal will exert to obtain drug when reinforcement is withheld as an index of drug-seeking behavior, and this behavior is thought to reflect wanting or craving that would precipitate relapse to drug use in humans (Berridge and Robinson, 1998; Wise, 2004). Drug addiction involves a profound sensitization in the neural processes that mediate drug-seeking behavior, and facilitates the incentive properties of drugs and drug-related stimuli as the addiction process proceeds (Stewart et al., 1984; Robinson and Berridge, 1993). Addiction-related changes in drug-seeking behavior can be measured in a variety of paradigms. In progressive ratio testing, addiction-related changes are indicated by an increase in the maximum number of drug-seeking responses an animal will perform to maintain drug self-administration (Mendrek et al., 1998; Piazza et al., 2000; Suto et al., 2003). In the reinstatement paradigm, addiction-related changes are reflected by an enhanced ability of environmental and pharmacological stimuli to trigger and maintain drug seeking in withdrawal (De Vries et al., 1998; Deroche et al., 1999; Sutton et al., 2000).

Addiction-related increases in drug-seeking behavior also are indicated by a vertical shift in the inverted U-shaped dose–response curve for drug self-administration when access to the drug is relatively unrestricted (Fig. 1). Thus, as the injection dose is lowered, addicted animals exert greater effort to maintain blood/brain drug levels when compared to non-addicted animals. However, neither the threshold dose for maintaining self-administration nor the dose maintaining peak self-administration rates is shifted leftward, as would occur with a generalized "pharmacological" sensitization to the drug. This caveat is explained by competing tolerance-like effects described below.

Following receipt of an intravenous drug injection, animals exhibit a pause in further drug-seeking behavior. The duration of the post-injection pause is tightly regulated by the amount of drug received such that increasing the injection dose also increases the length of the pause, thereby decreasing the number of injections taken over a given period of time. This inverse relationship produces a descending limb in the self-administration dose–response curve reflecting drug-induced inhibitory regulation of further drug intake. From a pharmacological viewpoint, addiction-related escalation in drug intake represents a form of tolerance to this inhibitory regulation, and results in a rightward shift in the descending limb of the curve (Fig. 1). A similar rightward shift is produced by antagonist pretreatment as animals compensate for surmountable receptor blockade by increasing their drug intake (Koob and Goeders, 1989). The increases in drug
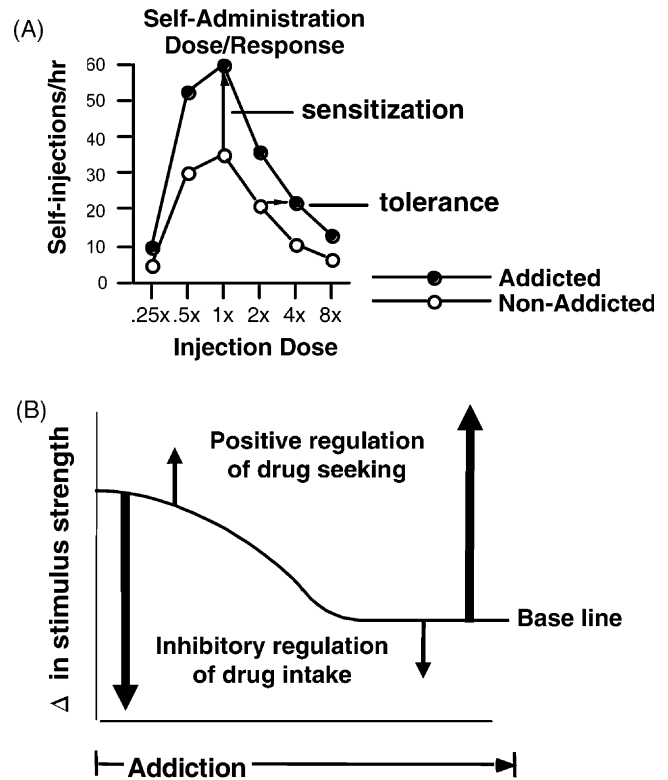

Fig. 1. (A) Comparison of the self-administration dose–response relationship in addicted versus non-addicted animals; (B) the addicted phenotype is indicated by a vertical and rightward shift in the inverted "U"-shaped dose–response curve typical of drug and alcohol self-administration. These alterations result from opposing changes in mechanisms regulating drug-taking and -seeking behaviors as the addiction process develops. Tolerance to inhibitory regulation of drug intake produces a rightward shift in the descending limb of the curve and escalating drug intake. Sensitization in mechanisms regulating drug seeking is reflected by a vertical shift in the peak rate of drug self-administration, an index of increased effort to maintain brain levels of drug as the injection dose is lowered. Sensitization in drug-seeking mechanisms also increases the propensity for relapse in withdrawal. Adapted from Self (in press) (Kaplan and Saddocks' Comprehensive Textbook of Psychiatary, 8th Ed., with permission).

intake reflect a shorter post-injection pause, and also resemble the effect of reducing the injection dose.

Accordingly, the combined effect of tolerance to inhibitory regulation of drug intake, and sensitization in processes regulating drug seeking, produces a rightward and vertical shift in the self-administration dose–response curve indicative of an addicted phenotype (Fig. 1). Similar behavioral alterations in human addicts are reflected by self-reports of increased wanting or craving, but reduced euphoria despite increases in the amount of drug consumed when compared to earlier stages of their drug use history. Differential alterations in these drug-taking and -seeking behaviors suggest that escalating drug intake (tolerance) and enhanced drug seeking (sensitization) result from mechanistically distinct phenomenon that should be

studied independently to gain better access to their underlying neurobiological causes. Several studies support this assertion by showing that drug-taking and -seeking behaviors are associated with different neurobiological responses, and these behaviors respond differently to neurobiological manipulation (e.g., (McGregor and Roberts, 1993; Carelli and Ijames, 2000; Olmstead et al., 2000; Foltin, 2001; Hutcheson et al., 2001a,b; Kantak et al., 2002).

A major goal of our studies is to identify critical "neuroadaptations" to chronic drug self-administration that functionally contribute to theses addiction-related changes in drug-taking and -seeking behaviors. These studies involve both forward (chronic drug use produces biological changes) and reverse (biological changes alter drug-taking and -seeking behaviors) experimental approaches to directly link neuroadaptations with addictive behavior. There is an enormous existing literature on neurobiological changes associated with chronic drug exposure, and so many of our studies have focused on neuroadaptations common to several classes of abused drugs and alcohol. More recently, we have used phenotypic differences in drug self-administration to identify pertinent neuroadaptations that are specifically associated with an addicted state. Finally, our current studies are searching for novel neuroadaptations that persist or arise over prolonged abstinence from chronic self-administration. This article reviews our past and present findings together with an integrative conceptual framework of the drug-addicted brain.

2. Regulation of drug-seeking behavior by D_1 and D_2 dopamine receptors

The mesolimbic dopamine system is a major neural substrate for the reinforcing effects of opiates, psychostimulants, ethanol, nicotine, and cannabinoids. In addition, the mesolimbic dopamine system is activated by exposure to drug-related environmental cues, stress, and other pharmacological stimuli that trigger relapse to drug seeking during withdrawal (Self and Newstler, 1998; Spealman et al., 1999; Stewart, 2000; Shalev et al., 2002; Phillips et al., 2003; Pruessner et al., 2004). The mesolimbic dopamine system consists of dopaminergic neurons in the VTA and their target neurons in forebrain regions such as the NAc. Dopamine release in the NAc is both sufficient, and in some but not all cases, necessary for environmental or pharmacological stimuli to induce relapse to drug-seeking behavior. Dopamine acts on two major classes of receptors that are primarily distinguishable by their opposite modulation of intracellular cyclic AMP (cAMP) formation (Lachowicz and Sibley, 1997). Striatal D_1 receptors (D_1 and D_5) are positively coupled to adenylyl cyclase

and stimulate cAMP formation, whereas striatal D_2 receptors (D_2, D_3 and D_4) are negatively coupled to the enzyme. Neurons intrinsic to the NAc express both D_1 and D_2 classes of dopamine receptors, and although there is evidence for co-localization in the same neurons (e.g., Aizman et al., 2000), D_1 and D_2 receptor expression levels substantially differ in distinct neuronal populations that project to different brain regions (Lu et al., 1998; Steiner and Gerfen, 1998; Aubert et al., 2000).

Agonists selective for either D_1 or D_2 receptors will support intravenous drug self-administration (Wise et al., 1990; Self and Stein, 1992; Caine and Koob, 1993; Weed et al., 1993; Weed and Woolverton, 1995; Self et al., 1996b), but co-activation of both receptors is necessary to support intra-NAc self-administration (Ikemoto et al., 1997). These results agree with numerous studies on the cooperative actions of D_1 and D_2 receptors on behavioral and physiological effects (Waddington et al., 1995; Hu and White, 1997; Hopf et al., 2003). In contrast, D_1 and D_2 receptors mediate opposing effects on drug-seeking behavior. Thus, while selective stimulation of D_2 receptors strongly induces cocaine- and heroin-seeking behavior in reinstatement paradigms, selective stimulation of D_1 receptors is virtually without effect, even at equipotent locomotor-activating doses (Wise et al., 1990; Self et al., 1996a; De Vries et al., 1999; Khroyan et al., 2000). Instead, stimulation of D_1 receptors completely attenuates cocaine seeking induced by cocaine priming injections and cocaine-related environmental cues, whereas stimulation of D_2 receptors facilitates cocaine-induced reinstatement (Self et al., 1996a; Khroyan et al., 2000; Alleweireldt et al., 2002). A similar D_1/D_2 dichotomy may also regulate craving responses in humans (Haney et al., 1998; Haney et al., 1999). These studies suggest that D_2 receptors play a primary role in drug-seeking behavior elicited by low doses of drugs, drug-related cues, stress, and other stimuli that activate the mesolimbic dopamine system. In contrast, high D_1 receptor "tone" inhibits drug-seeking behavior possibly by satiating reward pathways (Self, 1998). However, since blockade of either D_1 or D_2 receptors attenuates reinstatement of cocaine seeking (Weissenborn et al., 1996; Khroyan et al., 2000; Alleweireldt et al., 2002), D_1 receptors may have a permissive or enabling role in D_2 receptor-mediated drug-seeking responses.

Given the prominent role of D_1 and D_2 receptors in regulating cocaine-seeking behavior, the transition from non-addicted to addicted states could involve differential alterations in the sensitivity of these receptors to dopaminergic stimulation. We recently tested this hypothesis using higher preferred levels of stabilized cocaine intake in outbred Sprague–Dawley rats as an index of an addicted state (Edwards and Self, 2002). Animals with higher preferred levels of cocaine intake

exhibit a vertical and rightward shift in their self-administration dose–response curves when compared to animals with lower intake, similar to the phenotypic differences reported in previous studies (Ahmed and Koob, 1998; Piazza et al., 2000). Interestingly, high intake animals are substantially less sensitive or tolerant to D_1 receptor-mediated inhibitory regulation of cocaine seeking, but more sensitive to D_2 receptor-mediated reinstatement of cocaine seeking when compared to low intake animals (Edwards and Self, 2002). Tolerance to D_1 receptor-mediated inhibitory regulation of drug seeking could contribute to escalating drug intake associated with an addicted phenotype, since surmountable blockade of D_1 receptors also increases cocaine intake during self-administration (Koob et al., 1987). Conversely, sensitization in D_2 receptor responses could contribute to a propensity for relapse to drug seeking in withdrawal.

D_2 receptors inhibit adenylyl cyclase activity and protein kinase A (PKA)-mediated phosphorylation, and acute inhibition of PKA activity in the NAc triggers reinstatement of cocaine-seeking behavior (Self et al., 1998). Thus, NAc infusions of a membrane permeable and selective PKA inhibitor mimic the effects of D_2 receptor stimulation on cocaine seeking, suggesting that D_2 receptors could mediate cocaine seeking though direct inhibition of cAMP-dependent protein phosphorylation. In contrast, acute activation of NAc PKA activity with infusions of a selective PKA activator fails to attenuate cocaine seeking as found with D_1 receptor stimulation, suggesting that other D_1 receptor-mediated physiological responses, such as direct regulation of G protein-operated ion channels, mediates the inhibitory effect on drug seeking.

3. Up-regulation in NAc cAMP–PKA signaling produces addiction-like changes in drug-taking and -seeking behaviors

Although acute inhibition of NAc PKA activity facilitates cocaine-seeking behavior, chronic exposure to drugs as diverse as cocaine, morphine, heroin and ethanol up-regulates the cAMP–PKA signaling pathway in the NAc (Terwilliger et al., 1991; Striplin and Kalivas, 1993; Self et al., 1995; Unterwald et al., 1996; Freeman et al., 2001; Lu et al., 2003). These drug-induced neuroadaptations are characterized by decreased levels of $G_{i/o}$ proteins that inhibit cAMP formation, and by increased levels of adenylyl cyclase and PKA activity, all of which contribute to a generalized up-regulation in cAMP–PKA signaling. A critical question is whether these neuroadaptations contribute to changes in drug-taking and -seeking behaviors consistent with the addicted phenotype described above.

We found that experimentally mimicking these neuroadaptations in the NAc produces tolerance-like increases in cocaine and heroin self-administration (Self et al., 1994; Self et al., 1998), and rightward shifts in the dose–response for morphine conditioned place preference, another index of tolerance to the rewarding effects of opiates (M. Janik and D.W. Self, unpublished observations). Conversely, down-regulation of NAc cAMP–PKA activity decreases cocaine intake, and another study found decreases in ethanol consumption, suggesting that PKA-mediated protein phosphorylation plays a pivotal role in regulating preferred levels of drug or alcohol intake (Self et al., 1998; Wand et al., 2001). Taken together, these findings suggest that drug-induced up-regulation in NAc cAMP–PKA signaling represents an intracellular mechanism of tolerance to inhibitory regulation of drug intake, as animals compensate for a decrease in drug effects by escalating their drug consumption (Fig. 2).

Tolerance-like increases in drug intake require as little as 90 min of sustained PKA activation, suggesting that PKA mediates direct negative feedback independent of downstream regulation of gene expression. One possible mechanism could involve a PKA-mediated phosphorylation of D_1 receptors, leading to desensitization and/or down-regulation (Sibley et al., 1998; Gardner et al., 2001). Indeed, long-term cocaine self-administration reduces D_1-stimulated cAMP formation and down-regulates D_1 receptors (Graziella De Montis et al., 1998; Moore et al., 1998b). Furthermore, we recently found that experimental up-regulation in cAMP–PKA-dependent protein phosphorylation in the NAc (via intra-NAc cholera toxin infusions) reduces the ability of D_1 receptor stimulation to attenuate reinstatement of cocaine-seeking behavior, as indicated by a rightward shift in the dose–inhibition curve for a D_1 agonist (Whisler et al., 2003). These results suggest that drug-induced up-regulation in cAMP–PKA signaling in the NAc produces tolerance to D_1 receptor-mediated inhibitory regulation of drug-taking and -seeking behaviors (Fig. 2), similar to the changes in D_1 receptor sensitivity we found in animals exhibiting an addicted phenotype described above.

Recent studies have shown that drug-induced up-regulation in cAMP–PKA signaling occurs in both D_1- and D_2-containing NAc neurons (Shaw-Lutchman et al., 2002; Shaw-Lutchman et al., 2003). However, given that D_2 receptor stimulation and acute inhibition of NAc PKA activity triggers reinstatement of cocaine seeking, it seems paradoxical that drug-induced up-regulation in cAMP–PKA signaling would contribute to addiction-related enhancement in cocaine-seeking behavior. Nevertheless, we found that experimental up-regulation in NAc cAMP–PKA signaling facilitates reinstatement of cocaine seeking and locomotor responses induced by D_2 receptor stimulation, indicat-
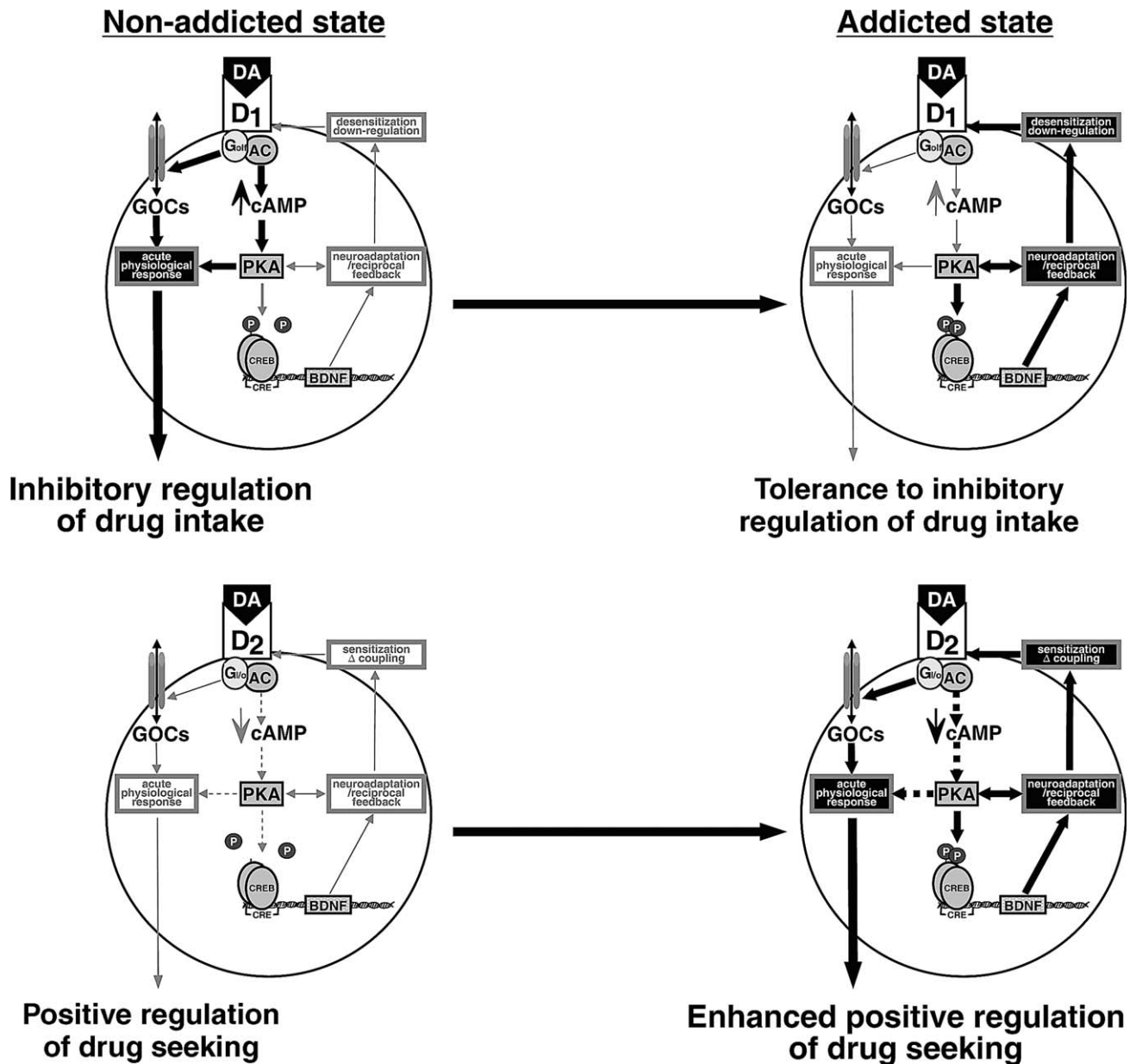
Fig. 2. Diagram depicting the role of cAMP/PKA up-regulation in the NAc in the bi-directional regulation of D_1 and D_2 receptor sensitivity and their ability to modulate drug-taking and -seeking behaviors. (Top) In a non-addicted state, cocaine-induced activation of D_1 receptors mediates a strong satiating influence that prolongs the duration of drug effects and limits overall drug consumption during self-administration. These acute effects are mediated by stimulatory G protein (G_{olf} in the NAc) that activate adenylyl cyclase (AC), cAMP formation and cAMP-dependent protein kinase (PKA), and also directly modulate G protein-operated ion channels (GOCs). As continued drug use transits to an addicted state, sustained up-regulation in cAMP–PKA signaling weakens D_1 receptor responses through reciprocal negative feedback pathways, possibly involving direct phosphorylation of D_1 receptors, or via CREB-regulated genes such as BDNF. Sustained PKA activity produces tolerance to D_1 receptor-mediated inhibitory regulation of drug intake leading to escalating drug consumption during self-administration binges. (Bottom) In contrast to D_1 receptors, D_2 receptors mediate relapse to drug seeking via coupling to inhibitory G proteins ($G_{i/o}$) that inhibit cAMP formation and PKA activity (dashed lines), and regulate other GOCs. In an addicted state, sustained up-regulation in PKA activity facilitates (rather than reduces) the ability of D_2 receptor stimulation to trigger drug seeking. This effect could involve enhanced D_2 receptor coupling to other $G_{i/o}$-coupled effectors such as GOCs, or greater acute inhibition of cAMP–PKA activity by D_2 receptor stimulation.

ing sensitization in D_2 receptor-mediated responses (Whisler et al., 2003). Therefore, drug-induced up-regulation in cAMP–PKA signaling in NAc neurons could directly augment the acute inhibition of PKA activity by D_2 receptor stimulation and/or facilitate D_2 receptor coupling to other signal transduction pathways (Fig. 2), despite substantial down-regulation in D_2 receptors in the NAc following chronic cocaine self-administration (Moore et al., 1998a; Nader et al., 2002). Given that chronic cocaine self-administration

also increases RGS-9, a negative regulator of D_2 receptor coupling in the NAc (Rahman et al., 2003), regulation of other possible targets involved in D_2 receptor coupling such as G protein-coupled receptor kinases could be involved (Gainetdinov et al., 2003). In any event, our results suggest that drug-induced up-regulation in cAMP–PKA signaling could act reciprocally in D_1-containing NAc neurons to weaken inhibitory regulation of drug seeking, while simultaneously enhancing positive regulation of drug seeking in D_2-containing neurons (Fig. 2). Our current studies are aimed at investigating this bi-directional regulation hypothesis by up-regulating cAMP–PKA signaling selectively in either D_1- or D_2-containing striatal neurons (Ruiz-Durantez et al., in press).

Although many of the behavioral effects of up-regulating cAMP–PKA signaling in NAc neurons can occur over a relatively short time frame, other long-term changes may involve PKA-mediated phosphorylation of cAMP-response element binding protein (CREB), and regulation of downstream gene targets such as brain-derived neurotrophic factor (BDNF). Our recent studies found that both acute cocaine administration and CREB-over-expression transiently increase BDNF protein levels in the NAc, suggesting that BDNF levels rise and fall following each self-administration binge (Graham and Self, 2003). We found that down-regulating NAc CREB levels with antisense oligonucleotides produces a downward shift in the cocaine self-administration dose–response curves consistent with a reduction in cocaine reinforcement, and opposite to addiction-related changes in self-administration (Whisler et al., in press). Conversely, intra-NAc infusions of BDNF produce addiction-like changes in cocaine self-administration several days after the infusions, including a vertical (enhanced drug-seeking) and rightward (tolerance to inhibitory regulation) shift in the dose–response curve (Graham and Self, 2003). Taken together, these results suggest that a PKA-CREB-BDNF cascade mediates enduring changes in both cocaine-taking and -seeking behaviors that could involve growth and structural changes in NAc neurons (Fig. 2). Further studies are needed to determine whether these effects also involve changes in the sensitivity of D_1 and D_2 receptors that regulate cocaine-taking and -seeking behaviors.

4. Drug-induced neuroplasticity in the mesolimbic dopamine system facilitates drug-seeking behavior

Dopamine neurons in the VTA and medium spiny neurons in the NAc both receive dense excitatory glutamatergic innervation that displays synaptic plasticity with repeated drug exposure (Fig. 3). Some of these changes emerge over time and endure for long periods
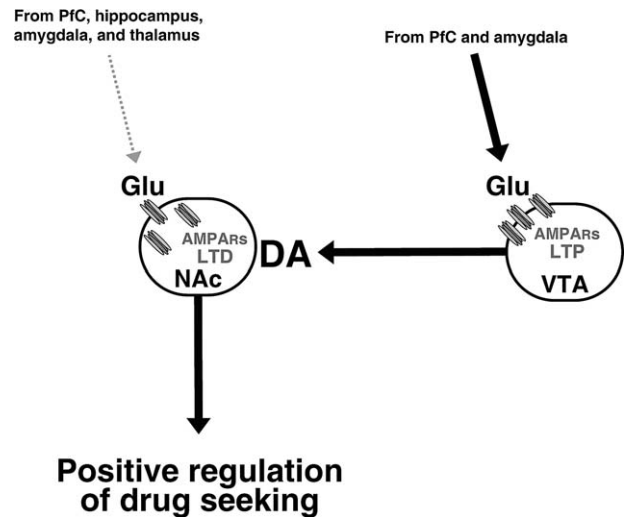

Fig. 3. Drug-induced neuroplasticity in the ventral tegmental area (VTA) and nucleus accumbens (NAc). Several drugs of abuse increase excitability of ventral tegmental area (VTA) dopamine neurons and produce long-term potentiation (LTP) in excitatory synapses, and this effect may involve up-regulation in the GluR1 subunit of AMPA glutamate (Glu) receptors. Experiments that mimic this GluR1 up-regulation suggest that potentiated excitatory synaptic input to VTA neurons contributes to addiction by increasing the pursuit of cocaine reinforcement, possibly by enhancing dopamine release (DA) in terminal regions such as the nucleus accumbens (NAc). In contrast to the VTA, chronic drug use reduces excitatory input to NAc neurons, including hypoactivity in cortical regions that project to the NAc, reduced levels of glutamate release in the NAc (pre-synaptic), and subsensitivity of AMPA glutamate receptor-mediated responses (post-synaptic) including long-term depression (LTD) in cortico-accumbal synapses. These changes create an imbalance favoring dopaminergic (D_2) input from the VTA over cortical and subcortical glutamatergic input to the NAc. Experiments that reverse deficits in AMPA-mediated input to NAc neurons restore inhibitory control over drug-seeking behavior, suggesting that these deficits contribute to positive regulation of drug seeking in addiction. PfC; prefrontal cortex.

of withdrawal, while others are more transient, but could be involved in the "induction" of long-term changes in the mesolimbic dopamine system (White and Kalivas, 1998; Wolf, 2002). One commonly reported effect is a transient enhancement in dopamine neuron excitability in the VTA that persists for 3, but not 14, days of withdrawal from repeated psychostimulant treatment (White et al., 1995; Zhang et al., 1997). Indeed, in vivo treatment with several drugs of abuse increases excitatory input while decreasing inhibitory input to VTA dopamine neurons (Jones et al., 2000; Saal et al., 2003). Even a single in vivo exposure to cocaine induces transient LTP (long-term potentiation) in excitatory synapses on VTA dopamine neurons, reflecting increased membrane expression of ionotropic alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) glutamate receptors (Ungless et al., 2001). These physiological changes are paralleled by an increased ability of intra-VTA AMPA infusions, but

not NMDA, to stimulate dopamine release in terminal regions of the nucleus accumbens after 3, but not 14, days of withdrawal (Giorgetti et al., 2001).

One potential mechanism for increased excitability of VTA dopamine neurons is an increase in the AMPA glutamate receptor subunit, GluR1, following chronic cocaine treatment (Fitzgerald et al., 1996; Churchill et al., 1999). Similarly, in humans, cocaine overdose is associated with VTA up-regulation in the GluR2 AMPA receptor subunit (Tang et al., 2003). In contrast, other studies fail to find regulation of AMPA receptors with repeated passive psychostimulant treatment (Lu et al., 2001), or find GluR1 increases selectively in animals that develop sensitized locomotor responses (Churchill et al., 1999). Another study failed to find increases in AMPA receptor subunits in withdrawal from chronic cocaine self-administration (Lu et al., 2003). However, we recently found large (~70%) increases in GluR1 protein levels in the VTA, but not adjacent substantia, after 1 day of withdrawal from chronic cocaine self-administration in rats, but substantially less (~35%) up-regulation in animals receiving cocaine passively by yoked injection (Choi et al., in press). In addition, chronic cocaine self-administration increases phosphorylated GluR1 levels by about 50%, possibly reflecting increased membrane expression of GluR1 that underlies LTP (Hayashi et al., 2000; Esteban et al., 2003).

Given that GluR1 over-expression in neurons leads to LTP-like increases in excitatory synaptic responses (Hayashi et al., 2000; Takahashi et al., 2003), we have used viral-mediated gene transfer in vivo to study the role of potentiated excitatory input to VTA neurons on drug-taking and -seeking behaviors. Over-expression of wild type GluR1 in VTA neurons facilitates cocaine-seeking behavior, as indicated when animals exert greater effort to obtain cocaine on a progressive ratio reinforcement schedule, but GluR1 has no effect on cocaine intake when access to cocaine is less restricted (Choi et al., 2003). In contrast, over-expression of a PKA-resistant GluR1 causes a downward shift in the self-administration dose–response curve, indicating a reduction in the reinforcing efficacy of cocaine, and a possible dominant negative effect on AMPA receptor trafficking mechanisms. Taken together, these results suggest that increased GluR1 levels and/or LTP in the VTA following chronic cocaine self-administration contribute to positive regulation of cocaine-seeking behavior (Fig. 3), whereas PKA-mediated GluR1 phosphorylation may be critical for this effect. Accordingly, increased excitability of VTA dopamine neurons could profoundly affect the ability of stimuli that activate this system to trigger relapse to drug seeking in withdrawal.

In contrast to the VTA, chronic cocaine use produces multiple deficits in excitatory glutamatergic input to the NAc, including sustained hypoactivity in frontal cortical regions that project to the NAc in abstinent human cocaine abusers (Volkow et al., 1992; London et al., 1999; Volkow and Fowler, 2000), reduced presynaptic and extracellular glutamate levels in the NAc (Keys et al., 1998; Bell et al., 2000; Baker et al., 2003), and subsensitivity in post-synaptic neurons to glutamate and AMPA receptor stimulation resembling long-term depression (LTD) in excitatory synapses (White et al., 1995; Thomas et al., 2001). LTD is associated with internalization of AMPA glutamate receptors and decreases in synaptic levels of AMPA receptor subunits (Heynen et al., 2000; Malinow and Malenka, 2002). Our studies suggest that restoring deficits in AMPA-mediated excitatory input to NAc neurons reduces the propensity for drug-seeking behavior. Thus, over-expression of GluR1 or GluR2 subunits in NAc neurons reduces cocaine seeking elicited by dopamine D_2 receptor stimulation and exposure to cocaine-related environmental cues (Sutton et al., 2003; Bachtell et al., in press). In addition, we recently found that over-expression of GluR1 or GluR2 in NAc neurons blocks the induction of locomotor sensitization with repeated cocaine treatments, but has no effect on the acute locomotor response to cocaine (Choi et al., 2002). These results suggest that experimental increases in excitatory input to NAc neurons prevents the development of sensitized responses that promote cocaine-seeking behavior, possibly by reversing or preventing natural LTD-like deficits that could underlie sensitization (White et al., 1995; Thomas et al., 2001).

In this regard, we have used a dominant negative mutant GluR1 (GluR1^{Q582E}) that inactivates endogenous AMPA receptors (Shi et al., 2001) to study the effects of drug-induced LTD in the NAc on addictive behavior. In contrast to wild type GluR1, over-expression of the dominant negative GluR1 mutant in NAc neurons facilitates sensitization of D_2, but not D_1, receptor-mediated locomotor responses, suggesting that reduced excitatory input to NAc neurons could contribute to addiction by facilitating positive regulation of drug-seeking behavior by D_2 receptor stimulation (Bachtell et al., in press). Thus, bi-directional regulation of synaptic plasticity in either the VTA (LTP), or the NAc (LTD) would both act to facilitate the propensity for relapse (Fig. 3).

However, this conclusion contradicts other studies showing that direct activation of NAc neurons via intra-NAc AMPA infusions induces cocaine-seeking behavior, and infusions of AMPA antagonists blocks this behavior (Cornish et al., 1999; Cornish and Kalivas, 2000). One possibility is that generalized activation of AMPA receptors disrupts inhibitory regulation of drug-seeking behavior emanating from cortical or subcortical glutamatergic input to the NAc. If so, then preserving endogenous synaptic activity would be important for bi-directional regulation of

addictive behavior by wild type and dominant negative GluR1. Alternatively, glutamatergic inputs may carry cognitive information relating to both activation and inhibition of drug-seeking behavior. In this case, phasic activation of glutamatergic inputs (as produced by agonist infusions) may be more related to transient activation by salient stimuli, whereas tonic up-regulation (as with GluR1 over-expression) may regulate background input and blunt signal gain with phasic activation thereby reducing the motivational response. Regarding the latter possibility, tonic elevations in extracellular glutamate levels also reduce reinstatement of cocaine-seeking behavior (Baker et al., 2003).

In contrast to drug-induced regulation of synaptic plasticity in the NAc, we found that extinction training during withdrawal from chronic cocaine self-administration leads to an up-regulation in both GluR1 and GluR2 AMPA subunits in the NAc shell (Sutton et al., 2003), the same subregion exhibiting LTD in cocaine withdrawal (Thomas et al., 2001). Extinction training diminishes cocaine-seeking behavior as animals learn that cocaine reinforcement is no longer available. Extinction training also reverses deficits in the NR1 subunit of *n*-methyl-D-aspartate (NMDA) receptors in the NAc shell produced by cocaine withdrawal, and increases the ratio of GluR1 to NR1 subunits (D. Simmons and D.W. Self, unpublished observation). Given that these extinction-induced increases parallel synaptic regulation of GluR1, GluR2, and NR1 protein levels by either LTP or reversal of LTD (Heynen et al., 2000), we hypothesize that extinction training also induces LTP (or reverses LTD) in cocaine withdrawal, leading to potentiation of excitatory synaptic input in cortico-NAc shell synapses. The possibility of activity-dependent synaptic regulation is further supported by the fact that a majority of NAc neurons are highly activated during extinction training (Fabbricatore et al., 1998), and extinction-induced up-regulation of GluR1 and GluR2 occurs despite decreases in mRNA levels for these subunits (Sutton et al., 2003).

Extinction-induced increases in the GluR1 subunit are positively associated with the level of extinction achieved during training (reflected by reduced cocaine seeking), suggesting that such experience-dependent GluR1 up-regulation acts reciprocally to facilitate extinction of cocaine-seeking behavior (Sutton et al., 2003). This conclusion is supported by the ability of either GluR1 or GluR2 over-expression in NAc shell neurons to facilitate extinction of cocaine seeking. Furthermore, either GluR1 or GluR2 over-expression during extinction training leads to prolonged attenuation of stress-induced relapse to cocaine seeking. These finings suggest that extinction-induced neuroplasticity in the NAc shell could represent a behavior-based approach for treatment that reduces the propensity for relapse by restoring excitatory synaptic input to NAc neurons.

5. Challenges for future research: the problem of persistence

It is important to note that most neuroadaptations inevitably revert to normal within a month or two following cessation of chonic drug or alcohol use. However, a high prevalence for relapse extends from months to years in abstinent drug abusers suggesting that drug-induced neuroadaptive responses cannot fully account for the persistence of addiction-related behavioral changes. One possiblity is that even transient changes in receptor signaling and gene expression can lead to relatively permanent changes in synaptic organization, such as dendritic arborization and formation of new synapses (Robinson et al., 2001). These structural changes would outlast many neuroadaptations in receptor signaling and gene expression, and even subtle changes involving numerous synapses in multiple brain regions would profoundly influence neural networks that regulate motivated behavior. Many enduring changes could directly influence the motivational response to drugs and environmental stimuli that trigger drug seeking, whereas other short-lived changes could facilitate incentive learning during chronic drug use and, thus, strengthen drug- and alcohol-related memories. Given the temporal dissociation between relatively short-lived drug-induced neuropadaptations and the persistence of behavioral changes, several current studies are aimed at (1) understanding the interaction between transient neuroadaptions and drug reinforcement (incentive learning) that may indirectly facilitate relapse in the long term, and, (2) identifying certain long-lasting or late-forming neuroadaptations in drug withdrawal that directly facilitate relapse to drug-seeking behavior. Such efforts are based on two conceptual models that illustrate the distinction between learning- and memory-based interactions in drug addiction (Fig. 4).

The first model is based on the direct influence of short-lived neuroadaptations to chonic drug use on the strength of drug reinforcement. In the initial phases of drug and alcohol use, self-administration is maintained primarily by the positive reinforcing effects of drugs that involve feelings of rush, high and euphoria. However, with the transition from recreational drug use to an addicted state, neuroadaptations produced by chronic drug use can lead to negative mood disturbances in early drug withdrawal such as dysphoria, anxiety, and depression (Koob and Le Moal, 1997, 2001). Although there is little evidence that negative mood associated with drug withdrawal contributes directly to drug-seeking behavior in animal studies
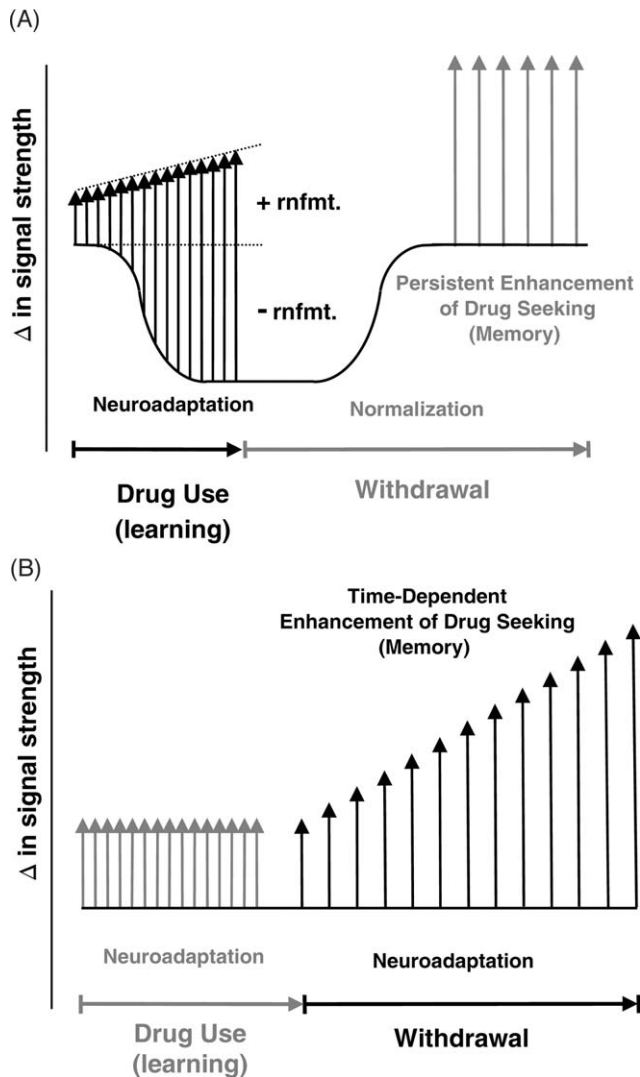
Fig. 4. Interaction between short- and long-term neuroadaptations to chronic drug use and mechanisms of learning and memory. (A) Short-term neuroadaptations can sensitize positive reinforcement mechanisms, but also facilitate negative reinforcement mechanisms by causing dysphoria, anxiety and depression in early withdrawal that are temporarily alleviated by drug use. Increases in both positive and negative reinforcement mechanisms would facilitate incentive learning (shown in black), leading to persistent increases in the motivational strength (salience) of drug-related memories even after short-term neuroadaptations normalize in long-term withdrawal (shown in gray); (B) Neuroadaptations that arise during withdrawal from drug use could directly facilitate the motivational strength of drug-related memories (shown in black), thereby increasing the propensity for relapse in prolonged abstinence. Adapted from Self (in press) (Kaplan and Saddocks' Comprehensive Textbook of Psychiatry, 8th Ed., with permission).

(Self and Nestler, 1998; Stewart, 2000), the neurobiological changes underlying these effects can augment the reinforcing efficacy of drugs of abuse. Thus, in addition to the positive reinforcing effects of drugs, the ability of drugs to alleviate the negative effects of drug withdrawal represents an additional source of reinforce-

ment (negative reinforcement) that would summate with positive reinforcement mechanisms to increase the overall reinforcing efficacy of drugs, and, hence, the strength of incentive learning (Fig. 4A). A recent study by Dickinson and colleagues supports this concept (Hutcheson et al., 2001a,b), where heroin-seeking behavior was markedly enhanced in animals that were allowed to alleviate opiate withdrawal symptoms by engaging in heroin self-administration, but not in equally dependent animals that were prevented from alleviating withdrawal. These changes could persist long after symptons of opiate dependence dissipate, and so incentive learning during drug use may have long-term consequences on the ability of drug-related memories to trigger drug-seeking behavior (Fig. 4A).

Thus, even transient neuroadaptations that temporarily enhance drug reinforcement would contribute to persistent craving and the propensity for relapse for prolonged periods of abstinence. In this regard, drug-induced up-regulation in the transcription factor $\triangle$FosB in the NAc could have similar long-term consequences on relapse to cocaine seeking. We found that transgenic mice over-expressing $\triangle$FosB in the striatum and NAc work harder to maintain cocaine self-administration on a progressive ratio reinforcement schedule, indicating an enhancement in cocaine reinforcement and possibly incentive learning (Colby et al., 2003). Future studies will determine whether this enhancement translates into a propensity for cocaine seeking in long-term abstinence when $\triangle$FosB levels return to normal, and whether it requires cocaine self-administration experience concurrent with increases in $\triangle$FosB (incentive learning). In contrast to cocaine-seeking behavior, $\triangle$FosB fails to increase cocaine intake when access to drug is less restricted, and this differs from the effect of experimentally up-regulating cAMP–PKA signaling in the NAc. These findings underscore the notion that addiction-related changes in drug-taking and -seeking behaviors can be differentially regulated by distinct neuroadaptations, and illustrate the importance of studying each neuroadaptation in the context of discrete aspects of self-administration behavior.

Another behavioral phenomenon that could reflect a direct facilitation of memory rather than incentive learning is a progressive increase in drug-seeking behavior that occurs in rats with progressively longer periods of "forced" abstinence (Tran-Nguyen et al., 1998; Grimm et al., 2001; Shalev et al., 2001). This effect is described as a "time-dependent" facilitation or "incubation" of drug-related memories that continues to increase over weeks to months following cessation of drug self-administration. Neurobiological changes that contribute to this phenomenon would arise or increase during drug withdrawal rather than during drug use (Fig. 4B). At present, there are only a few neurobiological changes that coincide with time-dependent facili-

Table 1
Neuroadaptations identified by microarray profiling of NAc after 6 weeks withdrawal from chronic cocaine self-administration

Regulated gene	Early cocaine withdrawal		Late cocaine withdrawal	
	mRNA	Protein	mRNA	Protein
Mu1 opioid receptor	Normal	Normal	↑	↑
A-kinase anchor protein 84	Normal	↑	↓	↑
Kv 4.3 K$^+$ channel	Normal	↑	↑	↓

tation of cocaine-seeking behavior, including parallel increases in extracellular dopamine levels in the amygdala, and withdrawal-induced increases in BDNF levels in several limbic brain regions (Tran-Nguyen et al., 1998; Grimm et al., 2003).

We have used microarray profiling in rats to indentify changes in gene expression in multiple limbic brain regions that coincide with time-dependent increases in cocaine-seeking behavior in withdrawal from chronic cocaine self-administration (Simmons et al., 2002). Table 1 summarizes some of the changes in gene expression in the NAc that have been verified at the protein level by Western blot. One change that could contribute to time-dependent increases in cocaine seeking is a late-forming increase in mu opioid receptor (MOR1) expression. MOR1 levels are normal after 10 days of withdrawal (early) from chronic cocaine self-administration, but increase by 6 weeks of withdrawal (late). NAc MOR1 levels begin to increase by 3 weeks and remain elevated for at least 12 weeks of withdrawal (D. Simmons and D.W. Self, unpublished observations). Studies are underway to determine the functional consequence of MOR1 up-regulation in the NAc on the propensity for relapse.

Another change identified by microarray profiling is an up-regulation in the A-kinase anchoring protein AKAP-84 that persists from early to late withdrawal (Table 1). Given that chronic exposure to cocaine (and other drugs) is known to up-regulate protein kinase A in the NAc, increases in AKAP-84 could target PKA up-regulation to specific cellular or synaptic compartments. Interestingly, up-regulation in AKAP-84 protein is accompanied by down-regulation in mRNA levels. Bi-directional regulation of mRNA and protein may represent compensatory adaptations in mRNA to changes in protein levels, or regulation of protein levels primarily through degradation rather than synthetic pathways. Similar bi-directional regulation in the Kv 4.3 potassium channel is reflected by increased mRNA and decreased protein levels 6 weeks after withdrawal from chronic cocaine self-administration. However, unlike regulation of AKAP-84 protein, which remains elevated throughout cocaine withdrawal, protein levels of Kv 4.3 are increased at early and decreased at late withdrawal in the NAc. These results suggest that cocaine withdrawal is a highly dynamic state character-

ized by induction of several withdrawal-induced changes while other more transient drug-induced changes dissipate.

In summary, although many studies have identified neuroadaptive responses to passive drug exposure, it is important to determine whether these neuroadaptations can be related specifically to addicted phenotypes using self-administration procedures. Moreover, while previous studies have provided substantial information on neuroadaptive responses to chronic drug exposure, the neuroadaptive response to drug withdrawal arguably is more pertinent to treatment, and should be a major focus of future work. It will remain challenging but necessary to establish critical cause–effect relationships between these neuroadaptive responses and addiction-related changes in drug-taking and -seeking behaviors. Finally, the ability of neuroadaptive responses to potentially influence drug-related learning and memory should be studied to fully appreciate their enduring consequences on the propensity for relapse.

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