The American Journal on Addictions, 18: 259–271, 2009

ISSN: 1055-0496 print / 1521-0391 online
DOI: 10.1080/10550490902925946

DISTINGUISHED SCHOLAR

Plasticity of Addiction: A Mesolimbic Dopamine Short-Circuit?

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The development of drug addiction progresses along a continuum from acute drug use to compulsive use and drug seeking behavior. Many researchers have focused on identifying the physiological mechanisms involved in drug addiction in order to develop effective pharmacotherapies. Neuroplasticity, the putative mechanism underlying learning and memory, is modified by drugs of abuse and may contribute to the development of the eventual addicted state. Innovative treatments directly targeting these drug-induced changes in brain reward components and circuits may be efficacious in reducing drug use and relapse. (Am J Addict 2009;18:259–271)

Addiction is a chronic illness characterized by compulsive drug seeking and use, despite the continued presence of negative personal health and social consequences.[1] How does addiction develop? A prevailing hypothesis suggested by similarities to models of synaptic plasticity is that addiction occurs because drugs of abuse are able to take control of normal brain reward circuits that provide reinforcement of behaviors related to survival (eg food, water and sex). While natural rewards activate the reward circuit until the survival-related behavior is learned,[2] drugs of abuse continue to stimulate the circuit upon repeated exposures. Studies of early phases of addiction often focus on the immediate short-term effects of drugs of abuse on brain circuitry with the idea that these initial drug-induced effects set in motion long-term changes responsible for late phases of addiction, such as craving, continued drug use and relapse. In this review, we focus on rapid, relatively short-term changes in properties such as neuronal firing rate and synaptic plasticity, induced by drugs of abuse in brain regions relevant to reward and reinforcement. We will then extrapolate these findings to later phases of addiction and discuss potential therapeutic targets.

Received December 12, 2008; revised February 2, 2009; accepted March 4, 2009.

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THE VENTRAL TEGMENTAL AREA AND ADDICTION

The study of the reward circuit often begins at the ventral tegmental area (VTA), a midbrain region that is the major site of dopamine neurons constituting the mesolimbic dopamine system.[3] The VTA is involved in the processing of natural reward-driven behaviors and it is an essential circuit mediating addiction.[4–8] This circuit provides information regarding the reward value of an action or stimulus to modify future behavior.[9] Dopaminergic neurons located in the VTA project to the nucleus accumbens (NAc) and dopamine release in the NAc is required for the rewarding properties of survival-related natural stimuli, such as food and mating opportunities.[10] Similarly, dopamine release is required for drugs of abuse to be perceived as rewarding,[11,12] suggesting that drugs of abuse and natural rewards affect the same reward circuitry. Furthermore, all addictive drugs trigger dopamine release from VTA neurons,[11] indicating a shared, common brain mechanism by which multiple different drugs of abuse may shape behavior.[10]

Studies in primates have demonstrated that dopamine neurons increase their firing rate when the animals are presented with reward-associated stimuli requiring behavioral responses.[13–15] Dopamine release from neurons with cell bodies in the VTA is also thought to encode information relating the expected value of a reward and the actual reward,[2] and so the firing rate of dopamine neurons may provide a predictive signal to shape behavior in order to obtain rewards. Not all drugs of abuse rapidly increase the firing rate of VTA dopamine neurons, most likely because of differing mechanisms of action. Thus, while drugs of abuse all trigger dopamine release in the reward circuit, the firing rate of dopamine neurons is not a required convergence point.

ACTIVITY OF DOPAMINE NEURONS IN VIVO AND IN VITRO

When recorded *in vivo* from anesthetized or paralyzed rats, VTA dopamine cells can be silent, or show irregular firing

and/or low bursting activity.[16–18] However, in freely moving animals, these cells show robust burst firing activity.[19] Bursts of action potentials recorded from these neurons exhibit spike-frequency adaptation and accommodation and, in most cases, the number of spikes in each burst (usually 2 or 3) is inversely correlated with the burst frequency.[20,21] Differences in burst firing activity between anesthetized and non-anesthetized rats are thought to result from the reduced levels of sensory information in the anesthetized condition.

Several lines of evidence suggest that the burst firing observed in DA neurons *in vivo* is driven by excitatory glutamatergic synaptic inputs arising from several nuclei including the prefrontal cortex (PFC), subthalamic nucleus, bed nucleus of the stria terminalis (BNST), and pedunculopontine tegmental nucleus (PPTg).[22] For instance, *in vivo* excitation or inhibition of PFC neurons increases or decreases bursting activity in VTA dopamine neurons, respectively.[23] *In vivo* bursting activity following PFC stimulation resembles the burst firing normally observed in DA neurons.[24,25] Similarly, *in vivo* electrical stimulation of the BNST drives bursting activity in dopamine cells.[26] Furthermore, either *in vivo* pharmacological stimulation of the PPTg or simultaneous stimulation of the PPTg and the hippocampal ventral subiculum induce bursting activity in VTA DA neurons, suggesting that coincident activity of specific excitatory afferents to the VTA can generate an amplified signal, increasing dopaminergic activity.[27] Finally, other studies *in vivo* have shown that direct application of NMDA onto DA neurons can induce bursting activity,[28–30] while pharmacological blockade of glutamate receptors in the VTA decreases burst firing in DA neurons.[31,32] Together, these findings indicate that the bursting activity recorded from VTA dopamine neurons *in vivo* is regulated by glutamatergic excitatory inputs arising from several brain regions.

In addition to glutamatergic afferents, the VTA receives GABAergic, cholinergic, noradrenergic, and serotonergic inputs from other brain regions.[33] Interestingly, it has been shown that *in vivo* infusion of muscarinic ACh receptor agonists onto VTA DA neurons can induce bursting activity[34] suggesting the possibility that the bursting activity of VTA dopamine neurons is in fact regulated by the interplay between glutamatergic and other afferents that release other neurotransmitters.

In contrast to the situation *in vivo*, most VTA dopamine neurons *in vitro* (tissue slices) exhibit regular, pacemaker firing activity when recorded using extracellular, intracellular (sharp microelectrode), or patch-clamp (cell-attached or whole-cell) recording techniques. In general, the resting membrane potential of VTA DA neurons is relatively depolarized, ranging from −40 to −60 mV, with typical firing rates of 1–7 Hz.[35,36] In addition, VTA dopamine cells express the hyperpolarization-activated inward current (I_h),[37,38] which is observed more rarely in VTA GABAergic neurons.[39] The non-bursting regular firing activity consistently reported in brain slices is thought to result from the severing of excitatory projections from PFC and other regions. Consistent with this idea, bath application of NMDA to brain slices can trigger rhythmic firing of DA cells.[29]

DRUGS OF ABUSE AND ACTIVITY OF VTA DA NEURONS

It has been suggested that bursting activity is more effective than tonic firing at enhancing transmitter release at synaptic terminals.[40,41] In the dopamine system, bursts of action potentials markedly enhance DA release in the NAc when compared with single spike activity.[42,43] Evidence from both *in vivo* and *in vitro* physiological studies have demonstrated that many drugs of abuse increase mesocorticolimbic activity, specifically enhancing dopamine release in target nuclei such as the NAc.[8,44] For instance, morphine increases the activity of VTA DA neurons by inhibiting neighboring GABAergic cells,[45] resulting in increased dopamine release in the NAc.[46] Likewise, nicotine both directly excites VTA DA neurons and increases excitatory synaptic transmission in the VTA.[47–50] Psychostimulants such as cocaine and amphetamine increase the amount of dopamine at synapses by blocking the DA transporter,[51,52] directly increasing vesicular release of dopamine[53] and by disinhibiting VTA DA cells.[54] Psychostimulants can also increase the bursting activity of DA cells *in vivo*, increasing DA release in the NAc.[55]

Why is this important? Experiments suggest that higher levels of DA release during bursting activity can turn on immediate early genes, thus producing lasting changes in DA neurons that could alter the output of this circuit in the long term. For example, increased DA release induces the expression of Fos-like immunoreactivity at dopaminergic synapses by acting on DA receptors.[56,57] Other experiments have linked the activation of DA receptors with increased expression in the NAc of Homer genes, another family of immediate early genes.[58] While Homer1a can be induced by psychostimulants, constitutively expressed Homer isoforms are also affected by psychostimulants like cocaine. During withdrawal from repeated cocaine administration, Homer1b/c isoforms are downregulated in the NAc[59] and cocaine sensitization is augmented. Similarly, the deletion of Homer1 and Homer2 genes produces a "pre-sensitized" state that can be reversed by restoring Homer expression in the NAc.[60] These findings suggest that an initial alteration in DA neuron firing rate by drugs of abuse may be linked to the development of addiction by expression of immediate early genes such as Homer.

Besides impacting gene expression, the firing rate of DA neurons may contribute directly to addiction. It has been argued that basal activity of DA neurons could be related to vulnerability to drugs of abuse. For example, rats classified as high responders to a novel environment readily acquired cocaine self-administration, while low responders did not.[61] Further examination of the high responding rats revealed an endogenously elevated basal firing rate and enhanced bursting activity of DA neurons as compared to low responding rats;[61] thus, basal activity of DA neurons might correlate with an inherent predisposition to drug administration or addiction. Psychostimulants such as cocaine and amphetamine rapidly decrease DA neuron firing rates, but this is followed by an

increase in firing rate, and withdrawal from these drugs also increases firing rate.[20] Other drugs of abuse, such as ethanol, morphine and Δ^9-THC, acutely increase DA neuron firing rate followed by a decrease in firing rate during withdrawal.[20] A recent study linking firing rate to addictive behavior found that preventing an increase in DA neuron firing rate caused by Δ^9-THC consequently blocked the rewarding effects of the drug.[62,63] Although alteration in DA neuron firing rate seems to be necessary for the development of addiction, such changes are transient. Thus, therapeutic intervention designed to prevent or reverse drug-induced changes in DA neuron firing rate may be ineffective if the neuroadaptations are rapidly transferred to other brain regions.

SYNAPTIC PLASTICITY IN THE VTA

In addition to changing firing rate, addictive drugs also acutely modify synaptic transmission in brain reward circuits. Multiple drugs of abuse persistently enhance neurotransmission at excitatory synapses on dopamine cells in the VTA,[64–68] while opioids and cocaine both persistently depress inhibitory synapses on dopamine cells.[69,70] These drugs appear to promote or block forms of plasticity that are candidate mechanisms of learning and memory in other brain regions, and therefore have the potential to influence long-term storage of reward-related memories that may lead to addiction.[71,72] Long-term potentiation or depression (LTP or LTD) is a long-lasting increase or decrease, respectively, in synaptic transmission. These cellular mechanisms are hypothesized to underlie information storage in the brain as they are rapidly established, maintained for long periods of time and strengthened by repetition. Nearly every excitatory synapse in the brain exhibits LTP, which suggests that this phenomenon is a universal mechanism that neurons can utilize for diverse functions.[73] The prototype and best characterized form of synaptic plasticity is NMDA receptor-dependent LTP, with an absolute requirement for NMDA receptor activation[74] that leads to postsynaptic calcium influx necessary to initiate LTP.[75] In many forms of LTP, continued maintenance of potentiated synaptic transmission results from an increase in the function and number of postsynaptic AMPA-type glutamate receptors.[76] Since excitatory synaptic transmission involving NMDA and AMPA receptors is key to synaptic plasticity, these receptors are hypothesized to play important roles in effects caused by drugs of abuse.

Several lines of evidence support the idea that synaptic plasticity is a good model for addiction. Changes in synaptic plasticity are observed following drug administration in parallel with behavioral changes. Importantly, in some cases the behavioral effects can be reversed when the changes in synaptic plasticity are reversed. Such studies provide strong evidence that drugs of abuse that are capable of shaping behavior create changes in synaptic plasticity in brain reward circuits.

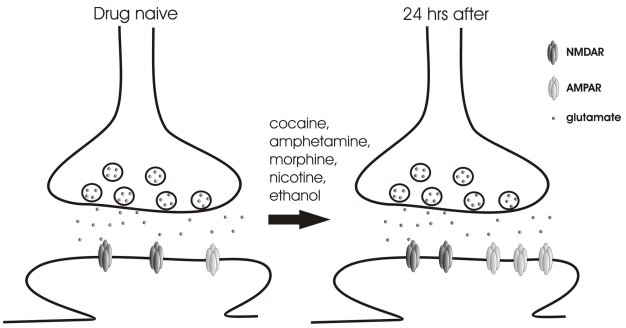

FIGURE 1. Glutamatergic synapse onto a VTA dopamine neuron before and 24 hours after single *in vivo* drug exposure. All addictive drugs of abuse tested potentiate this synapse by increasing the number and/or function of synaptic AMPARs on the dopamine neuron.

SYNAPTIC PLASTICITY OF EXCITATORY SYNAPSES: A COMMON MECHANISM IN ADDICTION?

The first study examining the effects of an addictive drug administered *in vivo* on synaptic plasticity found that a single exposure to cocaine potentiated excitatory synapses in the VTA measured 24 hours following drug administration (see Figure 1).[68] The potentiation was dependent upon NMDA receptors, which is consistent with the requirement of NMDA receptors for LTP at VTA synapses.[77,78] In addition, the enhanced synaptic transmission occurred via an increase in the number or function of AMPA receptors, without altering the contribution of the NMDA receptor.[68] This enhanced synaptic response persisted for 5 days after cocaine exposure, but was not detected 10 days following cocaine exposure, indicating the transient nature of changes in the VTA caused by drug exposure. Synaptic stimulation utilized to elicit LTP in drug-naïve brain slices was ineffective after cocaine exposure suggesting that cocaine treatment increased synaptic strength via the same mechanism as LTP. In agreement with this finding, cocaine exposure increases the density of dendritic spines on VTA DA cells, sites of contact for excitatory synapses that form and grow in parallel with LTP expression.[79]

A follow-up study showed that other drugs of abuse, including amphetamine, nicotine, morphine and ethanol, caused a similar increase in excitatory synaptic transmission (see Figure 1).[66] Interestingly, acute stress also increased synaptic strength in an NMDA receptor dependent manner, resulting in an increase in AMPA receptor number or function. A glucocorticoid receptor antagonist administered prior to the acute stress prevented the increase in synaptic strength. Importantly, the glucocorticoid receptor antagonist had no effect on cocaine-induced potentiation.[66] As stress can promote relapse in individuals abstaining from abused substances, this finding suggests the possibility of using glucocorticoid receptor antagonists to reduce the probability of relapse; however, this

has not yet been tested in human patients. Additionally, the extensive distribution of glucocorticoid receptors, both within and outside of the CNS, combined with potentially serious side-effects currently limits the therapeutic ability of such drugs unless highly specific or localized treatments could be substituted. Another study identified orexin A signaling in the VTA as a critical step for cocaine-induced synaptic plasticity and development of behavioral sensitization.[80] Blocking orexin signaling prevented the enhancement of excitatory synaptic transmission, and thus orexin receptor antagonists may prevent modifications to the brain reward pathway and possibly cocaine seeking behaviors.

Later studies differentiated the pathways involved in drug- versus stress-induced synaptic potentiation into a D1-like dopamine receptor dependent pathway for drugs of abuse and the glucocorticoid receptor dependent pathway for acute stress.[81] As reported in other forms of LTP, cocaine and acute stress required the GluR1 subunit of the AMPA receptor for the potentiation of excitatory synapses.[81] This finding raises the possibility that blockade of synaptic plasticity at synapses containing GluR1 subunits may prevent the establishment of drug-induced plasticity leading to addiction. Although GluR1-specific antagonists are not currently available and would suffer from the same spatial and temporal localization problems as glucocorticoid receptor antagonists, other cellular mechanisms required for synaptic plasticity could be targeted. The signaling cascades used for synaptic plasticity at VTA synapses may be different from those in other brain regions, offering the hope that selective drugs could be developed to interfere with addiction-related plasticity but not memory-related plasticity mechanisms.[82–84]

A potential strategy for reversing cocaine-induced plasticity has been demonstrated in principle by activation of a metabotropic glutamate receptor, mGluR1.[85] Cocaine not only enhances excitatory synaptic transmission, but also changes the postsynaptic receptor components by exchanging GluR2-containing AMPA receptors for GluR2- lacking AMPA receptors (see Figure 2a,b).[85] This seemingly slight change in subunit composition has profound effects on calcium permeability. GluR2-lacking AMPA receptors are highly permeable to Ca^{2+} while GluR2-containing AMPA receptors have almost no Ca^{2+} permeability.[86] As Ca^{2+} is a ubiquitous signaling molecule, the permeability differences translate into significant functional differences.

In contrast to the effect of cocaine, a form of LTD mediated by mGluR1 previously described at these synapses in the VTA involves swapping of native GluR2 lacking AMPA receptors for GluR2-containing AMPA receptors.[87] The possibility of dynamic AMPA receptor redistribution led the investigators to discover that mGluR1 activation initiating LTD reversed the cocaine-induced AMPA receptor plasticity (see Figure 2c).[85] Establishing mGluR-LTD required not only de novo synthesis of GluR2, but also selective replacement of GluR2-lacking AMPA receptors with newly synthesized GluR2-containing AMPA receptors.[88] Thus, cocaine enhancement of synaptic plasticity can be reversed by mGluR1 activation

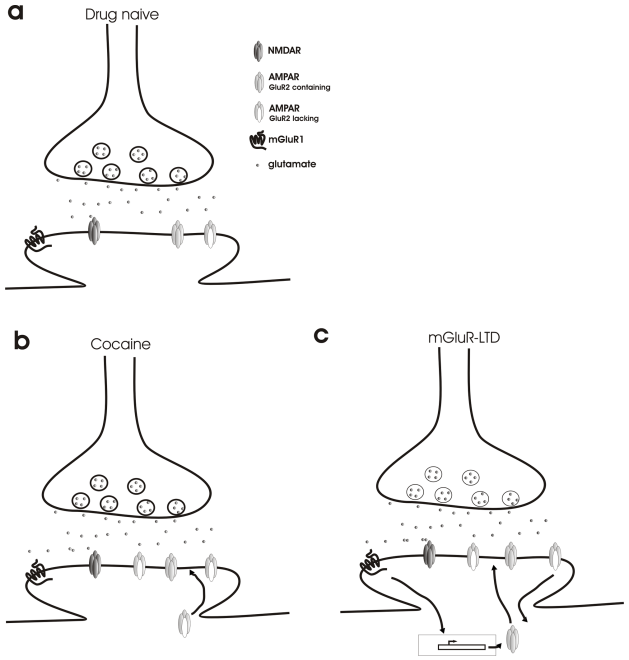

FIGURE 2. Reversal of cocaine-induced synaptic plasticity by mGluR activation. A. A drug-naïve glutamatergic synapse onto a VTA dopamine neuron. B. A single injection of cocaine triggers the insertion of GluR2-lacking AMPARs at the synapse. C. Following the cocaine-induced potentiation shown in (B), activation of mGluR1 removes newly inserted GluR2-lacking AMPARs and replaces some with GluR2-containing AMPARs.

leading to LTD and redistribution of AMPA receptors. An obvious advantage to this situation is that the plasticity can be modified after the initial cocaine-induced change has occurred, rather than merely preventing the initial alteration. However, given that most drug-induced synaptic changes in the VTA are transient, lasting less than 10 days, the utility of this approach may be limited to this brief period. Whether or not selective mGluR1 agonists exhibit efficacy in reversing behavioral phenotypes of addiction remains unknown. Targeting GluR2-lacking AMPA receptors in the NAc may also be therapeutically effective, as discussed in a later section.

As discussed above, all drugs of abuse including cocaine, amphetamine, morphine, nicotine and ethanol increased synaptic strength of excitatory synapses onto the same midbrain dopamine neurons despite the fact that these drugs have different mechanisms of action.[66] Since these findings are so strikingly similar, all addictive drugs might act through a common mechanism to alter brain reward circuitry in early development of addiction.

INHIBITORY SYNAPSES IN THE VTA ARE ALSO TARGETED BY DRUGS OF ABUSE

In addition to acting on excitatory neurotransmission, drugs of abuse can alter synaptic plasticity by modulating inhibitory

synapses in reward circuits. Sustained levels of inhibition may prevent potentiation by LTP-inducing stimuli, whereas reduction or elimination of inhibitory tone may permit previously sub-threshold stimuli to induce long-lasting changes in synaptic transmission. The important balance between excitatory and inhibitory inputs was clearly demonstrated in a study that utilized $GABA_A$ receptor antagonists to remove the influence of inhibitory inputs and revealed the ability to induce robust LTP that was precluded by inhibition.[70] Thus, modulation of the inhibitory network may provide a means to counter cocaine-induced changes in synaptic plasticity. Selective targeting of inhibitory neurotransmission in the VTA could prevent cocaine-induced changes in synaptic plasticity and possibly cocaine-seeking behavior. Indeed, GABA-enhancing drugs reduce cocaine self-administration in animal models,[89] although food responding was also decreased in this study suggesting that the reduction in self-administration was not specific to the rewarding properties of cocaine, but generalized to the reward circuit. Human clinical trials have demonstrated efficacy of a GABA-enhancing drug, gamma vinyl-GABA, in reducing cocaine use and reinstatement via a GABAergic mechanism.[90,91] When directly injected into the VTA, baclofen, a $GABA_B$ receptor agonist, was also effective in reducing cocaine self-administration in animal models of addiction, while muscimol, a $GABA_A$ receptor agonist, had no effect on cocaine self-administration.[92] These findings suggest that GABA-enhancing drugs reduce cocaine self-administration and reinstatement primarily via $GABA_B$ receptors and support the use of baclofen to treat cocaine craving,[93] and alcohol dependence.[94]

Pan and colleagues further explored the balance between excitation and inhibition to reveal that cocaine exposure enables an endocannabinoid-mediated form of synaptic plasticity to reduce inhibition.[95] Thus, in addition to GABA-enhancing drugs, CB_1 receptor antagonists locally acting in the VTA might be used to counter cocaine-induced changes. A necessary prerequisite for CB_1 receptor antagonists would be specific and local action to minimize undesired side effects caused by global inhibition of the receptor. These studies suggest that precisely controlling inhibitory neurotransmission may be a viable treatment for cocaine addiction.

In addition to cocaine, ethanol also targets inhibitory synapses in the VTA. Not only does ethanol directly increase the firing rate of VTA dopamine neurons[96] but it simultaneously inhibits the firing rate of VTA GABAergic neurons.[97,98] The first study to examine synaptic changes in the VTA after exposure to ethanol reported a transient enhancement of inhibitory neurotransmitter release onto dopamine neurons lasting at least 7 days.[99] This ethanol-induced synaptic plasticity may contribute to the development of alcoholism, as pharmacological manipulations that increase GABAergic inhibition facilitate ethanol consumption in rats,[100,101] whereas antagonists of $GABA_A$ receptors reduce ethanol drinking.[100,102] In addition to altering inhibitory circuits, ethanol enhanced excitatory neurotransmission in an ethanol self-administration study. DA neurons from animals trained to self-administer ethanol over several weeks expressed an increase in the strength of excitatory synapses compared to neurons from naïve animals.[103] Together these studies suggest that ethanol potentiates both inhibitory and excitatory transmission in the VTA, perhaps with differing time courses.

As indicated by ethanol-induced changes in synaptic plasticity, inhibitory inputs onto VTA dopamine neurons can undergo changes in synaptic strength similar to excitatory inputs. Recently, a form of LTP of GABAergic synapses onto VTA dopamine neurons (LTP_{GABA}) has been found[69] that mirrors the potentiation seen at excitatory synapses onto the same dopamine neurons.[77] Like LTP at excitatory synapses, LTP_{GABA} is NMDAR-dependent (see Figure 3). The simultaneous existence of excitatory and inhibitory synaptic plasticity of inputs onto dopamine neurons in the VTA suggests that competing mechanisms regulate neuronal excitability and firing rate. Since drugs of abuse potentiate excitatory synapses on VTA dopamine neurons, it is of interest to determine whether addictive drugs can also alter the strength of inhibitory synapses onto these neurons. Administration of morphine 24 hours prior to brain slice preparation completely blocked the ability of the inhibitory synapses to undergo LTP_{GABA} (see Figure 3).[69] A single administration of morphine therefore potentiates excitatory synaptic transmission[66] while at the same time preventing a complementary increase in inhibitory transmission that normally could have counterbalanced the increased excitation. Blockade of LTP_{GABA} by morphine could prevent long-term damping of dopamine neuron activity that might otherwise be able to reverse or prevent synaptic plasticity at excitatory terminals induced by drugs of abuse, and may

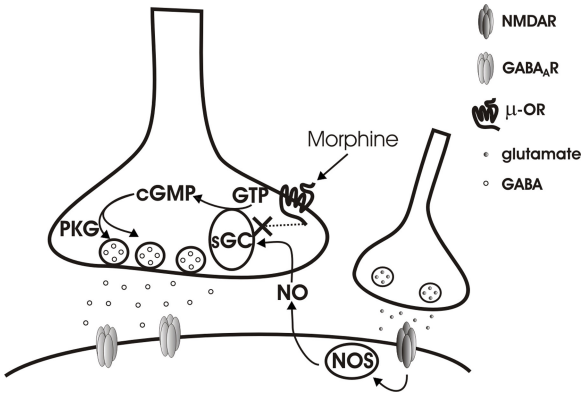

FIGURE 3. Mechanism of LTP_{GABA} at an inhibitory synapse onto a VTA dopamine neuron. Stimulation of glutamatergic afferents activates NMDARs on the dopamine neuron. Ca^{2+} influx activates nitric oxide synthase (NOS) to generate nitric oxide (NO). NO activates soluble guanylate cyclase (sGC) present in the presynaptic GABAergic terminal to produce cGMP. Through an unknown mechanism, activation of PKG results in an increase in GABA release. *In vivo* administration of morphine 24 hours prior to brain slice preparation blocks LTP_{GABA} at a stage between NO generation and activation of sGC.

contribute to the development of addiction. Perhaps enhancing LTP_{GABA} might be an effective therapeutic strategy to counter the increase in excitatory transmission caused by drugs of abuse, such as cocaine and ethanol. This hypothesis would be consistent with previous studies demonstrating reduced self-administration and reinstatement of drug seeking behavior by GABA-enhancing drugs such as gamma vinyl-GABA.[90,91]

The effects of other addictive drugs on LTP_{GABA} remains an intriguing question in the development of addiction. While repeated exposure to cocaine does not potentiate excitatory transmission more than a single exposure,[68,83] repeated administration of cocaine appears to reduce GABA-mediated inhibition.[70,95] Further work will be needed to ascertain whether cocaine exposure inhibits LTP_{GABA} similarly to a single exposure to morphine and whether multiple drug exposures differ in effect on LTP_{GABA}.

NICOTINE AND VTA SYNAPTIC PLASTICITY

In addition to the drugs of abuse discussed so far, nicotine can also alter synaptic plasticity of VTA dopamine neurons via mechanisms similar to LTP. Activation of presynaptic receptors on excitatory terminals by nicotine induced a long-lasting increase in excitatory neurotransmitter release onto dopamine neurons in the VTA.[50] In parallel, inhibitory neurotransmitter release onto dopamine neurons is reduced after a transient initial increase that subsides due to desensitization of the nicotinic receptors on the inhibitory terminals.[105] The loss of inhibitory neurotransmission effectively permits an increase in excitability of the dopamine neuron by eliminating the braking mechanism. This cumulative increase in excitability results from a crucial difference in desensitization of the nicotinic receptors present on excitatory versus inhibitory terminals.[105] Desensitization of the $\alpha4\beta2$ containing nicotinic receptors located on inhibitory GABAergic terminals occurs within several minutes, whereas nicotinic receptors containing $\alpha7$ subunits located on excitatory glutamatergic terminals are largely resistant to desensitization. The addictive nature of nicotine likely occurs as a result of its ability to increase synaptic strength and firing rate of midbrain dopamine neurons not only by increasing the strength of excitatory synaptic transmission, but also by decreasing inhibitory transmission.

While several treatment strategies exist for nicotine addiction, a high rate of relapse plagues individuals attempting to quit. Indeed, relapse rates may be as high as 80% and fewer than 6% of individuals who attempt to quit remain nicotine-free during the first year of abstinence.[105] Mainstream pharmacotherapies for smoking cessation include nicotine replacement therapy and bupropion.[106] Bupropion blocks noradrenalin and dopamine reuptake to elevate levels of these neurotransmitter during nicotine withdrawal, which may alleviate withdrawal symptoms. The most recently approved drug for smoking cessation is varenicline, a nicotinic $\alpha4\beta2$ partial agonist,[107] which is hypothesized to ameliorate craving and withdrawal symptoms while preventing the reinforcing effects of nicotine.[108] While current treatments focus on nicotinic receptors, it will be interesting to determine whether existing or novel treatments are able to prevent or reverse the changes in synaptic plasticity and firing rate of VTA dopamine neurons triggered by nicotine.

SYNAPTIC PLASTICITY AND MODELS OF ADDICTION

The development of behavioral sensitization and conditioned place preference, two experimental models of drug-induced behavioral changes hypothesized to be involved in development of addiction, require NMDA receptor activation, as NMDAR antagonists diminish these addiction-related behaviors. In order to further explore the role of NMDA receptors in addiction, two recent studies genetically deleted a necessary subunit of the NMDA receptor selectively in dopamine neurons of the VTA (see Table 1). Both groups confirmed that cocaine did not potentiate excitatory neurotransmission in the knockout animals, in contrast to wild-type controls;[113,114] however, their findings disagreed on the conditioned place preference behavioral measure, perhaps because of differential compensatory adaptations. Zweifel and colleagues[114] speculate that an increase in NMDA receptor number or function may be responsible for the increased drug craving that occurs during withdrawal as expression of NR1 increases not only after prolonged cocaine administration,[115] but also during extended withdrawal from cocaine.[116] Similarly, Engblom and colleagues report that activation of NMDA receptors is necessary for cue-induced reinstatement. If correct, preventing an increase in NMDA receptor number or function could be a useful strategy to improve drug abstinence.

In addition, Engblom and colleagues also engineered knockout animals lacking either the GluR1 or GluR2 subunits of the AMPA receptor in dopamine neurons (see Table 1).[114] Intriguingly, GluR1 deletion resulted in the failure of these animals to extinguish drug-seeking behavior when the previously drug-associated environment was then paired with a saline, rather than drug, injection.[113] Specific GluR1 activation may therefore assist in extinction of drug associated learning. However, GluR1 is necessary for the initial synaptic potentiation of AMPA receptor currents that is hypothesized to underlie the initiation of events leading to addiction.[81] Indeed, GluR1 enhanced the rewarding properties of morphine when expression levels were artificially increased in the VTA via viral infection, in one of the first studies to suggest the possibility of a link between a synaptic change (AMPAR subunit expression) and a behavioral change (time spent in drug-paired environment) relevant to addiction.[117] It is interesting that establishing behavioral sensitization or conditioned place preference was not dependent upon GluR1.[113] Perhaps the outcome of GluR1 activation, eg, addiction or extinction, depends upon the current state of the reward circuit. While postsynaptic receptors such as AMPAR and NMDAR are known to be important elements in these

TABLE 1. Effects of glutamate receptor subunit deletion on behavioral assays

Receptor	Subunit	Selectivity	Behavioral sensitization	CPP	Extinction	Reinstatement	Reference
AMPAR	GluR1$^{-/-}$	DA neurons	N.S.	N.S.	Absent	—	114
		Constitutive	N.S.	Absent	—	—	81
		Constitutive	—	Food – N.S. Cocaine – N.S.	—	—	111
		Constitutive	—	—	Reduced	Enhanced	112
	GluR2$^{-/-}$	DA neurons	N.S.	N.S.	N.S.	N.S.	114
		Constitutive	—	Food – Absent Cocaine – N.S.	—	—	111
NMDAR	NR1$^{-/-}$	DA neurons	N.S.	N.S.	N.S.	Absent	114
		DA neurons (inducible)	—	N.S.	N.S.	Absent	114
		DA neurons	Acute – N.S. Withdrawal – Reduced	Absent	—	—	115
		VTA (viral induction)	—	Absent	—	—	115
		Constitutive knock-down	Cocaine – Reduced Amphetamine – N.S.	N.S.	—	—	113
	NR2$^{-/-}$	Constitutive	—	Absent	—	—	110

N.S. – not significantly different from control; dashes indicate "not determined."

models of addiction, the ubiquitous expression of the same receptors throughout the brain presents serious challenges to developing pharmacotherapies based on modifying signaling of AMPARs or NMDARs. Treatments must be specific not only for the receptor subtype but also to the localized reward circuit.

While self-administration of drugs of abuse is undoubtedly a more complete model of addiction than passive, experimenter-controlled administration, a recent report highlights a surprising difference in the stability of changes in synaptic plasticity following drug administration. Chen and colleagues report that passive cocaine administration is insufficient to induce an increase in AMPAR/NMDAR ratio that occurs with self-administration of food, sucrose or cocaine.[118] Intriguingly, the persistence of the synaptic change depended on the reward that was self-administered. Food, sucrose and cocaine self-administration caused potentiation at VTA DA synapses that was maintained 7 days following abstinence from the reward;[118] however, at 21 days of abstinence only cocaine-administering animals still expressed an increased AMPAR/NMDAR ratio. Remarkably, this change in synaptic plasticity was maintained even after a 3 month abstinence period.[118] These findings strongly suggest that both natural rewards and drugs of abuse act through a common pathway to induce changes in behavior.

In contrast to the long-lasting alterations in synaptic strength caused by drugs of abuse, Stuber and colleagues report that reward learning transiently increases synaptic strength, but this vanishes once a reward association is learned.[119] Both dopamine release and synaptic strength increased during the reward learning protocols, implying that learning triggers synaptic plasticity in the same VTA DA neurons that are highly relevant to the development of addiction.[119] Whereas natural rewards produce transient synaptic modifications that readily permit subsequent reward learning, drugs of abuse such as cocaine instigate an enduring, maladaptive change in plasticity that might be thought of as "overlearning" the rewarding properties of the drug at the expense of natural rewards that are then hindered from forming appropriate new reward memories. These studies suggest that therapeutic interventions based on reversing changes in synaptic plasticity in the VTA would be required early to be effective in treating addiction, before storage of drug-associated information is distributed to other brain regions. The therapeutic window may even occur during the transition from recreational use to compulsive use—a time when users are unlikely to seek assistance.

ROLE OF THE NUCLEUS ACCUMBENS IN ADDICTION

Changes in firing rate or synaptic plasticity in VTA dopamine neurons are in general relatively short-lived, on the order of days.[64,120] The immediate alterations in signaling induced by drugs of abuse in the VTA may be communicated to other downstream targets in different brain regions responsible for long-term effects of addiction, such as craving and relapse. VTA activation by drugs of abuse may differ from that elicited by natural reinforcing stimuli in being more intense, involving a greater number of VTA neurons, or causing more persistent VTA neuron activation.

Drugs of abuse acting on VTA dopamine neurons induce dopamine release at terminals in the NAc, a brain region important in motivation, reward and attention.[121,122] The "addiction" signal therefore may be passed on from the VTA to the NAc. Indeed, drugs of abuse also influence synaptic plasticity in neurons in the NAc[84,123,124] via mechanisms similar to LTP and LTD found in the VTA. Most addictive drugs decrease the basal excitability level of medium spiny neurons, the most common type of neuron found in the NAc,[125] which may partly explain the decreased salience and response of substance abusers to naturally rewarding stimuli.[126,127]

SYNAPTIC PLASTICITY IN THE NAc

Unlike the situation in the VTA, a single exposure to cocaine does not alter synaptic plasticity at NAc spiny neurons; however, repeated cocaine exposure reduces long-term excitability of NAc neurons.[84] This finding is in accord with the hypothesis that addiction develops initially in the VTA following early drug exposure and progresses with repeated exposure to drug-related reinforcement in the NAc. Moreover, these data suggested that cocaine might trigger LTD at excitatory synapses in the NAc. A later study confirmed that LTD was absent in animals self-administering cocaine, as expected if cocaine administration had already saturated the LTD mechanism.[123] Further, the absence of LTD persisted for at least 21 days of abstinence after cocaine exposure suggesting that the persistent loss of LTD may parallel the time course of long-term addiction related behaviors, such as drug craving or relapse. Indeed, reinstatement of drug-seeking behavior appears to increase glutamate release in the NAc from neurons originating in the prefrontal cortex[128] and during extended withdrawal cocaine re-exposure enhances glutamate release that is likely necessary to overcome the decreased excitability of neurons in the NAc.[129–131]

Different forms of plasticity are induced by addictive drugs at synapses on NAc medium spiny neurons that together contribute to uncontrollable drug-seeking, a hallmark of addiction. A recent study by Kourrich and colleagues indicates that prior drug exposure to cocaine determines whether a subsequent exposure will increase or decrease excitatory neurotransmission in the NAc.[132] Less than 24 h after repeated cocaine exposure, excitatory synaptic transmission is reduced in the NAc. Extended drug-free periods after the last exposure promote enhanced excitatory transmission; however, even a single re-exposure to cocaine reverses the potentiation.[132] Currently the function of these alterations to NAc synaptic plasticity is unknown. The sensitivity of the circuit to cocaine exposure after a drug-free period may serve to focus the reward system on cocaine at the expense of natural reinforcers and therefore contribute to addiction. The increase in excitation following abstinence from repeated cocaine exposure may be an attempt to restore homeostasis after the initial depression caused by cocaine; however, the enhanced excitatory transmission might also contribute to drug craving, which intensifies during abstinence, and often results in relapse.

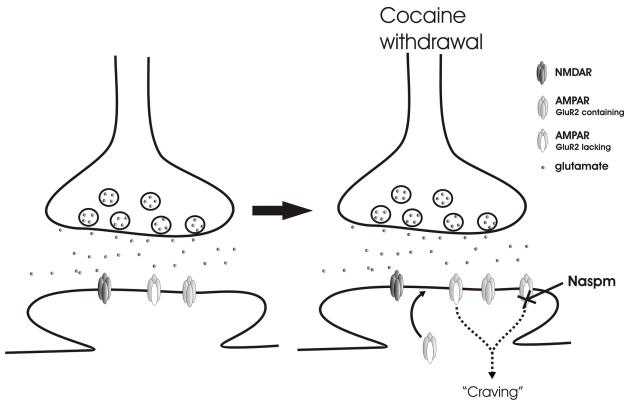

FIGURE 4. Glutamatergic synapse onto a NAc medium spiny neuron. Prolonged withdrawal from cocaine self-administration increases expression of GluR2-lacking AMPARs, which mediate the increase in cue-induced cocaine craving. Blockade of GluR2-lacking AMPARs with Naspm, prior to testing for cue-induced cocaine seeking, reduced cocaine craving, as measure by behavioral responses to a cocaine-associated cue.

It was recently reported that during prolonged withdrawal from cocaine, the number of AMPA receptors on medium spiny neurons is increased and the newly inserted receptors consist of different subunits that respond more robustly to glutamate release.[133] In NAc neurons, GluR2-lacking AMPA receptors are upregulated during cocaine withdrawal.[132,134,135] Moreover, these AMPARs participate in cue-induced cocaine seeking because a selective antagonist of GluR2-lacking AMPA receptors delivered into the NAc significantly reduced cued cocaine-seeking behavior (Figure 4).[133] These data suggest that selective AMPA receptor blockade may offer a therapeutic advantage to reduce drug craving and relapse. Selective and locally acting mGluR1 agonists and GluR2-lacking AMPA receptor antagonists are currently needed to determine whether modulation of AMPA receptor distributions will be an effective therapy for addiction.

A promising potential treatment for cocaine addiction is N-acetylcysteine, a cysteine prodrug used to increase cystine-glutamate exchange, resulting in an increase in extrasynaptic glutamate.[136,137] During withdrawal from repeated cocaine exposure, basal levels of extracellular glutamate are reduced.[129] Cystine-glutamate transport, which is primarily driven by substrate concentration gradients, is consequently impaired.[138] Normalization of cystine-glutamate exchange by N-acetylcysteine provides extrasynaptic glutamate to presynaptic mGluRs that reduce synaptic glutamate release by a feedback mechanism.[139] In a recent pilot study, N-acetylcysteine reportedly reduced cocaine craving in addicted subjects, possibly by acting as a brake on craving.[140] Investigation into the mechanism of action of N-acetylcysteine revealed that the cocaine-induced reduction of basal glutamate levels and enhanced glutamate in the presence of cocaine

were prevented by prior administration of N-acetylcysteine through the restoration of extrasynaptic glutamate levels and presynaptic mGluR function.[141] By preventing cocaine-induced plasticity, N-acetylcysteine reduced the number of self-administered cocaine infusions and drug-cued reinstatement. This study lends support to the hypothesis that changes in plasticity can underlie the development of addiction. However, it also illustrates that therapeutic treatments can be developed to alter neuronal function without directly affecting synaptic transmission or neuronal excitability, even if these parameters are the ones persistently altered by drug exposure. A better understanding of the relationship between drug-induced changes and behavior will help to separate those changes that would be therapeutically beneficial from those that should be prevented or reversed.

As previously mentioned, another family of signaling proteins implicated in cocaine-induced plasticity in the NAc is the Homer protein family. Homer proteins are involved in plasticity induced by multiple drugs of abuse by virtue of their ability to regulate glutamate receptors and neurotransmission. Short forms of Homer proteins serve as immediate early genes and are expressed in an activity-dependent manner. Long forms of Homer proteins are constitutively expressed and form multimers and protein complexes thought to be important in signal transduction at the synapse. Properties of glutamate transmission in Homer knockout mice exhibit striking similarities to wild-type animals undergoing withdrawal from repeated cocaine exposures, including facilitated extracellular glutamate release in the NAc in response to cocaine.[60] Ethanol induced changes in NAc plasticity were found to depend on Homer2, as repeated ethanol administration did not increase extracellular glutamate or dopamine levels in Homer2 knockout animals, in contrast to wild-type controls.[142] Furthermore, unlike their wild-type counterparts, Homer2 knockout mice did not develop conditioned place preference or sensitization to ethanol and importantly, the behavioral differences were reversed when Homer2 was virally overexpressed in the knockout animals.[142] A recent lesion analysis indicated that the NAc is important in the acquisition of ethanol conditioned place preference; however, a separate lesion of the amygdala also impaired acquisition suggesting that both sites are important in ethanol reinforcement.[143]

Similar to findings in the VTA, a single injection of morphine produces persistent changes in the NAc, including enhanced dopamine release in response to an electrical stimulus.[144] Morphine exposure differentially alters specific subtypes of glutamate receptors. For example, NMDA receptor subtypes NR1 and NR2A were upregulated following implantation of a 48 h morphine pellet,[145] whereas the AMPA receptor subtype GluR1 was decreased from cell membrane sites in response to repeated morphine administration.[146] Drug induced changes in glutamate receptors may underlie some effects of morphine on synaptic plasticity in the NAc. Synapses in the NAc can express both LTP and LTD[147] and these forms of plasticity have recently been found to be temporally altered by morphine. Following repeated morphine administration, LTP was impaired 12 h after the last morphine treatment, but not after a 4 day withdrawal period. Conversely, a stress-facilitated LTD was elicited 12 h after morphine termination, but was not expressed after a longer 4 day withdrawal period, demonstrating that the effect of morphine withdrawal on NAc synaptic plasticity (both LTP and LTD) changes with time.[148] These similar forms of plasticity in the VTA and NAc may carry reward-related information necessary for establishing addiction.

CONCLUSIONS

Currently it is unclear how changes in synaptic strength due to drug exposure translate into drug-associated memories and addiction; however, investigations into the acute effects of drugs of abuse on synaptic plasticity have revealed attractive targets for further investigation of possible therapeutic treatments, contributing to the understanding of plasticity and addiction. Recent investigations into the cellular mechanisms that occur during drug abuse highlight the complex nature of addiction and the difficulty in developing novel therapeutic targets for effective treatment. Changes that occur at the level of the synapse and corresponding changes in behavior are multifaceted and additional studies are necessary to increase our understanding of the connections between synaptic change and addiction-related behaviors. An unfortunate corollary is that development of novel treatments for drug dependence based on data from plasticity studies remains in early stages because of this limited understanding of the linkage between plasticity and clinically relevant behaviors. It is premature to speculate how effective pharmacological manipulation of synaptic plasticity in brain reward circuits will correspond to clinically relevant outcomes, at least until novel interventions are developed that can be wielded with more surgical precision. Perhaps then a combination of treatment modalities targeting cellular and synaptic changes along with behavior modification will provide needed improvements in managing addictive disorders.

This research and some of the viewpoints in this article were supported by grants DA024527 (Dr. Niehaus), Minority supplement to DA011289 (Dr. Cruz-Bermúdez), and DA011289 (Dr. Kauer) from the National Institutes of Health, Bethesda, Md.

We would like to thank Dr. Feresheteh Salmanzadeh Nugent (USUHS) for helpful comments and discussion on this topic and article.

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