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Review

Microdialysis and the neurochemistry of addiction

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Abstract

Drug addiction is a process beginning with the initial exposure to a drug of abuse, and leading, in some individuals, to chronic habitual use, and high rates of relapse. Microdialysis allows researchers to monitor the neurochemical changes that occur in the brain after the initial exposure to a drug, and the neurochemical changes that occur with repeated exposure. These changes in the brain are often referred to as drug-induced neuroplasticity, and the aim of this article is to review studies that have utilized microdialysis to increase our understanding of the neuroplasticity that occurs in the process of addiction. We will review how several neurotransmitter systems, including glutamate, GABA, the monoamines, and others, are altered after chronic drug exposure, and how microdialysis can be used to determine if putative treatments for addiction can reverse the drug-induced neuroplasticity in these systems. We will also briefly discuss our recent research using a known change in GABA neurotransmission that occurs during reinstatement of drug-seeking to screen for possible novel treatments to prevent relapse. Overall, microdialysis in combination with other behavioral and pharmacological techniques has greatly increased our understanding of addiction-related neuroplasticity, and provides a means for discovering new ways to prevent these changes and treat addiction.

Keywords: Microdialysis; Neuroplasticity; Addiction; Glutamate; GABA; Dopamine; Neuropeptide

Contents

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1. Introduction

Drug addiction is characterized by compulsive drug-seeking despite severe negative consequences. Increasingly, addiction research is coming to understand that this pathological compulsion develops through maladaptive learning processes where associations between the rewarding aspects of the drug and cues in the environment, come to take over behavior in such a way that addicts have a difficult time stopping drug use and experience high rates of relapse after abstinence. Long-lasting and possibly permanent changes in the brain, including alterations on the systems neurocircuitry level, underlie this maladaptive learning (Kalivas and Volkow, 2005; Kelley, 2004; Nestler, 2001). Microdialysis and other in vivo neurochemical techniques provide a means for understanding the neuroplasticity that occurs with chronic exposure to a drug of abuse, by allowing a comparison of neurochemical output in different brain regions at different stages of the addiction process, and in comparison to drug naïve animals.

Briefly, microdialysis is a technique where a semi-permeable membrane 1–2 mm in length is stereotaxically implanted into a discrete brain region. A dialysis buffer that is similar to cerebral spinal fluid in ionic composition is perfused through the membrane via tubing usually attached to a syringe pump. Substances in the brain that are in low concentration in the buffer and that are small enough to pass through the dialysis membrane (including all small molecule neurotransmitters and some peptides), will diffuse into the dialysis membrane and will flow through another tube for collection. The contents of the dialysis

Table 1
Summary of plasticity in neurotransmission after chronic cocaine or amphetamine

Drug administration/behavioral paradigm	Neurotransmitter monitored	Brain region	Effect	References
Psychomotor sensitization (early withdrawal)	Glutamate	mPFC	↑	Williams and Steketee (2004)
		Nac	NC	Robinson et al. (1997)
	GABA	mPFC	↑	Jayaram and Steketee (2005)
	Dopamine	mPFC		
		Nac	NC	Wolf et al. (1993); Segal and Kuczenski (1992a,b); Paulson and Robinson (1995); Kalivas and Duffy (1993); Hooks et al. (1994)
	Serotonin	Nac	↑	Parsons and Justice (1993)
		VTA	↑	Parsons and Justice (1993)
		DRN	↑	Parsons and Justice (1993)
Psychomotor sensitization (late withdrawal)	Glutamate	mPFC	NC	Williams and Steketee (2004)
		NAc	↑	Pierce et al. (1996); Reid and Berger (1996)
		VTA	↑	Kalivas and Duffy (1998)
	GABA	mPFC	NC	Jayaram and Steketee (2005)
		VP	↓	Torregrossa et al., unpublished observations
	Dopamine	NAc	↑	Robinson et al. (1988); Wolf et al. (1993); Paulson and Robinson (1995); Kalivas and Duffy (1993); Heidbreder et al. (1996); Hooks et al. (1994); Zapata et al. (2003)
	Acetylcholine	Striatum	↑	Bickerdike and Abercrombie (1997)
	Cholecystokinin	NAc shell	↑	Beinfeld et al. (2002)
Basal extracellular concentration after withdrawal	Glutamate	NAc	↓	Hotsenpiller et al. (2001); Baker et al. (2002, 2003); McFarland et al. (2003)
	GABA	NAc	↑	Xi et al. (2003)
	Dopamine	NAc (early withdrawal)	↑	Heidbreder et al. (1996)
		NAc (late withdrawal)	↓	Parsons et al. (1991); Heidbreder et al. (1996)
	Cholecystokinin	NAc shell	↑	Beinfeld et al. (2002)
Drug-primed reinstatement/ drug associated cue presentation	Glutamate	Nac core	↑	Hotsenpiller et al. (2001) (cues); McFarland et al. (2003) (drug reinstatement)
	GABA	VP	↓	Tang et al. (2005)
	Dopamine	NAc (drug reinstatement)	↑	Neisewander et al. (1996); McFarland et al. (2003)
		mPFC (one cue pairing)	↑	Lin et al. (2007)
		mPFC (multiple cue pairings)	↓	Lin et al. (2007)
		Amygdala	↑	Tran-Nguyen et al. (1998)

Key: Up arrow = increased extracellular concentration; down arrow = decreased extracellular concentration; NC = no change in neurotransmitter concentration. NAc = nucleus accumbens, mPFC = medial prefrontal cortex, VP = ventral pallidum, VTA = ventral tegmental area, DRN= dorsal raphe nucleus.

samples can then be determined by several methods, usually high pressure liquid chromatography (HPLC) coupled to a detector (fluorescent, electrochemical, etc.). Please see an excellent review by Parent et al. (2001) for a more complete description of in vivo microdialysis techniques in the rat.

In addiction research, animal models allow us to study neurochemical changes during the learning process (acquisition of self-administration), once learning is established and behavior is stable (maintenance of self-administration), once drug is no longer available (abstinence or extinction), and during relapse to drug use (reinstatement) (Bossert et al., 2005). Other animal models, such as psychomotor sensitization, which is the phenomena that repeated exposure to drugs of abuse results in a progressive increase in locomotor behavior, and conditioned place preference (CPP), where animals begin to prefer a certain context after repeated associations of that context with a drug of abuse, also allow researchers to understand the neuroplasticity associated with chronic drug exposure and learned associations between context and rewarding stimuli, respectively (Sanchis-Segura and Spanagel, 2006). The advantage of the combination of microdialysis and behavioral studies is that the relevance of changes in neurotransmitter output can be inferred from associated changes in behavioral output.

The focus of this review will be to examine the neurochemical studies that utilized microdialysis to increase our understanding of the neuroplasticity of drug addiction. We will concentrate on studies that have found neurochemical changes in mesocorticolimbic circuitry after chronic drug exposure, including the ventral tegmental area (VTA), frontal cortex, dorsal and ventral striatum, amygdala, and ventral pallidum (VP). Table 1 summarizes many of the neurochemical changes that occur after chronic cocaine or amphetamine. We will place particular emphasis on the neurochemistry of relapse using the reinstatement model, including recent studies using microdialysis to find new treatments to prevent relapse by reversing drug-induced neuroplasticity.

2. Glutamate

Changes in glutamatergic neurotransmission are associated with phenomena that are thought to underlie new learning, such as long term potentiation (LTP). Therefore, because addiction is thought to involve learned associations in mesocorticolimbic reward circuitry, much research over the past decade has focused on changes in glutamatergic systems. Glutamatergic projection neurons and neurons expressing glutamate receptors are found throughout the brain, including brain regions known to be important for the rewarding properties of drugs such as frontal cortical regions, the nucleus accumbens (NAc), and the amygdala. In addition, the addictive drugs phencyclidine (PCP) and ketamine have direct actions on glutamate receptors (NMDA receptor antagonists), while other drugs of abuse have indirect actions on glutamate systems. Recent research has demonstrated that chronic drug abuse can result in profound changes in glutamatergic activity.

One of the first studies demonstrating altered glutamatergic neurotransmission after chronic cocaine administration used the psychomotor sensitization model to show that animals expressing behavioral sensitization to cocaine had increased glutamate efflux in the NAc core after a cocaine challenge compared to both saline treated and non-sensitized cocaine-treated rats after 21 days of withdrawal (Pierce et al., 1996). The importance of glutamate neurotransmission in the NAc core for the expression of behavioral sensitization was further verified in this study by showing that AMPA in the NAc increased locomotor activity in sensitized rats only, while the AMPA antagonist CNQX prevented the sensitized motor response to cocaine. Likewise, Reid and Berger (1996) found that after 10 days of withdrawal cocaine-sensitized rats had a greater increase in NAc glutamate release after a cocaine challenge compared to saline treated rats. However, an extended withdrawal period may be necessary for this cocaine-induced glutamatergic neuroplasticity, after a 48 hour withdrawal. Robinson et al. (1997) did not see an increase in glutamate in the NAc after cocaine challenge. However, these researchers did observe an increase in aspartate at this time point, which may produce similar effects to glutamate. In addition, several studies have found that while a high dose of amphetamine did increase glutamate release in the NAc and the VTA, the increase was delayed and occurred regardless of previous amphetamine sensitization or vehicle pretreatment (Xue et al., 1996). In contrast, Kalivas and Duffy (1998) found that a cocaine challenge after 21 days of withdrawal increased glutamate release in the VTA selectively in cocaine-sensitized rats.

Cocaine has also been shown to increase glutamate release in the medial prefrontal cortex (mPFC) after a sensitization regimen and 1 day or 7 days of withdrawal in animals expressing behavioral sensitization, but not after 30 days of withdrawal, suggesting that glutamatergic neuroplasticity in the mPFC is more transient than in the NAc or VTA, but may be necessary for the development of sensitization (Williams and Steketee, 2004).

Microdialysis studies have also revealed that during withdrawal from a chronic cocaine regimen administered either non-contingently (Hotsenpiller et al., 2001; Baker et al., 2002, 2003) or via self-administration (McFarland et al., 2003), basal levels of glutamate in the NAc are reduced. However, it should be noted that Robinson et al. (1997) did not see a change in basal glutamate in the NAc after a 48 hour withdrawal, but this study did not use no-net flux microdialysis so it is difficult to determine the accuracy of this data. Baker et al. (2003) found that the decrease in basal levels of glutamate during withdrawal from chronic cocaine was due to disruptions in glial cystine/glutamate exchange. The cystine/glutamate exchanger exchanges one extracellular cystine for one intracellular glutamate. After chronic cocaine and withdrawal the affinity of the cystine/glutamate exchanger is reduced such that extracellular glutamate is reduced. It is unknown how quickly this phenomenon develops after cessation of chronic cocaine.

However, after presentation of cues or context previously associated with cocaine (Bell et al., 2000; Hotsenpiller et al., 2001), a cocaine-priming injection, or a stressor to reinstate cocaine-seeking (McFarland et al., 2003, 2004) glutamate release in the NAc is greatly increased compared to control animals. The increase in glutamate that occurs in the NAc core during cocaine-primed reinstatement only occurs in rats that learned self-administration and not in yoked-cocaine controls. Very high doses of cocaine are required to see increased glutamate in drug

naïve animals. In addition, the increase in glutamate release occurs regardless of whether the levers are present in the operant chamber, indicating that the increase in glutamate in the NAc during reinstatement is not simply due to lever pressing behavior (McFarland et al., 2003). Therefore, chronic exposure to cocaine results in long-term changes in glutamate neurotransmission in the NAc that may underlie relapse behavior.

Currently, few studies have been conducted to determine if other drugs of abuse cause similar changes in glutamate neurotransmission in the NAc. However, chronic binge alcohol consumption has been shown to increase NAc glutamate release to a greater degree on the 6th day of alcohol compared to the 1st, and a challenge injection of alcohol resulted in a sensitized glutamate release in the NAc in rats with previous chronic alcohol exposure (Szumlinski et al., 2007). In addition, chronic morphine treatment decreases basal levels of glutamate in the anterior cingulate cortex (ACC) immediatley after the last morphine treatment. Moreover, another injection of morphine further decreases glutamate, while naloxone increases glutamate release in the ACC of morphine dependent rats (Hao et al., 2005). Therefore, changes in glutamatergic transmission may be important for the development of addictive behaviors to multiple drugs of abuse.

3. GABA

Ultimately, glutamate in the NAc will affect the activity of GABAergic output neurons, and every brain region within the mesocorticolimbic circuitry contains GABAergic interneurons; therefore, understanding the effects of chronic drug use on GABAergic neurotransmission is critical for understanding the neuroplasticity of addiction. In the medial prefrontal cortex (mPFC) GABAergic interneurons can regulate dopaminergic and glutamatergic neurotransmission. Animals sensitized to cocaine show an increase in mPFC GABA release in response to a cocaine challenge after 1 or 7 days, but not 28 days withdrawal (Jayaram and Steketee, 2005). In addition, after cocaine sensitization the GABAB receptor agonist baclofen infused into the mPFC increases glutamate release in the mPFC and ipsilateral NAc and VTA. Saline treated animals show no increase in mPFC glutamate in response to baclofen (Jayaram and Steketee, 2004). Therefore, chronic cocaine alters the ability of GABA to regulate glutamate neurotransmission in the mPFC, and the cocaine challenge-induced increase in mPFC GABA release provides a possible mechanism for the increased activation of mPFC mediating behavioral sensitization and reinstatement behavior.

GABAergic neurotransmission in the NAc is also altered after chronic cocaine. Xi et al. (2003) studied the effect of chronic cocaine and 3 weeks withdrawal on basal levels of GABA in the NAc using no-net flux microdialysis. They found that repeated cocaine increased basal levels of GABA in the NAc, and that this was likely mediated by desensitization of GABAB receptors, as a GABAB antagonist further increased GABA, while a GABAB agonist decreased GABA output in the NAc. In addition, the GABAB antagonist increased glutamate and dopamine in the NAc only in cocaine-sensitized rats. However, in the VTA, 3 days after repeated amphetamine administration, signaling through GABAB receptors appears to be enhanced, as administration of a GABAB antagonist increases dopamine release to a greater extent than saline treated controls (Giorgetti et al., 2002).

GABAergic medium spiny neurons in the NAc send projections to the VP and the VTA. Several drugs of abuse including amphetamine (Bourdelais and Kalivas, 1990), cocaine (Caille and Parsons, 2006), morphine (Tang et al., 2005; Caille and Parsons, 2006), and a cannabinoid receptor agonist that mimics the effects of marijuana (Caille and Parsons, 2006) are known to acutely decrease GABA release in the VP of rats. Rats self-administering intravenous (i.v.) heroin also show a decrease in VP GABA occurring shortly after the first self-administered infusion (Caille and Parsons, 2004). However, rats self-administering i.v. cocaine (Sizemore et al., 2000) or receiving acute oral alcohol (Cowen et al., 1998), showed no change in VP GABA release.

Little research has been conducted to determine the long-term neuroplastic changes in VP GABA transmission in response to chronic exposure to a drug of abuse. However, Tang et al. (2005) demonstrated that cocaine-primed reinstatement behavior causes a decrease in extracellular GABA in the VP. This decrease in GABA also occurred in yoked-cocaine rats, but to a slightly lesser extent. The cocaine-induced decrease in GABA was shown to be mediated by endogenous enkephalin release because the mu opioid receptor antagonist CTAP blocked both the decrease in GABA in the VP and reinstatement behavior. This study implies that increased GABAergic neurotransmission in the VP may be a means of preventing reinstatement. Indeed, the GABAB receptor agonist baclofen has been used to treat human addicts with some success, and the mechanism of action may be to increase GABAergic inhibition of VP output neurons (Roberts 2005).

4. Dopamine

Classically, drug addiction research has focused on dopaminergic systems because all known drugs of abuse and natural rewards have been shown to acutely increase dopamine release in the NAc despite having diverse mechanisms of action. Dopaminergic cell bodies are located in the VTA and substantia nigra. The VTA projects to many brain regions known to be important for reward including the PFC, dorsal striatum, NAc, VP, hippocampus and amygdala (Hyman et al., 2006).

4.1. Dopamine and sensitization

In addition to the acute effects of drugs of abuse, dopaminergic neuroplasticity has been demonstrated in response to chronic drug exposure. Several studies have examined the effect of a challenge dose of an addictive drug after a sensitizing regimen of administration and varying periods of withdrawal. Robinson et al. (1988) found that behavioral sensitization to D-amphetamine resulted in an enhanced release of dopamine in the NAc in response to a challenge dose after 15–20 days withdrawal. Further, Wolf et al. (1993) found that chronic amphetamine administration resulted in behavioral sensitization at both early (3–4 days) and late (10–14 days) withdrawal, but an enhancement of amphetamine-induced dopamine release in the NAc was only observed in late withdrawal. Similarly, repeated

amphetamine administration directly into the VTA caused an enhancement of dopamine release in the NAc in response to a systemically administered amphetamine challenge after 2 weeks of withdrawal compared to animals treated with intra-VTA saline (Vezina, 1993). Segal and Kuczenski also showed no increase in NAc dopamine after short withdrawal from amphetamine (Segal and Kuczenski, 1992a) or cocaine (Segal and Kuczenski, 1992b). Likewise, Paulson and Robinson (1995) found that rats treated with chronic amphetamine showed increase dopamine release in both the dorsal and ventral striatum upon amphetamine challenge after 28 days, but not 3 and 7 days withdrawal. In addition, chronic high dose cocaine was shown to result in a diminished increase in dopamine in the NAc after 1 day withdrawal, with an enhancement in dopamine release in response to cocaine challenge after extended withdrawal (Kalivas and Duffy, 1993). However, it should be noted that behavioral sensitization occurred regardless of the length of withdrawal, indicating that the enhancement of accumbal dopamine release may not be necessary for the behavioral effects of chronic psychostimulant administration.

Basal levels of dopamine in the NAc may also change over the course of withdrawal as Heidbreder et al. (1996) found that 2 days after chronic cocaine, basal extracellular dopamine in the NAc is increased, but that basal dopamine levels gradually decreased over the course of withdrawal, and like other studies (see above), after 22 days withdrawal dopamine in the NAc was significantly increased in response to a cocaine challenge. Likewise, Parsons et al. (1991) found that basal dopamine in the NAc was reduced 10 days after chronic cocaine. The decrease in basal dopamine may occur earlier in withdrawal than reported by Heidbreder and colleagues as basal dopamine has also been shown to be reduced after 3 days of withdrawal from chronic cocaine, while dopamine release was increased in response to a cocaine challenge using more quantitative microdialysis and voltammetric techniques (Chefer and Shippenberg, 2002). Chen et al. (1996) also found that a cocaine challenge enhanced NAc dopamine release after 7 days withdrawal from chronic cocaine compared to drug naïve controls, but that this enhancement only occurred in response to systemically administered cocaine, not when reverse dialyzed directly into the NAc, indicating that the effect of cocaine challenge on dopamine efflux in the NAc requires the activity of other brain regions. However, it should be noted that direct amphetamine application to striatal slices does produce an enhancement of dopamine release in rats chronically treated with amphetamine compared to saline treated controls, indicating that there may be differences in the neurocircuitry required for dopamine sensitization in response to cocaine or amphetamine, or differences between striatal subregions (Kolta et al., 1985).

The majority of studies investigating the effects of chronic psychostimulants on the sensitization of dopaminergic neurotransmission utilize non-contingent injections of often high doses. However, it is important to know if the contingency and amount of drug taken that occurs with self-administration is capable of causing long-term changes in dopaminergic activity. Similar to studies using non-contingent cocaine injections, animals self-administering cocaine for 20 days showed enhanced dopamine release in the NAc in response to cocaine challenge after 21 days but not 24 hour withdrawal when compared to yoked-saline controls (Hooks et al., 1994). Likewise, Zapata et al. (2003) found that mice that self-administered cocaine or received yoked cocaine showed similar increases in NAc dopamine release after cocaine challenge. However, one study found that after cocaine self-administration dopamine efflux in response to non-contingent or contingent cocaine was reduced after 7 days withdrawal compared to 1 day withdrawal (Meil et al., 1995). The discrepancies in these findings are difficult to explain, but it is possible that a longer withdrawal period was necessary to see the cocaine challenge-induced increase in NAc dopamine.

Several studies have also shown differential effects of chronic drug administration on dopamine release in the shell and core subregions of the NAc. Sensitization to morphine, amphetamine, and cocaine produces an increase in dopamine release in the NAc core, but not the shell upon challenge injection after 10–14 days withdrawal (Cadoni and Di Chiara, 1999; Cadoni et al., 2000). However, amphetamine injected directly into the shell subregion produced a greater increase in local dopamine release after chronic cocaine at either early or late withdrawal compared to saline treated rats. However, in the core, amphetamine produced a reduced dopamine efflux in cocaine-treated rats at early withdrawal while there was no difference between saline and cocaine-treated rats in amphetamine-induced dopamine efflux at late withdrawal (Kalivas and Pierce, 1995). In addition, cues associated with cocaine self-administration presented non-contingently selectively increased dopamine release in the NAc core, with no effect in the shell (Ito et al., 2000). Therefore, the discrepant results of many of the studies mentioned above on the ability of chronic psychostimulant administration to sensitize NAc dopamine release could be the result of differential sampling from the core and shell subregions.

While much research on dopaminergic neuroplasticity in drug addiction has focused on cocaine and amphetamine effects in the NAc, there are some reports demonstrating similar changes in dopamine neurotransmission after repeated exposure to other drugs of abuse. First, repeated nicotine can produce behavioral sensitization and increased dopamine release when given as a challenge locally into the NAc or dorsal striatum (Shim et al., 2001) or systemically (Reid et al., 1998) in comparison to rats chronically treated with saline. Chronic MDMA also causes an increase in NAc dopamine release upon MDMA challenge after 12 days withdrawal (Kalivas et al., 1998a,b), while chronic morphine causes increased basal dopamine in the NAc and increased dopamine metabolites in the NAc after a morphine challenge (Johnson and Glick, 1993). Therefore, some dopaminergic neuroplasticity may be common to multiple drugs of abuse.

4.2. Dopamine and drug-associated cues

Drugs of abuse not only change how the brain responds to the drug over time, but addictive drugs also alter how the brain responds to cues and contexts associated with drug reward. Animals receiving chronic cocaine in a distinct context showed an enhancement of dopamine release in the NAc when a challenge dose of cocaine was given in that context compared to animals receiving cocaine in a novel context (Duvauchelle et al., 2000). On

the other hand, prefrontal cortical dopamine efflux was reduced in rats exposed to a context previously associated with multiple methamphetamine administrations, as opposed to animals receiving methamphetamine once in a context that showed an increase in PFC dopamine release when re-exposed to that context, suggesting that context-induced dopamine release in the PFC attenuates over repeated pairings with methamphetamine (Lin et al., 2007).

In addition, Ikegami et al. (2007) recently reported that limited pairings (12 times) of cues with cocaine administration resulted in increased dopamine release in the NAc when a cocaine challenge was given in the presence of the cues, while there was no alteration of dopamine release in the PFC. However, after extended pairings of cues with cocaine (40 times), a cocaine challenge in the presence of the cues enhanced PFC dopamine levels when compared to animals receiving a cocaine challenge and nonreinforced cues, while the NAc no longer showed a enhanced response to cocaine in the presence of cues. While this study may seem counter to the previously mentioned decrease in context-induced dopamine release in the PFC after repeated methamphetamine pairings (Lin et al., 2007), there was no drug challenge in the presence of the context in the methamphetamine study, suggesting that while PFC dopamine responses to the context decline, the PFC may remain hyper-responsive to drug challenge-induced dopamine release in the presence of cues. However, with extended training dopamine release in the NAc is less influenced by cocaine-associated cues.

Cues paired with morphine and nicotine have also been shown to alter dopamine release in the NAc and PFC. Three pairings of a cue with morphine or nicotine enhances dopamine release in the NAc shell and PFC in response to a morphine or nicotine challenge, while there is no potentiation of dopamine release in the core (Bassereo et al., 2007). Therefore, conditioning of cues with several drugs of abuse can enhance dopamine release on a subsequent pairing of the drug with that cue, though the amount of conditioning may differentially affect how the PFC and NAc respond to drug-associated cues. However, these results imply that it may be possible to pharmacologically reduce the dopamine response to conditioned cues to reduce the rewarding properties of drugs and the craving induced by drug cues.

4.3. Dopamine and reinstatement

While the studies reviewed above indicate that chronic drug exposure can lead to long-term changes in dopamine neurotransmission, behavioral sensitization of locomotor activity was often shown to occur regardless of whether or not dopamine release in the NAc was enhanced. Therefore, the importance of this dopaminergic neuroplasticity for the expression of addiction-related behaviors is unclear. A few studies have examined the dynamics of dopamine release during drug self-administration, extinction, and after cue or drug-primed reinstatement. Ranaldi et al. (1999) examined the amount of dopamine efflux in the NAc during D-amphetamine self-administration, extinction, and reinstatement. As expected, self-administered D-amphetamine increased dopamine release in the NAc, peaking about 10-15 minutes after infusion and dropping before the next lever press was initiated to produce another amphetamine infusion. The magnitude of dopamine increase after each infusion was slightly less in the self-administration group than in the yoked-amphetamine group. Dopamine levels were high at the start of the extinction phase and rapidly dropped despite continued lever pressing, then rapidly increased after an amphetamine-priming injection, consistent with the pharmacological effects of amphetamine. No comparison was made to a group receiving a priming injection with previous yoked-cocaine or saline experience to know if the magnitude of dopamine release in the NAc is different depending on previous experience, and if behavior is directed toward drug-seeking.

On the other hand, a similar study examined dopamine release in the NAc during extinction from cocaine self-administration (or yoked-saline), and after cocaine-primed reinstatement. Dopamine concentrations were stable during extinction and slightly higher in the yoked-saline group than the self-administration group. Upon cocaine-priming, self-administration animals robustly reinstated cocaine-seeking behavior while the amount of dopamine release was much less than yoked-saline animals receiving an acute cocaine injection (Neisewander et al., 1996). This study implies that increased dopamine release within the NAc is not associated with increased drug-seeking behavior. Likewise, McFarland et al. (2003) found that while a cocaine-priming injection increased dopamine release in the NAc, this effect occurred regardless of whether the animals reinstated lever pressing. However, in this study the amount of dopamine release was higher in the self-administration and yoked-cocaine groups than in the yoked-saline group after a cocaine-prime. The difference in the amount of dopamine release after cocaine-prime between cocaine and saline experienced groups is not clear. One possibility is that there are rostral–caudal differences in accumbal dopamine release as Neisewander et al. (1996) sampled from more rostral NAc.

In addition, dopamine neurotransmission is altered in the amygdala during extinction and cocaine-primed reinstatement after varying periods of time after cessation of cocaine self-administration. Animals returning to the self-administration chamber for extinction training after 1 day, 1 week, or 1 month of abstinence all increased lever pressing at the beginning of the extinction session, however, the animals who had 1 month of abstinence showed the greatest increase in behavior and was the only group to show an increase in dopamine release in the amygdala at the start of the extinction session. None of the groups showed a change in dopamine release during cue-primed reinstatement, but during cocaine-primed reinstatement, all groups showed increased dopamine release in the amygdala. However, the 1 month abstinent group showed the greatest increase in dopamine release and the greatest amount of reinstatement behavior (Tran-Nguyen et al., 1998). Therefore, dopamine release within the amygdala may be more important than within the NAc for mediating the magnitude of reinstatement behavior or possibly increased "craving" associated with long periods of withdrawal. However, it should be noted that activation of the amygdala is not necessary for cocaine-primed reinstatement (McFarland and Kalivas, 2001).

The necessity of dopaminergic neuroplasticity for reinstatement to other drugs of abuse has not been extensively studied. However, naloxone precipitated withdrawal in rats trained to

self-administer heroin has been shown to depress dopamine levels in the NAc while not producing reinstatement, whereas spontaneous withdrawal does initiate reinstatement behavior without reducing basal dopamine levels in the NAc (Shaham et al., 1996). In addition, alcohol associated cues can reinstate alcohol-seeking behavior, resulting in a brief increase in NAc dopamine release before cue presentation (anticipation of the session), while dopamine levels dropped after cue presentation, possibly due to the mismatch between reward expectancy (alcohol delivery) and reward outcome (no alcohol available), as rats presented previously unrewarded cues showed no drop in NAc dopamine (Katner and Weiss, 1999).

4.4. Summary

Overall, it appears that there is some dopaminergic neuroplasticity associated with chronic exposure to some drugs of abuse, but the majority of studies do not support a critical role for changes in dopamine neurotransmission for the expression of addiction-related behaviors like sensitization or reinstatement, though dopamine may encode information regarding stimuli associated with drug experience.

5. Other neurotransmitter systems

The majority of studies utilizing microdialysis to study the neuroplasticity of drug addiction have focused on glutamatergic, GABAergic, and dopaminergic neurotransmission; however, there is some evidence that chronic drug exposure alters the activity of other neurotransmitter systems that may be important to understand in order to find better treatments for addiction. For example, cocaine not only blocks the re-uptake of dopamine from the synapse but also blocks serotonin and norepinephrine re-uptake, nicotine alters the activity of cholinergic systems and endogenous opioid peptides have profound effects on reward pathways, much like the abused opiates morphine and heroin. Therefore, we will review some studies indicating neuroplasticity in other neurotransmitters systems associated with chronic drug exposure.

5.1. Serotonin and norepinephrine

Chronic cocaine administration results in an enhancement of serotonin release in response to a cocaine challenge in the NAc, VTA and dorsal raphe nucleus (DRN) 1 day after cocaine or saline administration in comparison to the saline-treated group, indicating sensitization to serotonin release in addition to dopamine after chronic cocaine treatment (Parsons and Justice, 1993). In addition, norepinephrine release in the mPFC has been shown to be important for morphine mediated reward. Acutely, morphine dose-dependently increases norepinephrine and dopamine release in the PFC and dopamine release in the NAc. Prefrontal cortical norepinephrine depletion prevents morphine induced dopamine release in the NAc and blocks morphine-induced CPP and reinstatement (Ventura et al., 2005). Therefore, norepinephrine activity in the mPFC is necessary for the expression of some aspects of morphine related reward.

5.2. Acetylcholine

Acetylcholine neurotransmission has also been shown to be altered after behavioral sensitization to amphetamine and nicotine. Striatal acetylcholine release is enhanced in response to an amphetamine challenge dose in a manner that progressively increases over the course of withdrawal akin to the progressive increase in locomotor sensitization (Bickerdike and Abercrombie, 1997). In addition, nicotine increases acetylcholine release in the PFC to a greater extent after repeated administration than after acute administration, and a nicotine challenge given after 3 days withdrawal enhanced PFC acetylcholine release over chronic saline treated controls and produced locomotor sensitization, again indicating that neuroplasticity in cholinergic systems may be important for the expression of addiction-related behaviors, particularly locomotor sensitization (Arnold et al., 2003).

Chronic opiate administration has also been shown to modulate cholinergic systems. Acutely, morphine reduces acetylcholine release in the NAc core and shell, and a morphine priming injection (at a dose that had no effect acutely), reduces NAc acetylcholine after 5 days withdrawal from repeated morphine exposure. However, after 15 and 35 days of withdrawal a morphine priming injection increases acetylcholine release in the NAc over chronic saline treated rats (Fiserova et al., 1999). Therefore, opiates may dynamically regulate cholinergic transmission, possibly correlating with presence or absence of withdrawal symptoms.

5.3. Peptides

Reward related brain structures also contain many different neuropeptides that are likely to be involved in addictive processes. Unfortunately, few studies have been able to directly measure changes in neuropeptide release in response to chronic drug exposure due to the technical difficulty in obtaining measurable quantities of peptides from microdialysis samples. A few studies have managed to use radioimmunoassays for the dectection of peptides in dialysis samples.

Acutely, a moderate dose of alcohol (Marinelli et al., 2005) and the cannabinoid agonist Δ^9-tetrahydrocannabinol (THC) (Valverde et al., 2001) increase release of the endogenous opioid enkephalin in the NAc, and chronic morphine increases enkephalin release in the periaqueductal gray (PAG) (Nieto et al., 2002), while acute ethanol, cocaine, and D-amphetamine increase the release of other endogenous opioids, the endorphins, in the NAc (Olive et al., 2001). Rats do not show an increase in enkephalin release in the VP after acute heroin administration, but do show a significant release of enkephalin after a second heroin injection (Olive and Maidment, 1998). Interestingly, mice receiving chronic morphine in the CPP paradigm showed an increase in enkephalin release in the NAc after being placed in the morphine-paired compartment and a decrease in NAc enkephalin after being placed in the saline-paired compartment. Unconditioned rats showed no change in enkephalin release when placed in either compartment (Nieto et al., 2002). These results imply that endogenous enkephalins in the NAc bidirectionally respond to the rewarding value of a particular context, possibly encoding the learned association

between the context and the reward or the drug "craving" that results from exposure to drug-associated cues.

Corticotropin Releasing Factor (CRF) is neuropeptide/hormone that is an important regulator of stress and the hypothalamic–pituitary–adrenal axis. CRF has also been implicated in the aversive effects of withdrawal from drugs of abuse. Indeed, rats self-administering cocaine during a 12 hour session tended to show decreases in dialysate concentrations of CRF in the central nucleus of the amygdala (CeA), while CRF concentrations were greatly enhanced during 12 hour withdrawal from cocaine self-administration (Richter and Weiss, 1999). Likewise, 2 week cannabinoid administration followed by antagonist induced withdrawal also produced a large increase in CRF in the CeA that corresponded to the observation of overt signs of withdrawal (Rodriguez de Fonseca et al., 1997). On the other hand, Richter et al. (1995) found that after a sensitizing regimen of cocaine or saline, a cocaine challenge produced an increase in CeA CRF only in cocaine-treated rats, suggesting a role of CRF in the expression of locomotor sensitization. Therefore, CRF may play a role in the sensitization process, while also mediating some of the negative effects of drug withdrawal. In addition, it is probable that the release of stress related peptides like CRF will depend on whether the drug is self-administered or given non-contingently.

Finally, the neuropeptide cholecystokinin (CCK), which is known to be localized in dopamine containing neurons, has also been successfully measured from microdialysis samples. Cocaine-sensitized rats have higher basal concentrations of CCK in the NAc shell and enhanced CCK release in response to a cocaine challenge in comparison to drug naïve rats (Beinfeld et al., 2002). Therefore, sensitization of CCK activity may also be important for the long-term behavioral effects of drug abuse. In the future, studies examining peptide neuroplasticity in reward related brain structures may yield new means for treating addiction that are likely to have fewer side effects than therapies that target neurotransmitter systems that have far-reaching effects on behavior like glutamate and GABA. Much research is being done to understand peptide regulation of reward related behavior; however, unfortunately, it is difficult to study peptides using microdialysis.

6. Reversal of neuroplasticity in the treatment of addiction

Microdialysis has revealed numerous changes in neurotransmission that occur after chronic exposure to drugs of abuse. However, the ultimate goal is to find better ways to treat addiction and prevent relapse. One approach for developing new treatments for addiction is to find ways of reversing the neuroplastic changes that occur over the course of chronic exposure to drugs of abuse. Microdialysis studies have revealed that there are important differences in neurotransmission depending on the particular drug used, the quantity of drug taken, and whether the subject is in early or late withdrawal. These factors should be taken under consideration when developing treatments, as a person who stopped taking drug 1 day before seeking treatment may need one type of treatment, while someone abstinent for weeks or months may need a different type of treatment to prevent relapse. Several studies have examined the ability of various treatments to reverse drug-induced neuroplasticity as determined by microdialysis, and we will review just a few of them here. In addition, we will discuss some of our recent efforts to find agents that reverse drug-induced GABAergic neuroplasticity in the VP.

6.1. Glutamate

Earlier in this review we discussed how chronic cocaine causes reduced basal glutamate in the NAc core after extended withdrawal, and that reinstatement causes a spike in glutamate in the NAc core that does not occur in controls (Baker et al., 2003; McFarland et al., 2003). Further studies have shown that it is possible to reverse this neuroplasticity by restoring activity of the cystine/glutamate exchanger. This was accomplished by administering the cysteine pro-drug *N*-acetylcysteine systemically or cystine directly into the NAc. *N*-acetylcysteine not only returns basal glutamate levels to normal, but also prevents cocaine-priming induced increases in glutamate and inhibits reinstatement behavior (Baker et al., 2003). These studies illuminate how microdialysis studies can be instrumental in understanding the neuroplasticity that occurs with chronic drug use and for finding ways to reverse this neuroplasticity to treat addiction and prevent relapse.

Table 2
Effect of peptide agonists and antagonists on extracellular GABA concentrations in the ventral pallidum

Receptor and compound investigated	Delta SNC80	DeltaNTI	Kappa U50488H	Kappa GNTI	NK 1 GR73632	NK 1 GR83224	NT 1 NT (8–13)	NT 1 SR48692
Change in VP GABA	No effect ($n=7$)	100 μM decreased** ($n=5$)	No effect ($n=5$)	No effect ($n=3$)	No effect ($n=6$)	No effect ($n=4$)	100 μM increased** ($n=7$)	No effect ($n=5$)

Table 2 summarizes the data from several studies conducted in adult male Sprague–Dawley rats where increasing doses of peptide receptor agonists or antagonists were reverse dialyzed into the ventral pallidum via an implanted microdialysis probe. The compounds listed across the top row are specific for each receptor listed (delta and kappa refer to the delta and kappa opioid receptors, NK1 refers to the Substance P neurokinin 1 receptor, and NT1 refers to the neurotensin 1 receptor), the first compound listed under each receptor type is a selective agonist, while the second compound listed is a selective antagonist. Each dose of compound was administered for 1 h and three samples were collected at each dose at 20 min intervals. The average extracellular concentration of GABA for the last two samples collected at each dose were compared to the average baseline concentration of GABA before drug was administered and repeated measures one-way ANOVA was performed to determine if there was any effect of drug treatment on the concentration of GABA. If there was a significant ($p<0.05$) effect, a Dunnett's post-hoc test was conducted to determine which dose of drug significantly altered GABA concentrations from baseline. Significant effects are listed above with the significant dose listed in its micromolar concentration. The delta opioid receptor antagonist naltrindole (NTI) significantly reduced GABA in the VP, while the neurotensin receptor agonist NT(8–13) significantly increased GABA in the VP, $^{**}p<0.01$.

In addition, Homer proteins are known to be regulated by cocaine, with acute cocaine increasing short forms of the protein (Brakeman et al., 1997) and withdrawal from repeated cocaine decreasing long forms of the protein in the NAc (Swanson et al., 2001). Restoration of long-forms of Homer proteins in the NAc by adeno-associated virus (AAV) mediated up-regulation of expression reversed the decrease in basal glutamate found after withdrawal from repeated cocaine, and blocked the increase in glutamate resulting from a cocaine challenge. Up-regulation of Homer protein expression also blocked the expression of locomotor sensitization to cocaine (Szumlinski et al., 2005). While it is unlikely that human addicts will be treated with AAV-Homer injection into the NAc, it may be possible to find other means of restoring NAc Homer expression after chronic cocaine in order to reverse the glutamatergic neuroplasticity that mediates some aspects of addiction.

In addition, there is promising pre-clinical research demonstrating that the cannabinoid receptor (CB1) antagonist AM251 can block cocaine-primed reinstatement, while not affecting sucrose reinstatment. Furthermore, while AM251 by itself increases glutamate release in the NAc, systemically administered AM251 prevents the cocaine-priming induced increase in NAc glutamate, providing a mechanism for the prevention of reinstatement behavior (Xi et al., 2006).

Finally, there is some evidence that the GABAB receptor agonist baclofen can prevent conditioned locomotion to stimuli previously paired with cocaine, and can prevent predatory odor-induced increases in glutamate in the NAc (Hotsenpiller and Wolf, 2003). This is an indication that baclofen may be able to prevent NAc responses to salient stimuli, which may be important for inhibiting drug-seeking behavior in response to conditioned cues.

6.2. GABA

Fewer studies have been aimed at reversing GABAergic neuroplasticity to treat addiction. While Xi et al. (2003) found that chronic cocaine increased basal levels of GABA in the NAc we are not aware of any studies that have determined if reversal of this increase in basal GABA can prevent cocaine-mediated behaviors, or conversely, if treatments known to block sensitization or reinstatement reverse changes in basal GABA. However, reversing the cocaine and opoid-induced decreases in GABA release in the VP has been evaluated. Tang et al. (2005) found that cocaine-primed reinstatement caused a decrease in GABA in the VP that could be blocked by intra-VP injection of the mu opioid receptor antagonist CTAP. CTAP in the VP also prevents reinstatement behavior. In addition, Caille and Parsons (2006) found that systemic administration of the cannabinoid CB1 receptor antagonist SR141716A prevented the morphine-induced decrease in VP GABA and inhibited heroin self-administration when administered directly into the NAc shell. SR141716A did not alter NAc dopamine release, or the cocaine-induced decrease in VP GABA. The CB1 agonist WIN55,212-2 also reduced GABA release in the VP in a mu opioid receptor dependent manner. Therefore, multiple drugs of abuse reduce GABA efflux in the VP, but it appears that a CB1 antagonist is only capable of inhibiting opioid-induced reward and decreases in VP GABA. Likewise, Xi and Stein (2000) found that increasing GABA concentrations in the VTA or VP blocked heroin self-administration in a GABAB receptor dependent manner.

6.3. Dopamine

The GABAergic system has also been shown to reverse some drug-induced changes in dopaminergic neurotransmission. The GABAB agonist baclofen prevents the increase in NAc shell dopamine release after acute nicotine, cocaine, or morphine (Fadda et al., 2003), and activation of GABA receptors by ethanol, lorazepam, or gamma-vinyl GABA inhibit cocaine-induced striatal increases in dopamine and cocaine-induced locomotor activity (Dewey et al., 1997).

Furthermore, the kappa opioid receptor agonist U-69593 administered in combination with cocaine for 3 days prevents the cocaine-induced increase in stereotypy and dopamine release in the NAc after 48 hour withdrawal (Heidbreder and Shippenberg, 1994). However, it is unknown if U-69593 given immediately before the challenge dose of cocaine can inhibit the increase in dopamine release and locomotor behavior.

7. Future studies

While this is not a comprehensive review of studies using microdialysis to find treatments that reverse the neuroplasticity associated with long-term drug use, there are many more studies investigating pharmacological treatments to prevent drug-induced changes in behavior, without examining changes in neurotransmission. However, microdialysis provides a fairly simple means of screening possible treatments for addiction by determining if they can reverse drug-induced changes in neurotransmission. Since we previously determined that cocaine-induced decreases in GABA in the VP are necessary for cocaine-primed reinstatement, we decided to use microdialysis to determine if other peptides co-localized in the projection from the NAc to the VP could regulate GABA output as has already been demonstrated by the endogenous enkephalins (Tang et al., 2005). Projection neurons from the NAc to the VP contain, in addition to enkephalin, neurotensin, substance P, and dynorphin (and others; Zahm et al., 1996). In addition, while Tang et al. (2005) determined that mu opioid receptors regulate GABA output from the NAc, enkephalin also binds (with higher affinity) delta opioid receptors, and the role of delta receptors in the regulation of GABA output was not determined. Therefore, we implanted drug naïve rats with microdialysis cannula aimed at the VP, reverse dialyzed increasing doses of selective agonists and antagonists for these peptide receptors, specifically the delta opioid receptor, neurotensin receptor (preferentially NTR1), the neurokinin 1 receptor (NK1), and the kappa opioid receptor. The extracellular concentration of GABA was then determined from each sample by high performance liquid chromatography (HPLC) with electrochemical (EC) detection (see methods from Tang et al., 2005). All experiments were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory*

Animals and were approved by the Medical University of South Carolina Animal Care and Use Committee.

The aim of these studies was to determine if any of the peptide receptors up-regulated GABA neurotransmission, as such a compound would be a good candidate for the reversal of cocaine-induced neuroplasticity in GABAergic neurotransmission. At the doses tested in these studies, the 8–13 peptide fragment of neurotensin (NT 8–13; neurotensin receptor agonist) was the only candidate that significantly [$F(3,27)=6.207$, $p=0.004$] increased extracellular concentrations of GABA in the VP (Table 2). The ability of neurotensin to regulate cocaine-induced neuroplasticity in GABA neurotransmission and cocaine-primed reinstatement behavior is still under investigation.

8. Conclusions

Understanding the long-term neuroplasticity in neurotransmission that occurs with chronic drug use is vital for understanding the process of addiction and can be invaluable for determining novel treatment strategies. While there are some fascinating examples of how pharmacological interventions can reverse plasticity in neurotransmission and block the expression of addiction-related behaviors, more research is needed to determine how chronic drug use alters neurocircuitry. In addition, potential therapeutics should be evaluated for both their ability to reverse the neuroplasticity that occurs with chronic drug use and to reverse addiction-related behaviors. In some instances, microdialysis can be utilized as a more efficient drug screen for therapeutics that reverse drug-induced changes in neurotransmission prior to conducting lengthy and costly behavioral experiments.

To date the majority of experimental evidence suggests that treatments that reverse the drug-induced increase in glutamate release in the NAc may have the most clinical utility. Reversing drug-induced decreases in GABA release in the VP, increases in dopamine in the amygdala, acetylcholine increases in the striatum, or CCK increases in the NAc shell are all intriguing possibilities for the treatment of addiction, and additional research is needed to determine if antagonism of these effects can block addiction-related behavior. Therefore, microdialysis is an important component of our technical armament for understanding drug-induced neuroplasticity and for finding novel treatment strategies.

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References

Arnold H, Nelson C, Sarter M, Bruno J. Sensitization of cortical acetylcholine release by repeated administration of nicotine in rats. Psychopharmacology 2003;165:346–58.

Baker D, Shen H, Kalivas P. Cystine/glutamate exchange serves as the source for extracellular glutamate: modifications by repeated cocaine administration. Amino Acids 2002;23:161–2.

Baker DA, McFarland K, Lake RW, Shen H, Tang XC, Toda S, et al. Neuroadaptations in cystine–glutamate exchange underlie cocaine relapse. Nat Neurosci 2003;6:743–9.

Bassereo V, De Luca MA, Di Chiara G. Differential impact of Pavlovian drug conditioned stimuli on in vivo dopamine transmission in the rat accumbens shell and core and in the prefrontal cortex. Psychopharmacology 2007;191:689–703.

Beinfeld M, Connolly K, Pierce R. Cocaine treatment increases extracellular cholecystokinin (CCK) in the nucleus accumbens shell of awake, freely moving rats, an effect that is enhanced in rats that are behaviorally sensitized to cocaine. J Neurochem 2002;81:1021–7.

Bell K, Duffy P, Kalivas PW. Context-specific enhancement of glutamate transmission by cocaine. Neuropsychopharmacology 2000;23:335–44.

Bickerdike MJ, Abercrombie ED. Striatal acetylcholine release correlates with behavioral sensitization in rats withdrawn from chronic amphetamine. J Pharmacol Exp Ther 1997;282:818–26.

Bossert JM, Ghitza UE, Lu L, Epstein DH, Shaham Y. Neurobiology of relapse to heroin and cocaine seeking: an update and clinical implications. Eur J Pharmacol 2005;526:36–50.

Bourdelais A, Kalivas P. Amphetamine lowers extracellular GABA concentration in the ventral pallidum. Brain Res 1990;516:132–6.

Brakeman PR, Lanahan AA, O'Brien R, Roche K, Barnes CA, Huganir RL, et al. Homer: a protein that selectively binds metabotropic glutamate receptors. Nature 1997;386:284–8.

Cadoni C, Di Chiara G. Reciprocal changes in dopamine responsiveness in the nucleus accumbens shell and core and in the dorsal caudate–putamen in rats sensitized to morphine. Neuroscience 1999;90:447–55.

Cadoni C, Solinas M, Di Chiara G. Psychostimulant sensitization: differential changes in accumbal shell and core dopamine. Eur J Pharmacol 2000;388:69–76.

Caille S, Parsons LH. Intravenous heroin self-administration decreases GABA efflux in the ventral pallidum: an in vivo microdialysis study in rats. Eur J Neurosci 2004;20:593–6.

Caille S, Parsons LH. Cannabinoid modulation of opiate reinforcement through the ventral striatopallidal pathway. Neuropsychopharmacology 2006;31:804–13.

Chefer VI, Shippenberg TS. Changes in basal and cocaine-evoked extracellular dopamine uptake and release in the rat nucleus accumbens during early abstinence from cocaine: quantitative determination under transient conditions. Neuroscience 2002;112:907–19.

Chen J, Marmur R, Paredas W, Pulles A, Gardner E. Systemic cocaine challenge after chronic cocaine treatment reveals sensitization of extracellular dopamine levels in nucleus accumbens but direct cocaine perfusion in nucleus accumbens does not: implications for the neural locus of cocaine sensitization. Life Sci 1996;58:139–46.

Cowen M, Chen F, Jarrott B, Lawrence AJ. Effects of acute ethanol on GABA release and GABAA receptor density in the rat mesolimbic system. Pharmacol Biochem Behav 1998;59:51–7.

Dewey SL, Chaurasia CS, Chen CE, Volkow ND, Clarkson FA, Porter SP, et al. GABAergic attenuation of cocaine-induced dopamine release and locomotor activity. Synapse 1997;25:393–8.

Duvauchelle C, Ikegami A, Castaneda E. Conditioned increases in behavioral activity and accumbens dopamine levels produced by intravenous cocaine. Behav Neurosci 2000;114:1156–66.

Fadda P, Scherma M, Fresu A, Collu M, Fratta W. Baclofen antagonizes nicotine-, cocaine-, and morphine-induced dopamine release in the nucleus accumbens of rat. Synapse 2003;50:1–6.

Fiserova M, Consolo S, Krsiak M. Chronic morphine induces long-lasting changes in acetylcholine release in rat nucleus accumbens core and shell: an in vivo microdialysis study. Psychopharmacology 1999;142:85–94.

Giorgetti M, Hotsenpiller G, Froestl W, Wolf ME. In vivo modulation of ventral tegmental area dopamine and glutamate efflux by local GABA(B) receptors is altered after repeated amphetamine treatment. Neuroscience 2002;109:585–95.

Hao Y, Yang JY, Guo M, Wu CF, Wu MF. Morphine decreases extracellular levels of glutamate in the anterior cingulate cortex: an in vivo microdialysis study in freely moving rats. Brain Res 2005;1040:191–6.

Heidbreder C, Shippenberg T. U-69593 prevents cocaine sensitization by normalizing basal accumbens dopamine. NeuroReport 1994;5:1797–800.

Heidbreder C, Thompson A, Shippenberg T. Role of extracellular dopamine in the initiation and long-term expression of behavioral sensitization to cocaine. J Pharmacol Exp Ther 1996;278:490–502.
Hooks M, Duffy P, Striplin C, Kalivas P. Behavioral and neurochemical sensitization following cocaine self-administration. Psychopharmacology 1994;115:265–72.
Hotsenpiller G, Wolf ME. Baclofen attenuates conditioned locomotion to cues associated with cocaine administration and stabilizes extracellular glutamate levels in rat nucleus accumbens. Neuroscience 2003;118:123–34.
Hotsenpiller G, Giorgetti M, Wolf ME. Alterations in behaviour and glutamate transmission following presentation of stimuli previously associated with cocaine exposure. Eur J Neurosci 2001;14:1843–55.
Hyman SE, Malenka RC, Nestler EJ. Neural mechanisms of addiction: the role of reward-related learning and memory. Annu Rev Neurosci 2006;29:565–98.
Ikegami A, Olsen CM, D'Souza MS, Duvauchelle CL. Experience-dependent effects of cocaine self-administration/conditioning on prefrontal and accumbens dopamine responses. Behav Neurosci 2007;121:389–400.
Ito R, Dalley JW, Howes SR, Robbins TW, Everitt BJ. Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. J Neurosci 2000;20:7489–95.
Jayaram P, Steketee JD. Effects of repeated cocaine on medial prefrontal cortical GABAB receptor modulation of neurotransmission in the mesocorticolimbic dopamine system. J Neurochem 2004;90:839–47.
Jayaram P, Steketee JD. Effects of cocaine-induced behavioural sensitization on GABA transmission within rat medial prefrontal cortex. Eur J Neurosci 2005;21:2035–9.
Johnson D, Glick S. Dopamine release and metabolism in nucleus accumbens and striatum of morphine-tolerant and nontolerant rats. Pharmacol Biochem Behav 1993;46:341–7.
Kalivas PW, Duffy P. Time course of extracellular dopamine and behavioral sensitization to cocaine. I. Dopamine axon terminals. J Neurosci 1993;13:266–75.
Kalivas P, Pierce R. Amphetamine produces sensitized increases in locomotion and extracellular dopamine preferentially in the nucleus accumbens shell of rats administered repeated cocaine. J Pharmacol Exp Ther 1995;275:1019–29.
Kalivas PW, Duffy P. Repeated cocaine administration alters extracellular glutamate in the ventral tegmental area. J Neurochem 1998;70:1497–502.
Kalivas PW, Volkow ND. The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry 2005;162:1403–13.
Kalivas P, Duffy P, White S. MDMA elicits behavioral and neurochemical sensitization in rats. Neuropsychopharmacology 1998a;18:469–79.
Kalivas P, Pierce R, Cornish J, Sorg B. A role for sensitization in craving and relapse in cocaine addiction. J Psychopharmacol 1998b;12:49–53.
Katner S, Weiss F. Ethanol-associated olfactory stimuli reinstate ethanol-seeking behavior after extinction and modify extracellular dopamine levels in the nucleus accumbens. Alcohol Clin Exp Res 1999;23:1751–60.
Kelley AE. Memory and addiction: shared neural circuitry and molecular mechanisms. Neuron 2004;44:161–79.
Kolta MG, Shreve P, De Souza V, Uretsky NJ. Time course of the development of the enhanced behavioral and biochemical responses to amphetamine aftger pretreatment with amphetamine. Neuorpharmacology 1985;24:823–9.
Lin SK, Pan WHT, Yeh PH. Prefrontal dopamine efflux during exposure to drug-associated contextual cues in rats with prior repeated methamphetamine. Brain Res Bull 2007;71:365–71.
Marinelli P, Bai L, Quirion R, Gianoulakis C. A microdialysis profile of Met-enkephalin release in the rat nucleus accumbens following alcohol administration. Alcohol Clin Exp Res 2005;29:1821–8.
McFarland K, Kalivas PW. The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 2001;21:8655–63.
McFarland K, Lapish CC, Kalivas PW. Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 2003;23:3531–7.
McFarland K, Davidge SB, Lapish CC, Kalivas PW. Limbic and motor circuitry underlying footshock-induced reinstatement of cocaine-seeking behavior. J Neurosci 2004;24:1551–60.
Meil W, Roll J, Grimm J, Lynch A, See R. Tolerance-like attenuation to contingent and noncontingent cocaine-induced elevation of extracellular dopamine in the ventral striatum following 7 days of withdrawal from chronic treatment. Psychopharmacology 1995;118:338–46.
Neisewander J, O'Dell L, Tran-Nguyen L, Castaneda E, Fuchs R. Dopamine overflow in the nucleus accumbens during extinction and reinstatement of cocaine self-administration behavior. Neuropsychopharmacology 1996;15:506–14.
Nestler EJ. Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci 2001;2:119–28.
Nieto MM, Wilson J, Cupo A, Roques BP, Noble F. Chronic morphine treatment modulates the extracellular levels of endogenous enkephalins in rat brain structures involved in opiate dependence: a microdialysis study. J Neurosci 2002;22:1034–41.
Olive M, Maidment N. Repeated heroin administration increases extracellular opioid peptide-like immunoreactivity in the globus pallidus/ventral pallidum of freely moving rats. Psychopharmacology 1998;139:251–4.
Olive MF, Koenig HN, Nannini MA, Hodge CW. Stimulation of endorphin neurotransmission in the nucleus accumbens by ethanol, cocaine, and amphetamine. J Neurosci 2001;21:184RC.
Parent M, Bush D, Rauw G, Master S, Vaccarino F, Baker G. Analysis of amino acids and catecholamines, 5-hydroxytryptamine and their metabolites in brain areas in the rat using in vivo microdialysis. Methods 2001;23:11–20.
Parsons LH, Justice JB. Serotonin and dopamine sensitization in the nucleus accumbens, ventral tegmental area, and dorsal raphe nucleus following repeated cocaine administration. J Neurochem 1993;61:1611–9.
Parsons LH, Smith AD, Justice JB. Basal extracellular dopamine is decreased in the rat nucleus accumbens during abstinence from chronic cocaine. Synapse 1991;9:60–5.
Paulson P, Robinson T. Amphetamine-induced time-dependent sensitization of dopamine neurotransmission in the dorsal and ventral striatum: a microdialysis study in behaving rats. Synapse 1995;19:56–65.
Pierce RC, Bell K, Duffy P, Kalivas PW. Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. J Neurosci 1996;16:1550–60.
Ranaldi R, Pocock D, Zereik R, Wise RA. Dopamine fluctuations in the nucleus accumbens during maintenance, extinction, and reinstatement of intravenous D-amphetamine self-administration. J Neurosci 1999;19:4102–9.
Reid M, Berger S. Evidence for sensitization of cocaine-induced nucleus accumbens glutamate release. NeuroReport 1996;7:1325–9.
Reid M, Ho L, Berger S. Behavioral and neurochemical components of nicotine sensitization following 15-day pretreatment: studies on contextual conditioning. Behav Pharmacol 1998;9:137–348.
Richter RM, Weiss F. In vivo CRF release in rat amygdala is increased during cocaine withdrawal in self-administering rats. Synapse 1999;32:254–61.
Richter RM, Pich EM, Koob GF, Weiss F. Sensitization of cocaine-stimulated increase in extracellular levels of corticotropin-releasing factor from the rat amygdala after repeated administration as determined by intracranial microdialysis. Neurosci Lett 1995;187:169–72.
Roberts DCS. Preclinical evidence for GABAB agonists as a pharmacotherapy for cocaine addiction. Physiol Behav 2005;86:18–20.
Robinson T, Jursen P, Bennett J, Bentgen K. Persistent sensitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by prior experience with (+)-amphetamine: a microdialysis study in freely moving rats. Brain Res 1988;462:211–22.
Robinson SE, Kunko PM, Smith JA, Wallace MJ, Mo Q, Maher JR. Extracellular aspartate concentration increases in nucleus accumbens after cocaine sensitization. Eur J Pharmacol 1997;319:31–6.
Rodriguez de Fonseca F, Carrera MR, Navarro M, Koob GF, Weiss F. Activation of corticotropin-releasing factor in the limbic system during cannabinoid withdrawal. Science 1997;276:2050–4.
Sanchis-Segura C, Spanagel R. Behavioral assessment of drug reinforcement and addictive features in rodents: an overview. Addict Biol 2006;11: 2–38.
Segal DS, Kuczenski R. In vivo microdialysis reveals a diminished amphetamine-induced DA response corresponding to behavioral sensitization produced by repeated amphetamine pretreatment. Brain Res 1992a;571:330–7.
Segal DS, Kuczenski R. Repeated cocaine administration induces behavioral sensitization and corresponding decreased extracellular dopamine responses in caudate and accumbens. Brain Res 1992b;577:351–5.
Shaham Y, Rajabi H, Stewart J. Relapse to heroin-seeking in rats under opioid maintenance: the effects of stress, heroin priming, and withdrawal. J Neurosci 1996;16:1957–63.
Shim I, Javaid JI, Wirtshafter D, Jang SY, Shin KH, Lee HJ, et al. Nicotine-induced behavioral sensitization is associated with extracellular dopamine release and

expression of c-*Fos* in the striatum and nucleus accumbens of the rat. Behav Brain Res 2001;121:137–47.

Sizemore G, Co C, Smith J. Ventral pallidal extracellular fluid levels of dopamine, serotonin, gamma amino butyric acid, and glutamate during cocaine self-administration in rats. Psychopharmacology 2000;150:391–8.

Swanson CJ, Baker DA, Carson D, Worley PF, Kalivas PW. Repeated cocaine administration attenuates group I metabotropic glutamate receptor-mediated glutamate release and behavioral activation: a potential role for Homer. J Neurosci 2001;21:9043–52.

Szumlinski KK, Abernathy KE, Oleson EB, Klugmann M, Lominac KD, He DY, et al. Homer isoforms differentially regulate cocaine-induced neuroplasticity. Neuropsychopharmacology 2005;31:768–77.

Szumlinski K, Diab M, Friedman R, Henze L, Lominac K, Bowers M. Accumbens neurochemical adaptations produced by binge-like alcohol consumption. Psychopharmacology 2007;190:415–31.

Tang XC, McFarland K, Cagle S, Kalivas PW. Cocaine-induced reinstatement requires endogenous stimulation of mu-opioid receptors in the ventral pallidum. J Neurosci 2005;25:4512–20.

Tran-Nguyen L, Fuchs R, Coffey G, Baker D, O'Dell L, Neisewander J. Time-dependent changes in cocaine-seeking behavior and extracellular dopamine levels in the amygdala during cocaine withdrawal. Neuropsychopharmacology 1998;19:48–59.

Valverde O, Noble F, Beslot F, Dauge V, Fournie-Zaluski MC, Roques BP. Delta9-tetrahydrocannabinol releases and facilitates the effects of endogenous enkephalins: reduction in morphine withdrawal syndrome without change in rewarding effect. Eur J Neurosci 2001;13:1816–24.

Ventura R, Alcaro A, Puglisi-Allegra S. Prefrontal cortical norepinephrine release is critical for morphine-induced reward, reinstatement and dopamine release in the nucleus accumbens. Cereb Cortex 2005;15:1877–86.

Vezina P. Amphetamine injected into the ventral tegmental area sensitizes the nucleus accumbens dopaminergic response to systemic amphetamine: an in vivo microdialysis study in the rat. Brain Res 1993;605:332–7.

Williams JM, Steketee JD. Cocaine increases medial prefrontal cortical glutamate overflow in cocaine-sensitized rats: a time course study. Eur J Neurosci 2004;20:1639–46.

Wolf M, White F, Nassar R, Brooderson R, Khansa M. Differential development of autoreceptor subsensitivity and enhanced dopamine release during amphetamine sensitization. J Pharmacol Exp Ther 1993;264:249–55.

Xi ZX, Stein EA. Increased mesolimbic GABA concentration blocks heroin self-administration in the rat. J Pharmacol Exp Ther 2000;294:613–9.

Xi ZX, Ramamoorthy S, Shen H, Lake R, Samuvel DJ, Kalivas PW. GABA transmission in the nucleus accumbens is altered after withdrawal from repeated cocaine. J Neurosci 2003;23:3498–505.

Xi ZX, Gilbert JG, Peng XQ, Pak AC, Li X, Gardner EL. Cannabinoid CB1 receptor antagonist AM251 inhibits cocaine-primed relapse in rats: role of glutamate in the nucleus accumbens. J Neurosci 2006;26:8531–6.

Xue CJ, Ng JP, Li Y, Wolf ME. Acute and repeated systemic amphetamine administration: effects on extracellular glutamate, aspartate, and serine levels in rat ventral tegmental area and nucleus accumbens. J Neurochem 1996;67:352–63.

Zahm DS, Williams E, Wohltmann C. Ventral striatopallidothalamic projection: IV. Relative involvement of neurochemically distinct subterritories in the ventral pallidum and adjacent parts of the rostroventral forebrain. J Comp Neurol 1996;364:340–62.

Zapata A, Chefer VI, Ator R, Shippenberg TS, Rocha BA. Behavioural sensitization and enhanced dopamine response in the nucleus accumbens after intravenous cocaine self-administration in mice. Eur J Neurosci 2003;17:590–6.