PERSISTENT EFFECTS OF SELF-ADMINISTERED COCAINE ON COGNITIVE FUNCTION AND BRAIN DOPAMINE RECEPTORS: THE CONSEQUENCES OF DIFFERENTIAL DRUG ACCESS

by

Lisa Anne Briand

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Doctoral Committee:

Professor Terry E. Robinson, Chair
Professor Kent C. Berridge
Professor Stephen A. Maren
Professor Martin Friedrich Sarter
Assistant Professor Geoffrey G. Murphy

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ABSTRACT

PERSISTENT EFFECTS OF SELF-ADMINISTERED COCAINE ON COGNITIVE FUNCTION AND BRAIN DOPAMINE RECEPTORS: THE EFFECTS OF DIFFERENTIAL DRUG ACCESS

By

Lisa Anne Briand

Chair: Terry E. Robinson

Drug addicts exhibit a number of cognitive deficits including deficits in frontocortical function and memory that persist long after the discontinuation of drug use. It is not clear, however, whether the cognitive deficits are a consequence of drug use, or are present prior to drug use, and thus are a potential predisposing factor for addiction. Characterizing the ability of drugs of abuse to lead to alterations in cognition and elucidating the molecular mechanisms that underlie these deficits may allow us to develop better treatment regimens for addicts. Animal models provide us with a means to examine the

effects of drugs of abuse on cognitive function and underlying dopamine systems however, the usefulness of these models is only as good as they accurately model the human condition. For decades, researchers have utilized relatively limited access self-administration procedures (1-3 hours of daily access for 1-3 weeks) to study addiction. However, more recently, researchers have started utilizing more extended access drug self-administration procedures that provide animals with either months of shorter daily self-administration sessions or longer daily access for 3-4 weeks. These extended access models have allowed experimenters to model the transition to compulsive drug use in animals and provide us with an excellent model with which to study the ability of cocaine to lead to deficits in cognitive function and alterations in ascending dopamine systems.

Specifically, the following questions were addressed: (1) How does differential access to cocaine self-administration alter sustained attention performance both during early and late withdrawal? (2) How does differential access to cocaine self-administration alter recognition memory performance following prolonged withdrawal? (3) Does cocaine self-administration lead to changes in prefrontal dopamine receptors? (4) Does cocaine self-administration lead to changes in striatal dopamine receptors? (5) Are there changes in the functional activation in mesolimbic and/or mesocortical dopamine systems following cocaine self-administration?

We report that extended access self-administration leads to deficits in both cognitive flexibility and recognition memory performance and these deficits persist during prolonged abstinence. In addition, we report that extended access cocaine self-administration leads to a persistent decrease in dopamine D2 mRNA and protein levels in the prefrontal cortex. In the striatum, we found that cocaine self-administration, both extended and limited access, leads to a persistent increase in the proportion of dopamine D2 high-affinity receptors, while having no effect on total D2 levels. Finally, we determined that extended access cocaine self-administration leads to a biphasic striatal rCBF response, with increases seen during early withdrawal and decreases seen following prolonged withdrawal. This work has important implications for the development of cocaine addiction treatment options, both for pharmacological and cognitive therapies.

Chapter I

Introduction

Drug addiction is one of our country's largest public health concerns, costing our taxpayers billions of dollars annually. Cocaine addiction is particularly troubling as there are currently no effective pharmacological treatments available for the 2.3 million cocaine users in the United States (Sofuoglu et al., 2006). The lack of effective pharmacological intervention is not for lack of attempts however. Clinical trials of over 50 compounds have been performed in the last five years alone, with abstinence rates (percentage of patients achieving 3 weeks of without drug use) ranging from 4-30%, similar to that seen with placebo (Kampman et al., 2003; Kampman et al., 2004; Dackis et al., 2005; Bisaga et al., 2006; Elkashef et al., 2006; Kampman et al., 2006). Although behavioral treatment regimens have reported slightly more successful abstinence rates of 30-60%, there is still a significant amount of the cocaine dependent population that is resistant to treatment (Rawson et al., 2002; Epstein et al., 2003; McKay et al., 2005; Rawson et al., 2006). Along with issues with efficacy of treatment, patient retention rates are also very low with drop out rates reaching as high as 60-80% (Carroll et al., 1991b; Carroll et al., 1994b; Sayre et al., 2002; Malcolm et al., 2005). As early drop out rates are associated with continued cocaine abuse, effective

treatments need to address both issues of efficacy and retention to ensure success (Simpson et al., 1999).

While overall cocaine dependence treatment success rates are fairly low, differential attrition and treatment success has been a focus of the clinical literature for the past decade (Aharonovich et al., 2003; Sofuoglu et al., 2006; Easton et al., 2007). In particular, deficits in cognitive function are predictive of poor treatment outcomes and low retention rates (Aharonovich et al., 2003; Aharonovich et al., 2006). As cocaine addicts have been shown to demonstrate deficits in a number of cognitive domains including attention, memory, problem solving, and decision-making (Ardila et al., 1991; Berry et al., 1993; Bolla et al., 1999; Bolla et al., 2000; Ainge et al., 2006), developing effective treatments for this population of cocaine addicts could play a large role in overall cocaine abuse relapse rates.

Characterizing these cognitive deficits and elucidating the molecular mechanisms that underlie them may allow us to develop better treatment regimens for addicts. Studies in human addicts do not allow us to determine whether these behavioral and neurobiological deficits precede drug use, and thus contribute to the propensity for addiction, or are a consequence of drug use. Although it is difficult to address this issue in humans, studies with non-human animals allow one to determine whether exposure to drugs of abuse are at least capable of producing changes in brain and behavior comparable to those seen in addicts.

Although the use of animal models can provide us with a more tightly controlled environment without the added complication of varied genetic backgrounds, it is also important that these studies attempt to mimic the human drug use patterns as closely as possible. The majority of studies on the effects of cocaine administration on behavioral and neurobiological outcomes have utilized experimenter administered cocaine or traditional self-administration procedures that result in stable, controlled drug intake. Although these models have been instrumental in studying the underlying neural systems that mediate the reinforcing and incentive motivational effects of drugs of abuse, they have not provided us with great insight into why some individuals undergo the transition to compulsive drug use and others are able to maintain controlled use. Recently, the use of more extended access drug self-administration procedures has allowed experimenters to model this transition to compulsive drug seeking (Ahmed and Koob, 1998; Paterson and Markou, 2003; Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Ferrario et al., 2005). Thus, the purpose of the studies presented in this dissertation is to explore the effects of extended access cocaine self-administration on cognitive function and the underlying dopamine systems that play a role in cognition in a rodent model.

This introduction will begin with a characterization of cognitive function in the human addict. The debate of whether these deficits precede addiction or are a result of drug use will be discussed. This will be followed with a discussion of the

deficits in cognitive function that have been demonstrated following chronic psychostimlant administration. Next the anatomy and function of the mesocorticolimbic dopamine system, focusing on the prefrontal cortex and striatum, will be described and neurobiological changes in the mesocorticolimbic dopamine system resulting from drug use in both human addicts and animal models will be presented. The introduction will conclude with a discussion of the experiments in the current dissertation and how they fit into the current literature.

Cognitive Deficits in Human Cocaine Addicts

Following the dramatic rise in cocaine use in the 1980s, researchers began to examine the effects of chronic cocaine on neurobehavioral functions, including alterations in cognition. The early literature on the effects of cocaine abuse on cognitive function was inconclusive, with some studies showing severe impairments (Ardila et al., 1991; Strickland et al., 1993) and others showing little to no effect (Bernal et al., 1994; Selby and Azrin, 1998). In fact, two studies actually found that cocaine use was associated with enhanced performance on some tasks (Manschreck et al., 1990; Hoff et al., 1996). The inconsistencies in these studies may be due to differences in the amount and frequency of drug use, differences in duration of abstinence, small sample sizes, lack of control groups, and/or lack of control for comorbid psychiatric conditions.

Overall, the literature suggests that the deficits seen in human addicts are fairly specific in nature rather than a more general deficit in intelligence or ability. The deficits described in the literature primarily fall into two general cognitive domains: so-called “executive functions”, including attention (Meek et al., 1989; Ardila et al., 1991; Beatty et al., 1995; Rosselli and Ardila, 1996), decision making (Bolla et al., 1998; Rogers et al., 1999; Grant et al., 2000; Bechara et al., 2001; Rogers and Robbins, 2001; Fishbein et al., 2005), and cognitive flexibility (Grant et al., 2000; Kelley et al., 2005), and memory, including working memory (Beatty et al., 1995; Verdejo-Garcia and Perez-Garcia, 2007), verbal memory (O'Malley et al., 1992; Mittenberg and Motta, 1993; van Gorp et al., 1999), and visuospatial memory (Meek et al., 1989; Berry et al., 1993; Hoff et al., 1996).

Executive Function Deficits

The initial study suggesting that human addicts show executive function deficits was done by Meek and colleagues (1989) on patients entering an inpatient treatment program. At admission, deficits were seen in more than two thirds of the patients, specifically in the executive domains of attention, abstraction, and working memory. While partial recovery was seen over the course of treatment, deficits were still apparent after the two-week abstinence period, particularly in the area of working memory. While this study examined polydrug users, Ardila, and colleagues (1991) focused on crack cocaine users and found specific deficits (compared to predetermined norms) on attention subtests. These deficits were correlated with the amount of drug use, suggesting a direct relationship between

cognitive impairment and cocaine use. This study was followed by a larger scale study comparing cocaine-dependent, polydrug-dependent, and control subjects and found that both cocaine- and polydrug-dependent groups exhibited attentional impairtments compared to the controls (Rosselli and Ardila, 1996).

Although previous work had suggest a correlation between drug intake and extent of cognitive impairment, Bolla and colleagues (1999) performed an elegant study that not only examined the relationship between drug intake and cognition but also controlled for duration of abstinence. In this study, patients' use was confirmed by positive drug screen immediately prior to admittance and cognitive testing occurred following 4 weeks of abstinence. Cocaine users showed dose dependent decrements in performance on a battery of tests of executive function [including the Wisconsin Card Sort Task (WCST) and the Interference version of the Stroop Task] with heavy users showing persistent deficits following a month of withdrawal.

Along with these more traditional neuropsychological tests, such as the WCST, recent work has begun to examine cocaine addicts' performance on decision-making or risk taking tasks, such as the Iowa Gambling Task (Grant et al., 2000; Bechara et al., 2001). These studies have shown that cocaine addicts make a higher proportion of disadvantageous choices and make significantly more perseverative errors than controls in both early stages of abstinence (Grant et al., 2000) and following longer withdrawal periods (Bechara et al., 2001).

Although the studies discussed above represent only a fraction of the work done on cognitive deficits in cocaine addicts, they clearly demonstrate that addicts exhibit deficits in a variety of types of executive function, and characterizing these deficits may be critical for the development of effective treatment strategies.

Memory Deficits

The early studies showing deficits on executive function also demonstrated deficits in memory function in cocaine addicts (Meek et al., 1989; Ardila et al., 1991). In addition, Manschreck and colleagues (1990) found that crack cocaine addicts demonstrate specific deficits on tests of delayed recall compared to controls. While this study did not control for duration of abstinence, work by Berry and colleagues (1993) examined cocaine users during both acute withdrawal (within 72 hours of drug use), and following a two week withdrawal period. At both withdrawal time points cocaine addicts exhibited decreased verbal memory relative to controls. The studies mentioned above in conjunction with many others (O'Malley et al., 1992; Mittenberg and Motta, 1993; Kelley et al., 2005), demonstrate a well-documented deficit in verbal memory in cocaine addicts.

Although many studies have examined verbal memory function in cocaine addicts, only a few studies have examined other memory domains. For example,

Hoff and colleagues (1996) have demonstrated deficits in spatial memory in crack cocaine users following varied periods of abstinence (mean=24 days). Researchers have also demonstrated deficits in visual memory (Gillen et al., 1998) during early withdrawal from cocaine as well as specific deficits in nonverbal declarative memory following longer periods of abstinence (van Gorp et al., 1999). Although a thorough study examining a variety of memory domains following different durations of abstinence is needed, it is clear that human addicts demonstrate a variety of memory deficits that seem to persist following prolonged withdrawal.

Taken together, the evidence for deficits in both memory and executive function suggest that cocaine addicts present with a variety of specific cognitive deficits that are relevant to their ability to participate in treatment programs (Aharonovich et al., 2003). A more comprehensive understanding of these deficits could help guide more appropriate treatment programs designed based upon a patients specific cognitive profile.

Cause vs. Consequence

Although it is clear from the human literature that cocaine addicts exhibit cognitive deficits, it is not clear whether these behavioral deficits precede drug use, and thus contribute to the propensity for addiction, or are a consequence of drug use. Dissociating the risk factors for addiction and the consequences of

drug use is important for the development of targeted enrichment programs for those individuals that are particularly vulnerable to addiction as well as investigating the underlying neurobiology of addiction-related neuroadaptations.

Recently, there have been a number of risk factors that have been characterized. Most frequently researchers and clinicians have focused on psychosocial and genetic risk factors, commonly examining children of substance abusers (Clark et al., 1998; Kirisci et al., 2005). Numerous studies have shown that children who have a parent, particularly a father, with substance abuse disorder (SUD) are at a higher risk to develop drug problems (Tarter, 1988; Tarter et al., 1998; Blackson et al., 1999; Clark et al., 2005; Kirisci et al., 2005). Despite the evidence that substance abuse runs in families (Merikangas et al., 1998), the genetic contribution of this risk factor is not well established. Early work done by Vaillant (1966) examining narcotic addicts treated in the early 1950s found that 24% of the addicts had a relative that was also suffering from substance addiction. In an analysis of monozygotic (MZ) twins reared apart, Grove and colleagues (1990) found that although there was significant heritability of substance abuse, there was only a 36% concordance rate in twins. While Gynther et al. (1995) found no significant heritability of substance abuse when comparing MZ to dizygotic (DZ) twins, Tsuang et al. (1996) did find a significantly higher concordance in MZ twins demonstrating a concordance rate of 63%. It is clear that although there may be a genetic component to addiction, the disorder can only be partially attributed to heritability.

Twin studies also allow researchers to examine the effects of shared environment on addiction outcome. Van den Bree and colleagues (1998) found that shared environmental influences accounted for 33% of the variance in male cocaine addicts and up to 93% of the variance in female addicts. However, in the twin study mentioned above, Tsuang et al. (1996) found that shared environment accounted for only 28% of the variance in their sample of male twin pairs.

While twin studies demonstrate that although both genetics and environment play a role in the development of addiction, the identification of more specific risk factors, such as temperament and cognitive function, may also be useful. Aggression is very prevalent in children that are at high risk for substance abuse (Tarter, 1988; Giancola et al., 1996a; Giancola et al., 1996b), and longitudinal studies have demonstrated that aggressive behavior in childhood is a precursor to adolescent drug use and delinquency (Brook et al., 1992; Brook et al., 1995). Hyperactivity, impulsivity and "sensation-seeking" have also been shown to be predictors of substance abuse (Galizio et al., 1983; Aytaclar et al., 1999; Moeller et al., 2001; Wagner, 2001). Along with these personality traits, studies have examined cognitive function in high-risk individuals and found that children in high-risk groups have lower scores on tests of executive function (Giancola et al., 1996a; Giancola et al., 1996b).

While this provides evidence that some alterations in cognitive function may precede drug use, there is also research that suggests that drug abuse can lead to decreased cognitive function. A longitudinal study by Brook and colleagues (2002) demonstrated that adolescent drug use led to a decrease in cognitive function when individuals were tested in their twenties. Along with this longitudinal study, studies of adult addicts have demonstrated a correlation between the severity of cognitive deficits and the amount of cocaine use, indicating that more drug use leads to more severe deficits (Bolla et al., 1999; Bolla et al., 2000).

Together these studies indicate that cognitive deficits may not only represent a risk factor predisposing individuals to become addicts but also a consequence of drug abuse. Further work is needed to determine the extent of the deficits resulting from drug abuse as opposed to those that preexist use.

Cognitive Function in Preclinical Models of Stimulant Abuse

Although longitudinal studies can provide insight into how drugs of abuse alter cognitive function, they are often prohibitively expensive and time consuming. Preclinical models provide researchers with a more efficient means to examine the effects of drugs of abuse on cognitive function while controlling for preexisting differences.

Although very little work has been done examining cognitive function following cocaine administration in non-human primate models, Jentsch and colleagues (2002) have demonstrated deficits in reversal learning following both acute and repeated experimenter administered cocaine in Vervet monkeys. In conjunction with this study, more extensive experimental evidence in rodents suggests that cognitive deficits can be a consequence of stimulant administration rather than solely a risk factor. Both experimenter-administered (Crider et al., 1982; Kondrad and Burk, 2004; Schoenbaum et al., 2004; Belcher et al., 2005a, 2006; Burke et al., 2006) and self-administered (Dalley et al., 2005a; Dalley et al., 2005b; Kantak et al., 2005) psychostimulants can produce transient decreases in attention performance, recognition memory, and fear extinction. In a study of selective attention, Crider and colleagues (1982) found that an amphetamine binge administration procedure led to a decrease in rats' ability to ignore irrelevant stimuli. Similarly, Kondrad and Burk (2004) found that amphetamine challenge following an escalating dose regimen of amphetamine led to an increase in false alarms in a signal detection task. In addition, Fletcher and colleagues (2005; 2007) demonstrated that repeated amphetamine administration leads to deficits on both an attentional set-shifting task and 5-choice serial reaction time task (5-CSRTT).

Similar to deficits seen in human addicts, experimenter-administered cocaine has produced persistent deficits in reversal learning tasks mediated by the orbital frontal cortex in both rats (Schoenbaum et al., 2004) and monkeys (Jentsch et

al., 2002) as well as deficits in fear extinction learning (Burke et al., 2006). Chronic experimenter-administered methamphetamine has also been shown to lead to persistent deficits in recognition memory (Belcher et al., 2005b; Belcher et al., 2006). However, despite the overwhelming evidence that self-administration of psychostimulant drugs leads to cognitive deficits within the first few days after the discontinuation of drug treatment, these deficits do not persist unless animals are given a drug challenge (Dalley et al., 2005a; Dalley et al., 2005b; Kantak et al., 2005). Dalley and colleagues (2005b) examined the effects of amphetamine self-administration on a 5-CSRTT and found a decrease in performance accuracy within the first 3-5 days following amphetamine self-administration. Similar results were found in a later study examining both cocaine and heroin self-administration (Dalley et al., 2005a). It is important to note that following both amphetamine and cocaine self-administration the animals in these studies exhibited significant performance deficits (in the form of increased omissions and an increase in response latency) and therefore the decrease in attentional accuracy may be primarily due to a generalized acute withdrawal syndrome rather than a specific attention deficit such as seen in human addicts.

So, why is it that preclinical models seem to be unable to mimick the deficits seen in human addicts using self-administration paradigms? It is possible that the persistent cognitive deficits seen in cocaine addicts are not in fact a consequence of drug use. Alternatively, the cocaine self-administration procedures used in the existing studies may not accurately model human

addiction. Thus far, self-administration studies have largely used limited access procedures (Dalley et al., 2005a; Dalley et al., 2005b; Kantak et al., 2005) that may not produce some of the key changes in brain and behavior that characterize addiction.

Extended Access Cocaine Self-Administration

Since their inception in the 1960s (Weeks, 1962; Thompson and Schuster, 1964; Pickens and Harris, 1968; Pickens and Thompson, 1968), intravenous drug self-administration procedures have been the gold standard for examining the reinforcing properties of drugs of abuse and their neurobiological consequences, as well as assessing factors influencing potential vulnerability to addiction. Animals have been shown to self-administer almost all drugs with human abuse potential (Weeks, 1962; Pickens and Harris, 1968; Pickens and Thompson, 1968; Lang et al., 1977; Takahashi and Singer, 1979; Carroll et al., 1981), and while the majority of these studies have been done in rodents, a variety of species have been studied using this paradigm (Thompson and Schuster, 1964; Pickens and Harris, 1968; Deneau et al., 1969; Risner, 1975; Risner and Jones, 1975; Balster et al., 1976; Criswell and Ridings, 1983). As some of the earlier studies examining uncontrolled access to self-administration demonstrated that animals continue to take drugs until they overdose (Bozarth and Wise, 1985), the majority of the studies have utilized limited access self-administration procedures that result in fairly stable drug intake after acquisition.

As human addicts undergo a transition to uncontrolled drug use rather than maintaining stable intake, more recently, research has focused on developing animal models that also lead to a loss of controlled drug intake. While there has been some effort to mimic human use patterns using experimenter administered drug procedures such as the work done by Mary Jeanne Kreek and colleagues utilizing chronic binge cocaine administration (Unterwald et al., 1994; Tsukada et al., 1996; Unterwald et al., 1996; Schlussman et al., 1998; Zhang et al., 2003), the use of extended access self-administration procedures have been most successful in modeling the transition to uncontrolled drug use. Wolffgramm and Heyne (1995) examined animals given access to oral self-administration of varying concentrations of ethanol over the course of 30-50 weeks, followed by a forced abstinence period (4-9 months) and challenge exposure period. This procedure resulted in groups of animals that were characterized as either ethanol-addicted or controlled drinkers. Comparable results have been found with oral amphetamine self-administration (Heyne and Wolffgramm, 1998).

Along a similar vein, Deroche-Gamonet et al. (2004) examined rats given daily cocaine intravenous self-administration access (40 min on + 15 min off x 3 per day) over the course of 3 months and divided them into high (HRein) and low (LRein) reinstatement groups. After examining these animals' drug taking behaviors it was evident that the HRein animals exhibited an increase in drug seeking during the drug free periods, an increase in drug seeking after punishment, and an increase in progressive ratio breakpoint responding that was

not seen in the LRein animals. These two studies elegantly demonstrate that when given long enough access certain animals that presumably exhibit a specific vulnerability profile develop addiction-like behaviors.

Although the above procedures provide us with a model to examine the addicted animals' brain and behavior, they rely on individual differences between animals and therefore do not control for preexisting differences that may lead to addictive vulnerability. Extended access procedures that compare animals given longer daily access to animals given shorter daily access do not rely on individual differences but rather experimenter manipulation to create "addicted" animals. Although this differential access procedure does not control for individual differences, it does not use them are the primary means to differentiate addicted animals from drug resistant ones. Utilizing a 6-hour access cocaine self-administration procedure, Ahmed and Koob (1998) demonstrated that over the course of 12-22 daily test sessions rats escalated their intake both within the first hour and over the course of the total session as compared with animals given 1-hour daily access. This escalation of intake is accompanied by an upward shift in the dose-response curve for cocaine and facilitated escalation following prolonged abstinence. The pattern of intake in animals given extended access is different than those given limited access as the intake of the long access animals is significantly higher during the first hour as compared with the 5 additional hours, indicating the animals are drug "loading" in the beginning of the session. This increase in intake during the first hour will continue for up to 2

months in long access animals even if they are returned to a limited access schedule (Ahmed and Koob, 1999).

In addition to producing uncontrolled drug intake, this extended access self-administration procedure leads to an increase in intracranial self-stimulation (ICSS) reward thresholds (Ahmed et al., 2002), similar to the effect of opiate withdrawal (Schaefer and Michael, 1986; Kenny et al., 2006), indicating that perhaps the extended access animals may exhibit a unique withdrawal syndrome that is not seen following limited access self-administration. While this decrease in ICSS reward threshold might be seen as a decrease in the sensitivity to reward or an unwillingness to work for smaller rewards, this is inconsistent with the upward shift in the cocaine dose-response curve see in the initial Ahmed and Koob study (Ahmed and Koob, 1998) as well as their finding that extended access cocaine leads to an increase in the animals' sensitivity to a dopamine antagonist (Ahmed and Koob, 2004).

Following Koob and colleagues demonstration of this escalation phenomena utilizing not only cocaine, but heroin and methamphetamine extended access self-administration as well (Ahmed et al., 2000; Kitamura et al., 2006), numerous other labs have utilized similar protocols to further characterize the effects of extended access self-administration on behavior. Consistent with extended access cocaine self-administration producing an sensitization of reward processing, Patterson and Markou (2003) demonstrated that extended access

animals display a higher breakpoint, particularly at the lower doses tested, compared to 1-hour limited access animals. In addition, Mantsch et al. (2004) and Knackstedt and Kalivas (2007) found that extended access animals exhibit an increase in drug-primed reinstatement as compared to limited access animals. Work in our lab has also demonstrated that animals given extended access to cocaine self-administration exhibit an increase in behavioral and structural plasticity associated with drug addiction (Ferrario et al., 2005).

In further support of the use of the extended access model, Vanderschuren and Everitt (2004) examined the ability of a extended access cocaine self-administration paradigm to lead to compulsive drug seeking in the face of adverse consequences. Following limited cocaine self-administration exposure, the presentation of an aversive CS (a tone previously paired with shock) led to a conditioned suppression of drug seeking, while after extended access self-administration experience animals do not exhibit conditioned suppression of responding. In addition, Pelloux and colleagues (2007) have shown that following extended access cocaine self-administration, animals will continue drug-seeking even when seeking responses intermittently lead to punishment (mild foot-shock).

Together these studies clearly demonstrate that extended access cocaine self-administration leads to a number of key diagnostic criteria including (but not limited to), escalation of intake, increased motivation for drug, drug-seeking

behavior when the drug is known to be unavailable, and drug-seeking despite adverse consequences. Therefore the use of similar procedures may yield cognitive deficits similar to those seen in human addicts, not yet found utilizing standard self-administration procedures.

Ascending Dopamine Projection Systems

The development of animal models to dissociate the effects of stimulant drugs on cognitive function from the effects of preexisting deficits that may lead to addiction also provide us with a means to examine the neurobiological systems involved in the dissociation. In the following section, I will describe the functional anatomy of the mesocorticolimbic dopamine system and how it is related to both addiction and cognitive function.

The mesocorticolimbic dopamine system is often divided into the mesocortical system, consisting of ascending dopamine projections from the ventral tegmental area (VTA) to the prefrontal cortex (PFC), and the mesolimbic system, consisting of ascending dopamine projections from the VTA to the nucleus accumbens (NAc) (see Figure 1).

Prefrontal Cortex

The traditional definition of prefrontal cortex consists of cortical regions near the frontal poles that receive input from mediodorsal thalamus. The PFC is made up

of interconnected subdivisions that send, receive and integrate input from nearly all sensory and motor cortical regions as well as subcortical regions. Individual subdivisions have been determined to be involved in distinct domains of working memory and attention with individualized processing and storage mechanisms (Goldman-Rakic, 1996). Visuospatial memory, as required for recalling your location in space, following maps, and performing delayed response tasks, is primarily localized to the dorsolateral prefrontal cortex (Fuster and Alexander, 1971; Kubota and Niki, 1971; Friedman and Goldman-Rakic, 1988; Funahashi et al., 1989; Verin et al., 1993; Friedman and Goldman-Rakic, 1994), while facial and object recognition memory is dependent upon the inferior prefrontal cortex (Wilson et al., 1993; Courtney et al., 1996; Adcock et al., 2000). In contrast, verbal working memory, including semanic encoding and retrieval, is localized to more inferior, anterior, and/or insular prefrontal regions (Raichle et al., 1994; Demb et al., 1995; Fiez et al., 1996).

Not all of these working memory domains are dependent on dopamine however. Research on dopamine in the PFC has determined that its primary role is in tasks that are localized to the dorsolateral prefrontal cortex in humans and primates (Sawaguchi and Goldman-Rakic, 1994; Muller et al., 1998; Winterer and Weinberger, 2004). It is important to note that the rodent PFC does not contain projections from the mediodorsal thalamus to dorsolateral regions of the cortex, therefore, functional and anatomical studies have suggested that the medial and

orbital frontal cortices serve homologous functions in these animals (Kolb, 1990; Birrell and Brown, 2000; Dalley et al., 2004).

The first evidence that dopamine might be the key neurotransmitter involved in visuospatial working memory tasks was presented by Brozoski and colleagues (1979). They demonstrated that prefrontal dopamine depletion in monkeys led to deficits on a delayed-response task equivalent to those seen after PFC lesions. Since this seminal work, much work has been done to elucidate the role of dopamine in PFC mediated cognitive functions. Along with additional studies demonstrating the role of prefrontal dopamine on delayed alteration performance (Simon et al., 1980; Stam et al., 1989), other cognitive functions relying on prefrontal dopamine have been identified. Work from the laboratory of John Salamone has determined that dopamine depletion in the mPFC of rats leads to a decrease in both active avoidance and performance on a differential reinforcement of low rates (DRL) task aimed at examining inhibitory control (Sokolowski et al., 1994; Sokolowski and Salamone, 1994). Another measure of inhibitory learning, prepulse inhibition (PPI), is also impaired following prefrontal dopamine depletion (Bubser and Koch, 1994).

Along with dopamine depletion studies, microdialysis studies have also provided evidence for a role of prefrontal dopamine in cognition. During the performance of a delayed-alternation task, monkeys exhibit an increase in dopamine in the PFC that is not seen during the performance of a sensory control task (Watanabe

et al., 1997). Similar results have been seen in rodents performing both discrimination learning (Yamamuro et al., 1994) and set shifting tasks (Stefani and Moghaddam, 2006).

In examining dopamine function in general and, in particular, the effects of dopamine on the PFC, it is important to take into account the different dopamine receptor subtypes. Dopamine receptors are divided into two main subtypes, D1-like and D2-like, and although both are localized within the PFC, their cellular distribution differs. D1 type receptors are localized at the postsynaptic terminals of pyramidal cells whereas D2 receptors are primarily located on GABAergic interneurons, with a much smaller proportion of these receptors localized on pyramidal neurons (Khan et al., 1998). These differences in localization, along with the contrasting effects on downstream signaling cascades (Stoof and Kebabian, 1984), contribute to the functional differences between these two receptor subtypes.

Disruption of the D1 receptor system via antagonist administration both systemically and directly into the monkey dlPFC disrupts performance on delayed alternation tasks (Sawaguchi and Goldman-Rakic, 1991, 1994). Similar results have been seen with prelimbic administration of D1 antgonists in the rat (Seamans et al., 1998). On the 5-CSRTT a D1 receptor antagonist has a differential effect in high vs. low performing animals, decreasing performance in high accuracy animals and increasing performance in low accuracy animals

(Granon et al., 2000). A similar biphasic response to a D1 antagonist was seen in rodents tested on a delayed version of the radial arm maze (Floresco and Phillips, 2001). This would suggest that there is an inverted U-shaped relationship between D1 activation and performance. Electrophysiological studies have determined that the primary role of D1 receptors seems to be to enhance and stabilize task-related activity in PFC neurons (Sawaguchi et al., 1988; Williams and Goldman-Rakic, 1995).

In contrast to the effects of prefrontal D1 receptors, prefrontal D2 receptors do not seem to be directly involved in delayed alteration performance (Sawaguchi and Goldman-Rakic, 1991, 1994). However, there is evidence that these receptors play a central role in working memory. Experiments in both humans and monkeys have shown that systemic D2 receptor antagonists lead to deficits in spatial working memory (Mehta et al., 1999; Von Huben et al., 2006). While these studies do not isolate the effect to D2 receptors in the PFC, work in mice has demonstrated that D2 antagonist injections directly into the PFC disrupt spatial working memory performance (Rinaldi et al., 2007).

Along with their role in spatial working memory, prefrontal D2 receptors are also critically involved in other executive functions, such as behavioral flexibility. Behavioral fleixibility, or the ability to alter behavior in response to changing environmental conditions, is disrupted by lesions of the PFC in humans (Owen et al., 1991; Owen et al., 1993), monkeys (Dias et al., 1996), and rodents (Birrell

and Brown, 2000). At the receptor level, local PFC antagonism of D2 receptors, but not D1 receptors, leads to an inability to change discrimination strategies (Floresco et al., 2006). Similar results have also been found following haloperidol administration in healthy human subjects (Tost et al., 2006).

Taken together, these results clearly demonstrate a specific role for prefrontal dopamine systems in cognitive function, dissociable into D1 and D2 components. Later on in the introduction, as I present data on how drugs of abuse alter the structure and function of this brain region, it will become even more clear how an accurate understanding of both normal prefrontal dopamine function and how drugs of abuse alter this system could be critical to understanding relapse and addiction.

Nucleus Accumbens

The nucleus accumbens (NAc) is part of a group of nuclei called the basal ganglia, which subserve a wide variety of behavioral functions. Driven, in part, by hypotheses by Wise and colleagues (Wise, 1984; Wise and Bozarth, 1985; Wise and Rompre, 1989) among others (Robinson and Becker, 1982; Dackis and Gold, 1985; Broderick, 1986; Koob, 1992), the primary focus of work on the nucleus accumbens in the last 30 years has been on the dopamine projections to this region from the VTA and their involvement in drug reward. Under normal conditions, the mesolimbic dopamine system is believed to mediate the effects of natural rewards such as food, water, and sex (Hernandez and Hoebel, 1988; for

review, see Kelley, 1999). In the case of addiction, drugs of abuse seem to "hijack" this system and push it into overdrive (Wise and Rompre, 1989; Kelley and Berridge, 2002).

The exact role of dopamine in reward is not clear however. For decades, dopamine was thought to represent the hedonic value of rewards. For example, dopamine depletion in the NAc by 6-hydroxydopamine (6-OHDA) decreases cocaine self-administration (Roberts et al., 1977; Pettit et al., 1984; Caine et al., 1993). Dopamine antagonists administered directly into the NAc have also been shown to increase cocaine self-administration behavior, presumably indicating a compensatory mechanism (Robledo et al., 1992; Caine et al., 1995). Furthermore, acute cocaine, amphetamine, nicotine, opiate, and ethanol administration (either experimenter-administered or self-administered) all lead to an increase in extracellular dopamine in the NAc (Di Chiara and Imperato, 1988; Hurd et al., 1990; Wise et al., 1995; Bradberry et al., 2000). These studies supported the hedonia hypothesis, or the idea that dopamine represents the hedonic value of a stimulus (Wise and Bozarth, 1985), however there is ample evidence to refute this theory.

Contrary to the hedonia hypothesis, hyperdopaminergic DAT knockdown mice do not exhibit increased "liking" reactions in response to food reward (Pecina et al., 2003). In addition, in a recent paper by Hnasko and colleagues (2005), dopamine-deficient (DD) mice were found to exhibit normal morphine conditioned

place preference. These studies in conjunction with both additional animal studies (Berridge et al., 1989; Berridge and Robinson, 1998) and human studies (Leyton et al., 2002; Leyton et al., 2005; Evans et al., 2006) clearly demonstrate that dopamine does not represent the hedonic impact of rewards (for review see Berridge, 2007).

Based on evidence from electrophysiological examination of ventral tegmental dopamine neurons, Wolframm Shultz and colleagues developed a learning hypothesis of dopamine action (Schultz, 1998; Schultz and Dickinson, 2000; Schultz, 2001; Waelti et al., 2001). This theory is based on evidence that dopaminergic neurons seem to obey prediction error models, such that dopamine neurons only respond to rewarding stimuli when they are unexpected. According to learning theories of addiction, drugs lead to excessive dopamine, which in turn leads to "overlearning" of drug seeking behaviors (Everitt et al., 2001; Montague et al., 2004; Redish, 2004). However, dopamine is not necessary for reward related learning. Siobhan Robinson and colleagues (2005) determined that dopamine was not necessary to learn goal-directed behavior in the form of an appetitive T-maze task however it is necessary for reward seeking. In support of this, hyperdopaminergic DAT knockdown mice do not exhibit enhanced Pavlovian or operant reward-related learning (Cagniard et al., 2006a).

While dopamine seems to not consistently code for hedonic reactions or reward-related learning, it is consistently related with drug "wanting' or seeking.

Hyperdopaminergic mice, while not exhibiting an increase in “liking” or learning, do exhibit an increase in drug seeking or drug “wanting” (Pecina et al., 2003; Cagniard et al., 2006a; Cagniard et al., 2006b) supporting Robinson & Berridge’s theory that dopamine mediates incentive salience attribution, leading to an increase in motivational drive, or “wanting” (Robinson and Berridge, 1993; Berridge and Robinson, 1998; Robinson and Berridge, 2000; Robinson and Berridge, 2003; Berridge, 2007).

While there is converging evidence that NAc dopamine is involved in incentive arousal, the specific roles of the two subdivisions of the NAc, the core and the shell, are unclear. Although this is partially do the difficultly in selectively manipulating these two areas, there are studies that have attempted to parse the two apart. While there is some evidence to suggest that it is the NAc shell that is more critically involved in the incentive salience attribution rather than the core (Parkinson et al., 1999; Wyvell and Berridge, 2000; Corbit et al., 2001), there is also ample evidence that the opposite is true (Alderson et al., 2001; Hall et al., 2001; Hutcheson et al., 2001). In an elegant review of the literature, Di Chiara (2002) hypothesizes that DA in the NAc shell serves to facilitate Pavlovian learning by promoting the acquisition of incentive stimuli that predict drug and as NAc shell DA release does not habituate after repeated stimulation this can lead to excessive incentive attribution in addiction. Dopamine release in the NAc core however exhibits sensitization and although the exact role of this sensitized

response is not entirely clear it may more critically involved in instrumental responding (Di Chiara, 2002).

Just as taking into account the role of the different subregions of the NAc is important in understanding the function of mesolimbic dopamine, it is also necessary to consider the two dopamine receptor subtypes. In the NAc, as well as other parts of the striatum, there are distinct populations of medium spiny neurons (MSNs) that express D2 receptors (co-localized with enkephalin) projecting to the ventral pallidum and D1 receptors (co-localized with substance P) projecting to both the ventral pallidum and the VTA (Lu et al., 1998). Although both D1 and D2 receptors appear to be involved in the behavioral changes resulting from cocaine and other psychostimulant drugs (Koob et al., 1987; Woolverton and Virus, 1989; Fontana et al., 1993; Caine et al., 1999; Caine et al., 2000), activation of D1 receptors appears to attenuate drug seeking in reinstatement models whereas activation of D2 receptors has the opposite effect (Self et al., 1996; Khroyan et al., 2000).

While the role of NAc dopamine in reward outlined above has been studied extensively, in the context of this dissertation it is also important to mention its role in cognitive function. Although some contend that lesions of the ventral striatum lead to performance deficits, i.e. increased response latencies, rather than cognitive deficits (Cole and Robbins, 1989), lesions of the nucleus accumbens have been shown to lead to a number of cognitive deficits that are

not primarily performance related. These deficits include impaired learning of a spatial discrimination task and impaired reversal learning (Annett et al., 1989), an impairment in PPI performance, indicating a decrease in sensorimotor inhibition (Kodsi and Swerdlow, 1994), impaired contextual fear conditioning (Riedel et al., 1997), a decrease in Pavlovian approach behavior in an autoshaping task (Parkinson et al., 2000), and an increase in perserverative responding on the 5-CSRTT (Christakou et al., 2004). Many of these impairments, including a decrease in reversal learning and spatial discrimination are thought to be dependent on dopamine depletion in the NAc (Taghzouti et al., 1985).

In examining the role of specific dopamine agonists in the NAc on cognitive function, Pezze and colleagues (2007) found that a D1 receptor antagonist infused into the NAc decreased accuracy and increased omissions on a 5-CSRTT, while an agonist had the opposite effect. In contrast, Pattij and colleagues (2007) found that D1 receptor antagonists infused into either the NAc core or shell led to a decrease in premature responding on the 5-CSRTT, while NAc core infusions lead to an increase in performance accuracy and NAc shell infusions led to an increase in omission rate. This would suggest that although D1 receptors in the NAc play a role in cognitive function, there are differential contributions of these receptors within the two subregions of the NAc.

Along with examining the effects of intra-accumbal infusions of D1 receptors, these two studies also examined the effects of D2 antagonist administration.

While a D2 receptor antagonist led to a similar decrease in response accuracy in the 5-CSRTT as seen with D1 antagonists, they also led to an increase in food retrieval latency. However, administration of a D2 receptor agonist had no effect on accuracy but increased perserverative responding (Pezze et al., 2007). While Pattij and colleagues (2007) found similar results in regards to the increase in latencies, they also found that D2 receptor antagonists infused into either the core or the shell led to an increase in omission rate. This would suggest that D2 receptors in the NAc play a key role in performance related activity. Just as the decrease in response accuracy in the 5-CSRTT cannot be attributed to performance deficits, Ploeger and colleagues (1994) demonstrated that intra-accumbal infusions of haloperidol, a d2 antagonist, lead to decreased acquisition of spatial learning in a Morris water maze task.

Together, these studies provide us with ample evidence that the dopamine system in the ventral striatum plays a key role in cognitive function that includes, but is not limited to, a role in the performance of cognitive tasks. As the NAc receives projections from the PFC and these neurons synapse on the same MSNs that receive dopaminergic input (Hara and Pickel, 2005), understanding this interaction could further our understanding of cognitive deficits resulting from addiction and dopamine-related disorders.

Caudate-Putamen

Although the caudate-putamen is the target region of the nigrostriatal dopamine projection system, primarily studied for its role in movement disorders, rather than the mesolimbic dopamine projection system, it also plays a role in reward processing. Recently, dopamine release in the caudate-putamen, also referred to as the dorsal striatum, has been implicated as a mechanism underlying cue-induced drug craving (Volkow et al., 2006). This theory is based primarily on PET imaging studies in human addicts demonstrating that viewing drug related stimuli leads to increased radiotracer displacement, presumably reflecting an increase in dopamine release (Volkow et al., 2006; Wong et al., 2006). Along with cue-induced craving this area has also been implicated in stress-induced craving in human cocaine addicts (Sinha et al., 2005).

Along with these clinical studies, animal work supports the involvement of dorsal striatum dopamine in cue-induced craving and reinstatement. For example, extracellular dopamine is increased dorsal striatum of rodents responding under a second-order schedule of reinforcement (Ito et al., 2002). Vanderschuren and colleagues (2005) have demonstrated that dorsal striatum dopamine is necessary for second-order responding. In further support of the role of the dorsal striatum in relapse, a recent study by See and colleagues (2007) showed that inactivation of the caudate-putamen attenuates drug seeking after prolonged abstinence.

The role of the dorsal striatum in cue-induced craving and relapse may be due to its role in stimulus-response learning. Lesions of the caudate have been know to lead to memory deficits in a number of different tasks involving the pairing of a stimulus with a reinforced response since the 1970s (Green et al., 1967; Schwartzbaum and Donovick, 1968; Allen and Davison, 1973; Hannon and Bader, 1974; Prado-Alcala et al., 1975; Levine et al., 1978; Sanberg et al., 1979). In particular, it seems to be the lateral caudate, or more specifically, the dorsolateral caudate, that is most involved in the acquisition of stimulus-response pairing (Reading et al., 1991; Adams et al., 2001; Kantak et al., 2001; Featherstone and McDonald, 2004b, a, 2005). The role of the dorsolateral caudate in stimulus-response learning is mediated by dopamine in this region as dopamine depletion leads to similar deficits as lesions (Neill et al., 1974; Carli et al., 1989).

In addition to the role of the dorsal striatal dopamine in reinstatement and stimulus-response learning, it has also been implicated in executive functions. Early work by in monkeys by Battig and colleagues (Battig et al., 1960) and work in cats by Ivan Divac (Divac, 1968, 1972) determined that lesions of the caudate lead to impaired delayed response and delayed alternation performance. A number of studies have demonstrated similar results in rats (Oberg and Divac, 1975; Divac et al., 1978; Mogensen et al., 1987; Dunnett et al., 1999). In addition to these delayed alternation deficits, lesions of the dorsal striatum lead to

decrease short-term memory performance on a delayed-match-to-sample task (Dobrossy et al., 1995, 1996) as well deficits on a serial visual discrimination task (Trueman et al., 2005). While these lesion studies do not allow us to isolate these impairments to a neurotransmitter system, Collins and colleagues (2000) have demonstrated that dopamine depletion in the caudate leads to similar deficits in delayed alternation as seen with caudate lesions. Along with these deficits, this study also demonstrated that dopamine depletion leads to deficits on extradimensional shifting in an attentional set-shifting task (Collins et al., 2000). Despite the evidence that dopamine in the caudate is critical for many cognitive tasks, there is also evidence that dopamine depletion in the caudate leads to an enhanced ability to ignore distraction in attentional set shifting tasks (Crofts et al., 2001).

As all three ascending dopamine systems, mesolimbic, mesocortical and nigrostriatal, seem to play a role in addiction, gaining a better understanding of how these systems are altered by drugs of abuse may lead to better relapse prevention.

Alterations in Dopamine Transmission and Dopamine Receptors within the Prefrontal Cortex and Striatum in Human Addicts

Although the information we are able to obtain from clinical populations is limited, both imaging and postmortem studies have provided us with some insight into

differences between the brains of addicts and healthy adults. With the exception of a few global changes, such as a decrease in cortical grey matter volume (Bartzokis et al., 2000), most of the work has focused on either the frontal cortex or the striatum.

Frontal Cortex

At the most global level, the brains of cocaine addicts exhibit structural differences from healthy controls, such as a decrease in gray matter volume in the prefrontal cortex (Liu et al., 1998; Fein et al., 2002; Matochik et al., 2003) and the orbitofrontal cortex (OFC) (Franklin et al., 2002; Matochik et al., 2003; Sim et al., 2007). At a more functional level, numerous studies have examined functional activation in the frontal cortex of cocaine addicts, utilizing regional cerebral blood flow (rCBF), glucose utilization, and functional magnetic resonance imaging (fMRI) procedures. Volkow and colleagues (Volkow et al., 1988) reported a decrease in rCBF in the PFC of cocaine addicts that persisted after 10 days of abstinence. This finding has been replicated by both by the Volkow lab (Volkow et al., 1993) and others (Strickland et al., 1993; Bell et al., 1994; Ernst et al., 2000; Browndyke et al., 2004) and the decrease in frontal rCBF has been extended out to 3-4 months of abstinence (Volkow et al., 1993). Decreased rCBF has also been reported in the OFC of cocaine addicts (Bolla et al., 2003). This persistent decrease in frontal cortical rCBF is accompanied by a persistent decrease in glucose metabolism in the frontal cortex of abstinent cocaine addicts (Volkow et al., 1991; Volkow et al., 1992).

Along with these general measurements of functional activation, researchers have examined markers of dopamine function in the frontal cortices of human addicts. Although no differences have been reported utilizing imaging techniques, postmortem studies indicate that cocaine addicts have a decrease in the number of dopamine transporter (DAT) binding sites in the PFC (Hitri et al., 1994). There is also postmortem evidence for a decrease in dopamine metabolites in the PFC of cocaine addicts, suggesting a decrease in dopamine levels in these subjects (Little et al., 1996).

Taken together, these studies support the theory that cocaine addicts exhibit a "hypofrontality", or decrease frontal cortex function (Goldstein and Volkow, 2002). This decrease in frontal cortex function may be dopamine mediated but human studies have not allowed us to determine this conclusively.

Striatum

Contrary to what is seen in the frontal cortex, there appears to be an increase in the metabolic activity of the striatum following chronic cocaine use, however this increase does not appear to be as persistent (Volkow et al., 1991). This is the only study to demonstrate general alterations in functional activation in human addicts, as most of the focus of this work has been on more specific changes in the dopamine system.

The most widely cited difference in the striata of cocaine addicts is a decrease in D2 receptor availability first noted by Volkow and colleagues (1990) and replicated by her own lab and others (Volkow et al., 1993; Martinez et al., 2004). This decrease in receptor availability is believed to represent a decrease in total striatal D2 receptor number. While the original studies by Volkow and colleagues (1990; 1993) did not differentiate between dorsal and ventral striatum, it is important to note that a more recent study by Martinez et al. (2004) determined that the decrease in D2 receptor availability is present in both the nucleus accumbens and the caudate-putamen.

Along with these changes in D2 receptor availability, an increase in DAT binding is also seen in human addicts. Malison and colleagues (1998) demonstrated that during acute withdrawal (96 hours or less since last cocaine adminstration) there is a 20% increase in DAT binding. This is consistent with postmortem studies of cocaine abusers demonstrating increased DAT mRNA (Little et al., 1998) and studies of both cocaine abusers and cocaine overdose victims that have found increased DAT binding compared with controls (Little et al., 1999; Qin et al., 2005). This increase in transporter number has been determined to reflect an increase in functional activation of the DAT as measured in postmortem synaptasomes (Mash et al., 2002). In addition to this alteration in terminal transporter function, there also seems to be a change in vesicular transporter. Little et al. (2003) found a decrease in VMAT immunoreactivity in the striata of cocaine abusers. The decrease in VMAT may reflect damage to dopaminergic

nerve terminals but as there is little evidence to support cocaine induced neurotoxicity, more work will need to be done to determine if this is the case.

Work by Hurd and Herkenham (1993) examining the gene expression profiles of striata from human cocaine addicts determined that addicts exhibited an increase in enkephalin mRNA in the caudate-putamen and a decrease in dynorphin mRNA in the putamen. As mentioned earlier, enkephalin is colocalized with D2 receptors while dynorphin (and substance P) is colocalized with D1 receptors. Therefore, this would suggest that there may be an upregulation of D1 receptors and corresponding decrease in D2 receptors, however receptor mRNA studies have not yielded this pattern of results (Meador-Woodruff et al., 1993).

Although the focus of the current dissertation is on dopamine related changes following drugs of abuse, it is important to note that postmortem studies of cocaine addicts have also found alterations in glutamate (Tang et al., 2003; Hemby et al., 2005), norepinephrine (Mash et al., 2005), and serotonin neurotransmitter systems (Little et al., 1996) in the striatum and prefrontal cortex. Brain changes have also been detected in human addicts in regions that I have not focused on, such as the amygdala (Makris et al., 2004).

As mentioned previously, these results from human studies do not allow us to parse apart cause vs. consequence. It is important to examine the results from preclinical studies in nonhuman primates and rodents to help determine whether

these alterations in mesocortical and mesolimbic systems are a result of drug abuse or reflect pre-existing vulnerabilities.

Alterations in Dopamine Transmission and Dopamine Receptors within the Prefrontal Cortex and Striatum Following Chronic Psychostimulant in Animals

Due to the varied drug administration procedures, withdrawal times, and testing parameters, it is difficult to form a cohesive picture from the large number of animal studies that have examined the effects of drugs of abuse, or even specifically cocaine, on dopamine transmission and dopamine receptors in the terminal regions of the ascending projection pathways. In the current section I will attempt to synthesize the literature on the effects of chronic cocaine on dopamine transmission and receptors in the prefrontal cortex and the striatum, providing

Prefrontal Cortex

Due to the expense of doing terminal procedures in monkeys, there are very few studies using molecular techniques to examine the underlying changes in dopamine transmission resulting from chronic cocaine administration. Therefore, researchers examine measures of "functional activation", such as glucose utilization and rCBF, to infer how drugs may change cortical brain regions involved in cognitive function. The one such study that has examined the

prefrontal cortex in monkeys following chronic cocaine self-administration found a decrease in prefrontal glucose metabolism as a result of drug administration (Beveridge et al., 2006).

While neurochemical and electrophysiological studies are more prevalent, rodent studies have also examined glucose utilization following repeated cocaine administration. Knapp and colleagues (2002) demonstrated that after 8 days of withdrawal, animals that had received repeated cocaine injections showed a decrease in glucose metabolism in the ventromedial orbital frontal cortex. However, similar studies looking at both cocaine and amphetamine administration have not replicated this result (Pontieri et al., 1995; Tsai et al., 1995).

To gain further understanding of how chronic cocaine changes the prefrontal cortex, a number of studies have utilized electrophysiology to determine how the firing rates of pyramidal neurons are altered during withdrawal from psychostimulants. The results of these studies also seem to be somewhat inconsistent, however. Following short term (5 days) *in vivo* cocaine administration and 3 days of withdrawal, slice cultures of mPFC neurons reveal an increase in membrane excitability driven by a decrease in voltage-gated K+ currents (VGKC), which in turn is driven by an upregulation of D1 receptor signaling (Dong et al., 2005; Nasif et al., 2005). The idea of an increase in PFC excitability is also supported by work looking at synaptic plasticity in mPFC slices

following a similar 5-day treatment regimen and 3 day withdrawal period. In studies by Huang and colleages cocaine treated animals exhibited a facilitation of long-term potentiation induction (Huang et al., 2007a) and impaired long-term depression (Huang et al., 2007b).

Though the above evidence would suggest that the PFC is actually hyperactive following cocaine abuse, there is evidence that supports the hypoactivity hypothesis that was developed around the clinical data. *In vivo* electrophysiological studies in rats undergoing chronic cocaine self-administration found that after multiple weeks of cocaine exposure there was a decrease in basal activity in prefrontal neurons as measured prior to the self-administration session (Sun and Rebec, 2006). This may indicate that the previous studies did not utilize a long enough cocaine administration regimen or a fundamental difference between the effects of contingent and noncontingent cocaine on prefrontal function.

While these studies provide insight into alterations in prefrontal cortical function following cocaine administration, they do not tell us about specific changes in the dopamine system in this brain region. Electrophysiological studies have examined the effects of dopamine receptor antagonists on prefrontal firing following chronic cocaine administration, however. Nogueira and colleagues (2006) looked at prefrontal neurons in anesthetized animals following 7 days of cocaine and 2-4 weeks of withdrawal and found that D2 receptor antagonists at

low doses failed to increase current-evoked excitability in cocaine treated animals (as seen in saline animals) and actually had the opposite effect at higher doses. This decrease in responsivity to dopamine agents is supported by work demonstrating a decrease in the dopamine responsiveness in mPFC neurons of animals that have undergone repeated amphetamine treatment (Peterson et al., 2000).

In addition to these electrophysiological studies, a number of neurochemical studies have also examined the effects of chronic psychostimulants on prefrontal dopamine transmission and dopamine receptors. When examining basal changes in prefrontal dopamine transmission, Karoum and colleagues (1990) demonstrated that during long term withdrawal (1-12 weeks) from repeated cocaine there is a decrease in dopamine and its metabolites in the frontal cortex. Similar results have been found following chronic amphetamine administration (Hedou et al., 2001). In addition to this decrease in extracellular dopamine, chronic cocaine administration leads to an increase in dopamine turnover (Meiergerd et al., 1997). Together these studies suggest that there is a decrease in basal dopamine transmission in the mPFC following repeated psychstimulant treatment.

Along with the changes in basal dopamine transmission, repeated psychostimulants have also been shown to decrease prefrontal dopamine responsivity. Repeated cocaine administration has been shown to abolish shock-

induced prefrontal dopamine release (Sorg and Kalivas, 1993) as well as decrease cocaine-induced prefrontal dopamine release (Sorg et al., 1997). It is not entirely clear whether this decrease in prefrontal dopamine responsivity lasts throughout prolonged withdrawal, however. In animals given a 4 day regimen of experimenter-administered cocaine, followed by either 1, 7, or 30 days of withdrawal, cocaine-induced dopamine overflow in the mPFC was decreased during early withdrawal (both 1 and 7 days) and after more prolonged withdrawal (30 days) was increased relative to controls (Williams and Steketee, 2005a). There is evidence that this initial decrease in dopamine overflow during acute withdrawal may be due to a decrease in dopamine release rather than clearance (Williams and Steketee, 2005b).

Along with these examinations of dopamine release and clearance, many studies have examined the effects of cocaine on dopamine receptors and dopamine transporters. While binding studies have yielded changes in D2 receptor binding, they are much more transient than those seen in human and nonhuman primate studies, and the results are contradictory. Studies utilizing experimenter-administered cocaine have demonstrated decreases in D2 receptor binding (Kleven et al., 1990) while self-administration studies have yielded the opposite effects, demonstrating an increase 20 minutes after cocaine administration that is gone after 2 weeks (Ben-Shahar et al., 2007). The results in rodents on the effects of chronic cocaine on DAT binding are a bit unclear as well, with short access self-administration leading to an increase in DAT density following a 14-

day withdrawal period while long access self-administration did not (Ben-Shahar et al., 2006). This would suggest that the increase in DAT binding seen in human addicts may not be critically involved in addiction, as it is seen even after modest drug administration.

While there are some conflicting results, the studies of the effects of chronic psychostimulants on PFC function provide evidence for hypofrontality resulting from drug abuse. As most of the studies mentioned here utilized experimenter-administered drug, it is possible that more consistent results would be found utilizing self-administration procedures that more closely parallel human drug use patterns. As any alterations in the underlying circuitry of the PFC could potentially lead to changes in cognitive functions that rely on processing in this area, a more full understanding of the effects of psychostimulant drugs on PFC neurons and, more specifically, dopamine in the PFC could help in our understanding of these cognitive deficits and potentially lead to more effective therapies.

Striatum

Though there is still relatively little work on the effects of chronic cocaine dopamine transmission in the striata of nonhuman primates, there is some evidence that the alterations in the dopamine system seen in human imaging studies may be a result of drug use. Following chronic cocaine self-administration, Nader and colleagues (2002) found a decrease in D2 receptor binding and an increase in D1 receptor binding in the striata (all striatal regions

rostral to the anterior commissure) of rhesus monkeys. This decrease in D2 receptor binding in self-administering monkeys was also seen in a previous study by the same group (Moore et al., 1998a). However, when D1 receptor binding is examined in the NAc alone, Moore and colleagues (1998b) report an increase following chronic cocaine self-administration.

Along with these binding studies, *in vivo* PET studies in monkeys have also demonstrated a decrease in striatal D2 receptor availability that developed after 1 week of cocaine self-administration and persisted following up to 1 year of abstinence (Nader et al., 2006). In examination of another aspect of the striatal dopamine system, Letchtworth and colleagues (2001) demonstrated that following long-term (up to 1.5 years) cocaine self-administration leads to an upregulation of DAT binding sites in both the nucleus accumbens and the caudate-putamen that follows an initial downregulation in DAT binding in these areas following short-term (5 days) self-administration.

To further add to our knowledge of how chronic psychostimulants alter dopamine transmission, studies in rodents have allowed researchers to use more invasive techniques to measure dopamine release. Early studies examining the effects of repeated amphetamine in rat striatal slices found that drug animals exhibited an increase in amphetamine-evoked dopamine release compared to saline controls following more than a week of withdrawal (Robinson and Becker, 1982; Wilcox et al., 1986). This increase in stimulated dopamine release following repeated

amphetamine is not limited to just amphetamine-evoked release however, as similar results have been seen with both KCl and electrical field stimulation-evoked dopamine release (Castaneda et al., 1988). This increase in evoked release can be generalized across psychostimulants as repeated cocaine administration leads to an increase in amphetamine-evoked DA in the nucleus accumbens *in vitro* (Clay et al., 1998).

Similar increases in evoked dopamine release have been seen *in vivo*, utilizing microdialysis in freely moving animals, with repeated amphetamine producing an increase in amphetamine-evoked dopamine release in the ventral striatum (Robinson et al., 1988). Repeated cocaine administration has also been shown to lead to an increase in psychostimulant-evoked dopamine release in the dorsal striatum (Akimoto et al., 1989; Akimoto et al., 1990) as well as the ventral striatum (Kalivas and Duffy, 1990; Ng et al., 1991). This alteration in evoked dopamine is not only seen with non-contingent cocaine administration, but also following chronic cocaine self-administration (Pettit et al., 1990).

Chronic psychostimulant administration not only leads to changes in stimulated dopamine transmission but also basal transmission. Repeated amphetamine administration has been shown to cause a long-lasting (up to 12 days) increase in dopamine metabolism, indicated by an increase in the dopamine metabolite DOPAC (Robinson and Camp, 1987). This increase in dopamine metabolism is seen in both the dorsal and ventral striatum and is still present after one month of

withdrawal (Paulson and Robinson, 1996). This increase in metabolism could be responsible for reports that chronic cocaine leads to a decrease in basal levels of extracellular dopamine in the ventral striatum during prolonged withdrawal (Rossetti et al., 1992) and the dorsal striatum in early withdrawal (Clay et al., 1998). However, as many other studies have not seen these basal differences it is not entirely clear how or if chronic psychostimulants alter basal dopamine concentrations (Robinson et al., 1988; Jones et al., 1996).

In addition to these studies examining dopamine transmission, numerous studies in rats have examined the effects of chronic psychostimulants on dopamine receptors in the striatum. Although the nonhuman primate binding studies seem to be consistent with what has been shown in the human literature, this is not the case in regards to the rodent literature. Studies examining the effects of experimenter-administered cocaine on D2 receptor binding in rats are mixed, with reports of increases (Trulson and Ulissey, 1987; Peris et al., 1990), decreases (Goeders and Kuhar, 1987; Kleven et al., 1990; Tsukada et al., 1996; Maggos et al., 1998), and no change (Lim et al., 1990; Alburges et al., 1993). While these divergent results may be due to differences in drug treatment regimen (i.e. dose, length of treatment, length of withdrawal) or autoradiography ligands, there is also another possibility.

Recent work has demonstrated that the dopamine D2 receptor exists in two interconvertible affinity states for dopamine. The high-affinity state ($D2^{High}$) is the

more functionally relevant state as dopamine preferentially binds to it over the receptors in the low-affinity state (Seeman et al., 2005). Repeated psychostimulant administration has been shown to cause a dramatic increase in the proportion of $D2^{High}$ receptors while having no effect on the total D2 receptor population (Seeman et al., 2002; Seeman et al., 2007). This increase in $D2^{High}$ receptors may explain why repeated psychostimulant treatment yields rats that are hypersensitive to the psychomotor and motivational effects of direct D2 agonists (De Vries et al., 2002; Edwards et al., 2007). As the current ligands used in human and non-primate imaging studies do not discriminate between the low- and high-affinity states of the D2 receptor, more studies will need to be done before we can determine whether this apparent hypersensitivity is relevant to the human condition.

Although it is possible that MSNs may be hypersensitive to dopamine, repeated cocaine administration seems to yield a decrease in the sensitivity of these neurons to glutamate release from PFC projections. White and colleagues have demonstrated that repeated cocaine or repeated amphetamine decreased the excitatory effect of glutamate on NAc MSNs after 3 days of withdrawal (White et al., 1995). This finding that chronic cocaine leads a decrease in NAc excitability is supported by evidence that repeated cocaine decreases AMPA/NMDA excitatory postsynaptic potential ratios at PFC-NAc synapses (Thomas et al., 2001). This study also suggests that this decrease in excitability is long lasting, as the decrease in AMPA/NMDA ratios was present after 10-14 days of

withdrawal. As stimulation of D2 receptors leads to a decrease in excitability in the NAc (West and Grace, 2002; Tseng and O'Donnell, 2004; Perez et al., 2006), this may be a potential mechanism by which this decrease in glutamate responsivity occurs.

Similarly to what has been found in the PFC, much of the work done on the effects of repeated cocaine in the striatum has utilized experimenter administered drug and as many differences have been seen between contingent and non-contingent drug administration (Broadbear et al., 1999; Kippin et al., 2006; Lecca et al., 2007), the relevance of these studies to human condition is not absolute. It will be important in future studies to examine striatal function utilizing not only self-administration procedures but extended access procedures that yield many symptoms of addiction that are characteristic of human addicts (Ahmed and Koob, 1998; Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004). As striatal function, and specifically dopamine in the striatum, is involved in a variety of cognitive functions, understanding how psychostimulant drugs alter this function could contribute to our understanding of deficits caused by drug abuse.

The Purpose of the Present Dissertation

The purpose of the following series of experiments is to examine the ability of cocaine to cause deficits in cognitive function and changes in the neurobiological circuits underlying cognition. As the issue of causality cannot be addressed in

clinical studies and the animal literature has not yet been able to generate deficits similar to those seen in human addicts utilizing contingent cocaine administration, many believe that these deficits may pre-exist addiction and play a role in addictive vulnerability. While there may be cognitive deficits that do precede addiction, we believe that the lack of persistent effects seen in the preclinical literature is due to the drug administration procedures utilized rather than an inability of cocaine to cause cognitive deficits. Thus, to this end, the aim of this dissertation is to utilize an extended access cocaine self-administration procedure to study the effects of cocaine on cognitive function and associated dopamine systems. As extended access cocaine self-administration has been shown to yield a number of other symptoms characteristic of human addiction, it is hypothesized that persistent cognitive deficits will be seen in rats similar to those seen in human addicts. Furthermore, we believe that alterations in the dopamine systems underlying these cognitive functions will be seen following extended access cocaine self-administration and that these alterations may further educate us on the mechanisms underlying drug-induced cognitive deficits.

Therefore the aim of Chapter II was to examine the effects of differential access cocaine self-administration on sustained attention performance. In addition, *in situ* hybridization and Western Blot analyses were conducted to examine cocaine-induced alterations in the underlying mesocortical dopamine circuitry. The goal of the experiments in Chapter III was to extend our findings on the effects of extended access cocaine on attentional function and examine the

ability of cocaine to cause deficits in recognition memory performance. As deficits in memory function are seen in human addicts, we hypothesized that the extended access cocaine self-administration animals would exhibit persistent memory deficits.

As discussed earlier, the effects of cocaine on striatal dopamine systems is not entirely clear, as there is a discord between the human and animal literatures. The experiment in Chapter IV was aimed at proposing a potential resolution to this controversy. Striatal tissue was taken from animals with differential access to cocaine self-administration and levels of both total D2 receptor binding and D2 high-affinity receptor binding were conducted. It is hypothesized that cocaine self-administration will lead to an increase in $D2^{High}$ receptors while not changing the total receptor density and that this increase will be more pronounced in the extended access group.

The fourth and final experiment, Chapter V, was aimed at examining changes in functional activation patterns following extended access cocaine self-administration in an attempt to further elucidate changes in mesocortical and mesolimbic dopamine systems throughout the course of withdrawal. To explore this issue we utilized a unique autoradiographic technique to measure basal changes in rCBF in mesocortical and mesolimbic brain regions.

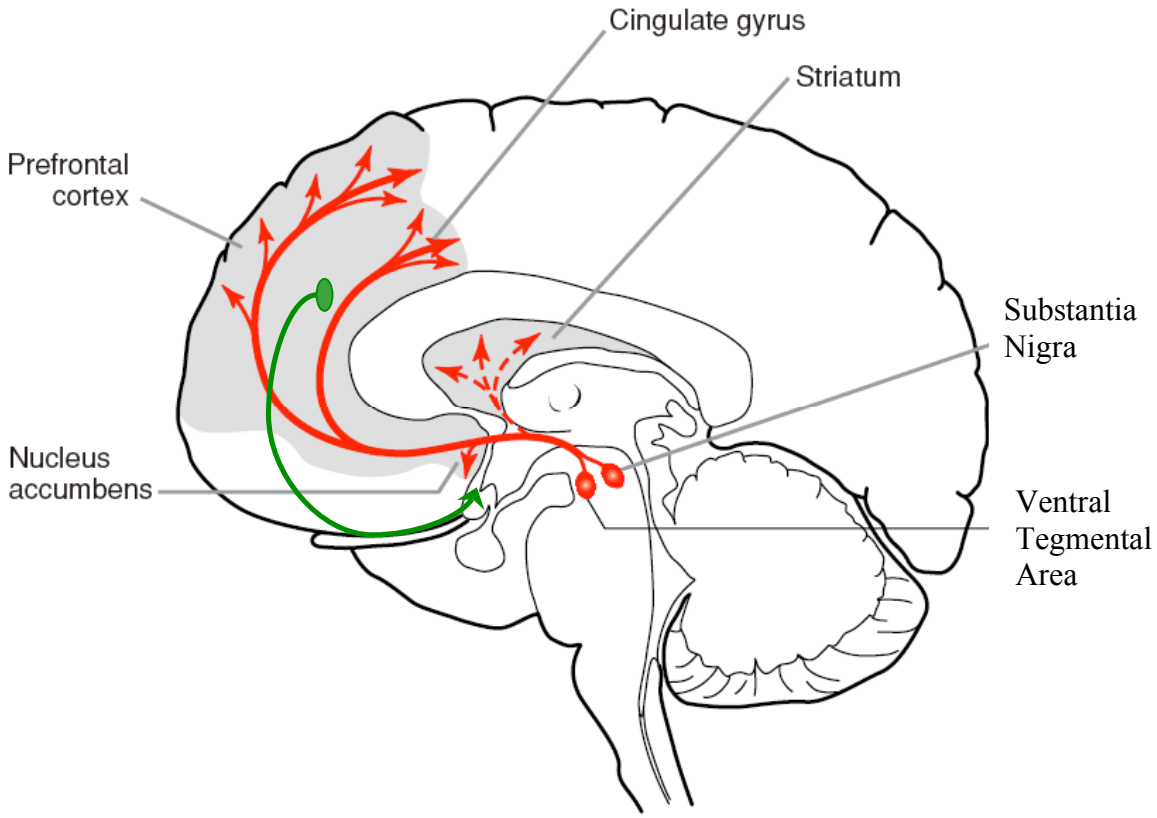


Figure 1.1. Diagram of ascending dopamine projection systems in the rat. The red arrows indicate dopamine projections from the ventral tegmental area to the nucleus accumbens (mesolimbic pathway) and the prefrontal cortex (mesocortical pathway) as well as dopamine projections from the substantia nigra to the caudate-putmen, or dorsal striatum (nigrostriatal pathway). The green arrow indicates glutamatergic descending projections from the prefrontal cortex to the nucleus accumbens.

CHAPTER II

PERSISTENT ALTERATIONS IN COGNITIVE FUNCTION AND PREFRONTAL DOPAMINE D2 RECEPTORS FOLLOWING EXTENDED, BUT NOT LIMITED, ACCESS TO SELF-ADMINISTERED COCAINE

Summary

Drug addicts have deficits in frontocortical function and cognition even long after the discontinuation of drug use. It is not clear, however, whether the cognitive deficits are a consequence of drug use, or are present prior to drug use, and thus are a potential predisposing factor for addiction. To determine if self-administration of cocaine is capable of producing long-lasting alterations in cognition, rats were allowed access to cocaine for either 1 hr/day (short access; ShA) or 6 hrs/day (long access; LgA) for 3 weeks. Between 1 and 30 days after the last self-administration session we examined performance on a cognitively demanding test of sustained attention that requires an intact prefrontal cortex. The expression of dopamine D2 receptor mRNA and D2 receptor protein levels in the prefrontal cortex were also examined. Early after the discontinuation of drug use LgA (but not ShA) animals were markedly impaired on the sustained

attention task. Although the LgA animals improved over time, they continued to show a persistent pattern of performance deficits indicative of a disruption of cognitive flexibility up to 30 days after the discontinuation of drug use. This was accompanied by a significant decrease in both DA D2 mRNA and D2 receptor protein in the prefrontal cortex of LgA (but not ShA) animals. These findings are consistent with the hypothesis that in addicts repeated cocaine use can potentially produce persistent deficits in cognition and brain function, and illustrate the usefulness of extended access self-administration procedures for studying the neurobiology of addiction.

Introduction

Cocaine addicts have deficits in cognitive function, including deficits on tests of short-term memory, attentional control, and decision-making, and these are evident even long after the discontinuation of drug use (Miller, 1985; Hoff et al., 1996; Rosselli and Ardila, 1996; Bolla et al., 1999). Many of these cognitive functions are known to require the prefrontal cortex, and structural and functional imaging studies suggest that indeed, prefrontal cortical function is impaired in addicts (Volkow et al., 1988; Volkow et al., 1991; Goldman-Rakic, 1995; Robbins, 1996; Bolla et al., 1998; Liu et al., 1998; Ernst et al., 2002; Bolla et al., 2003; Matochik et al., 2003; Bolla et al., 2004; Kosten et al., 2006). It is not clear, however, whether these behavioral and neurobiological deficits precede drug use, and thus potentially contribute to the propensity for addiction, or are a

consequence of drug use. It is difficult to address this issue in humans, but studies with non-human animals allow one to determine whether exposure to a drug of abuse is capable of producing changes in brain and behavior comparable to those seen in addicts.

Studies using non-human animals have shown that when psychostimulant drugs are administered by an experimenter they can produce both acute and persistent deficits in cognitive function (Crider et al., 1982; Jentsch et al., 2002; Kondrad and Bruk, 2004; Schoenbaum et al., 2004; Burke et al., 2006). For example, Schoenbaum *et al.* (2004) reported that experimenter-administered cocaine produces persistent reversal deficits on an odor discrimination task mediated by the orbital frontal cortex in rats, and similar deficits have been seen in non-human primates (Jentsch et al., 2002). However, experiments in which animals were allowed to self-administer cocaine have not revealed persistent cognitive deficits. The self-administration of psychostimulant drugs results in cognitive deficits during the period of acute withdrawal, for a few days after the discontinuation of drug treatment, but these do not persist unless animals are given an additional drug challenge (Crider et al., 1982; Kondrad and Burk, 2004; Dalley et al., 2005a; Dalley et al., 2005b; Kantak et al., 2005). It remains possible, therefore, that the persistent cognitive deficits seen in cocaine addicts are not in fact a consequence of drug use. Alternatively, the self-administration procedures used thus far may not accurately model the addictive state.

Preclinical studies involving self-administration have largely used limited access procedures (Dalley et al., 2005a; Dalley et al., 2005b; Kantak et al., 2005), but these may not produce some of the key changes in brain and behavior that characterize addiction. Recent reports suggest that extended access procedures are required to produce many symptoms characteristic of addiction, including escalation of drug-intake (Ahmed and Koob, 1998; Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Ferrario et al., 2005). We hypothesized, therefore, that extended (but not limited) access to self-administered cocaine may produce persistent cognitive deficits, and associated changes in prefrontal cortical dopamine function. This hypothesis is tested here.

Materials and Methods

Subjects. One hundred and thirty-eight male Wistar rats (Harlan, Indianapolis, IL) weighing 200-225g at the start of the experiment were individually housed in square plastic hanging cages (8 x 9 x 8 cm). The animals were housed in a temperature and humidity controlled room with a 14:10 light/dark cycle, with water available ad libitum. Animals were food restricted throughout the experiment to maintain at least 90% of their free feeding body weight. All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA).

Apparatus. Behavioral training, testing, and drug administration took place in 16 operant chambers measuring 22 x 18 x 13 cm (Med Associates, St. Albans, VT, USA) located inside larger sound-attenuating chambers. For the sustained attention task, each operant chamber was equipped with a panel consisting of one light (2.8 W), two retractable levers, and a food pellet dispenser, all located on the front wall. A house light (2.8 W) was located close to the ceiling of the back wall. The floor of the chamber consisted of 19 stainless steel rods (4 mm in diameter) spaced 1.5 cm apart (center-to-center). For the drug self-administration procedure, each operant chamber had two nose poke holes equipped with cue lights. A tone (2900 Hz) was also available inside of the chamber. The floor of the chamber consisted of a solid piece of black plastic placed over the stainless steel rods. Med-PC for Windows software (v. 1.1, Med Associates) controlled all signal presentation, lever operation, pellet delivery, drug delivery, tone presentation and data collection via a Pentium PC.

Experiment I: Within-Subjects Study.

Sustained Attention Task Training. For a detailed description of sustained attention procedures see McGaughy & Sarter (1995). The task required four phases of training. *Phase I.* All animals were shaped to lever press for food (45-mg banana flavored pellet, Bioserv, Frenchtown, NJ) utilizing a fixed ratio 1 (FR1) schedule such that one lever press resulted in the delivery of one food pellet. During this stage both of the levers were extended into the box at the

beginning of each session and remained extended for the duration of the session. Training continued until the animals had at least three days of 120 reinforced lever presses. *Phase II.* After reaching this criterion, rats were then shaped to discriminate between signals (illumination of the central signal light for a period of 1 s) and non-signals (no illumination). For this training phase the start of each individual trial was indicated by the extension of both levers into the box. Response on one lever was reinforced if it followed a signal, while response on the other lever was reinforced if it followed a non-signal. The assignment of levers was counter-balanced across subjects to minimize side-bias. To increase the salience of the signal light and facilitate acquisition of the appropriate response, the house light was not illuminated during this training step. Signal and non-signal trials were presented pseudo-randomly with a mean intertrial interval (ITI) of 9±3 s. Four responses were possible during this second phase of training: *hit,* defined as the correct response to a signal; *miss,* the incorrect response to a signal; *correct rejection,* the correct response to a non-signal; and *false alarm,* the erroneous response to a non-signal (i.e., a claim for a signal following a non-signal; see Figure 2.1 for schematic). If the animal responded incorrectly, the previous trial was repeated up to three times. If a rat continued to respond incorrectly, it received a forced-choice trial in which only the correct lever was extended into the box while the signal was presented. Forced-choice trials also served to impede the generation of a side bias. Trials in which the rats failed to emit a response within 4 s of lever insertions were counted as omissions. Animals reached criterion for this portion of training when they responded

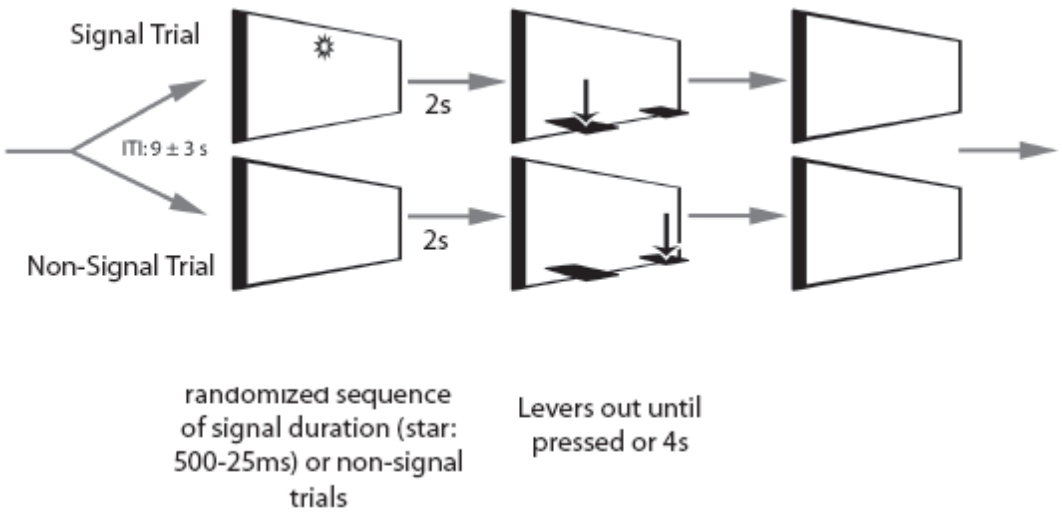


Figure 2.1. Schematic illustration of the of the sustained attention task. Correct responses on signal trials (hits) and non-signal trials (correct rejections) were rewarded (arrows) and incorrect responses (misses and false alarms, respectively) were not.

correctly to 65% of the signal and non-signal trials for five consecutive days. *Phase III.* For the third stage of training the 1.0 s signal was replaced with three different signal lengths of 500, 50, and 25 ms, equally distributed across the session. Following correct responding to 65% of the 500 ms and non-signal trials, the final stage of training began. *Phase IV.* During the final stage of training, the house light was illuminated; this is a critical step in the acquisition of the sustained attention task as it forces animals to limit competitive behaviors and to closely monitor the intelligence panel in order to detect signal events. The final task consisted of three blocks of 54 trials, with nine trials of each signal length and 27 non-signal trials. The signal and non-signal trials were pseudo-randomized. Animals reached criterion when their performance became asymptotic at a level of at least 70% hits on the longest (500 ms) signal length trials, 70% correct rejections on the non-signal trials, and less than 30% omissions.

Surgical Procedures. Individual animals reached performance criterion on the sustained attention task at different rates, and therefore cocaine self-administration and subsequent attention testing occurred at different times for each animal. After reaching criterion on the attention task, animals were anaesthetized with ketamine and xylazine anesthesia (77:1.5 mg/ml, intraperitoneal [IP], at 0.1 mL/100g of body weight) and a silicone catheter (Plastics One, Roanoke, VA) was inserted into the right jugular vein and passed subcutaneously to exit from the animals' back (see Caine et al., 1993). Animals

were allowed to recover from surgery for a minimum of 3 days prior to drug administration. Catheters were flushed daily with 0.1 mL of gentamicin (50 mg/kg, in 0.9% sterile bacteriostatic saline).

Cocaine Self-Administration. Rats were transported from their home cage to an operant chamber 6 days a week for 4 weeks, where they were allowed to nose-poke for cocaine (0.4 mg/kg/infusion in 50 µL of saline administered over 1.6 s) on a continuous reinforcement schedule (FR1) with a time-out of 20 s. A training session commenced with the illumination of the "active" nose-poke hole stimulus light. Responding in this hole resulted in drug delivery. Responding in the other nose-poke hole, designated inactive, had no consequences. Rats were initially trained during daily one hour sessions for a total of six days. Animals that did not acquire stable self-administration behavior (at least 5 infusions each day for 3 consecutive days) were removed from the study. After the initial 6-day training period animals were divided into two groups: a long access group (LgA) and a short access group (ShA). These groups were balanced according to both the amount of drug they administered during the first week of training and their performance during attention training. Animals in the ShA group continued to receive one-hour test sessions, whereas animals in the LgA group received six-hour test sessions for an additional 16-17 sessions. A third group of rats (no-drug control group) received sham surgery and were transported each day to a novel test room where they were placed into Plexiglas chambers, similar to the operant chambers.

Sustained Attention Testing. One day following four weeks of drug self-administration (or transport in the no-drug control groups), animals were retested on the sustained attention task (n=12-16). Animals were tested again two weeks after the last self-administration session, followed by daily retraining for 1 week following the test on day 14.

Experiment II: Between-Subjects Study.

Experiment II utilized a between-subjects design to control for potentially confounding effects of repeated testing on the performance on the attention task in Experiment I. This study also examined performance on the sustained attention task one month after the last self-administration session to determine the persistence of any drug-induced cognitive deficits. Along with the behavioral analysis, Experiment II was also designed to examine changes in the expression of dopamine D1 and D2 receptor mRNA in the prefrontal cortex.

The methods for sustained attention task training and drug self-administration were identical to those in Experiment I.

Sustained Attention Testing. Following the completion of self-administration, animals in the No Drug, ShA, and LgA groups were divided into two more groups. One group (n=5-6) was tested on the attention task 1 day following the

last self-administration session and one group (n=6-8) was tested 30 days following the last self-administration session.

Tissue Collection. Three days following their respective sustained attention testing session (at days 4 & 33) all animals were decapitated and their brains removed and frozen in isopentane (-40 to -50°C) on dry ice. The brains were then stored at -80°C. Coronal brain sections (10 µm) were cut on a cryostat at 200 µm intervals, thaw mounted on Superfrost/Plus slides (4 sections per slide, Fisher Scientific, Pittsburgh, PA) and stored at -80°C until further processing.

In Situ Hybridization. For detailed methodology of the *in situ* hybridization techniques used see Kabbaj et al. (2000). Following fixation, sections were hybridized with ^{35}S-labeled cRNA probes produced using standard in vitro transcription methodology. Prefrontal cortex sections were processed for *in situ* hybridization of dopamine D1 and D2 receptor mRNA (480-mer). The probes were diluted in hybridization buffer and slides were coverslipped and incubated overnight at 55°C. Following rinses and dehydration, slides were exposed to x-ray film (Kodak Biomax-MR, Eastman Kodak, Rochester, NY) for 2 weeks. The specificity of the hybridization signal was confirmed with sense probe controls (data not shown).

Quantification. Autoradiographs were digitized using a Scanmaker 1000XL scanner (Microtek, Carson, CA) and the magnitude of the signal of the

radioactive probes was determined using Scion Image (Scion Corporation, Frederick, MD). A macro was used to automatically determine signal above background in each brain region (written by Dr. S. Campeau, University of Colorado, Boulder, CO). A background value was obtained from a white matter region such as the corpus callosum in each section and only values exceeding the background by 3.5 times the standard deviation were included in the analysis. Results are expressed as the mean integrated density of signal pixels divided by the total number of pixels in the selected region. The person quantifying was blind to group assignments. Anatomical regions were identified and subdivided for densitometric analysis according to the stereotaxic atlas of Paxinos and Watson (Paxinos and Watson, 1998). More specifically, the cingulate, prelimbic, and infralimbic subregions of the PFC at 2.7mm anterior from bregma were quantified. As the same pattern of results was found in all three subregions, we collapsed these regions into a single region referred to as the prefrontal cortex.

Experiment III. D2 Receptor Protein

Experiment III was designed to examine the effects of limited vs. extended access to self-administered cocaine on levels of DA D2 receptor protein in the prefrontal cortex. The methods for drug self-administration were identical to those in Experiment I.

Tissue Collection and Sample Preparation. Three or thirty days following their final self-administration session animals were decapitated and their brains removed. The entire prefrontal cortex was dissected, fast frozen on dry ice and kept at -80°C until further processing. Frozen brain tissue was homogenized (1:10, wt/vol) in cold 50 mM Tris-HCl buffer, pH 6.8, containing 2% SDS, 1 mM EDTA, and various protease inhibitors as previously reported (Garcia-Fuster et al., 2007). Aliquots of total homogenate were mixed with equal volumes of electrophoresis loading buffer, denatured and stored at –20°C until use. Protein concentrations were determined by the BCA Protein Assay Reagent (Pierce Biotechnology, Rockford, IL, USA).

Western Blot Analysis. For western blot analysis, 33 μg of each protein sample (determined to be within the linear range for immunobloting of D2DR) was separated by electrophoresis on 10% acrylamide/bisacrylamide minigels and transferred electrophoretically to nitrocellulose membranes. The membranes were subsequently blocked for 1 hour at room temperature in phosphate buffered saline (PBS, in mM: 137 NaCl, 2.68 KCl, 10.14 Na_2HPO_4, 1.76 KH_2PO_4) containing 0.2% Tween-20, 0.5% bovine serum albumin (BSA), and 5% non-fat dry milk. Incubation with the primary antibodies (mouse anti-dopamine D2 receptor, 1:400 dilution, Santa Cruz Biotechnology, Inc., Santa Cruz, CA; mouse anti-β-actin, 1:10,000 dilution, Sigma-Adrich, St. Louis, MO) was performed overnight at 4°C. After extensive washing in PBS the membranes were incubated with the secondary antibody, horseradish peroxidase-linked anti-mouse IgG,

(1:5,000 dilution) in blocking solution at room temperature for 1 h. Immunoreactivity of target proteins was detected with the ECL Western Blot Detection system (Amersham International, Buckinghamshire, UK) and visualized by exposure to Hyperfilm ECL film (Amersham) for 60 s to 60 min. The autoradiograms were then quantified by densitometric scanning (IOD) using Image J software. Each protein sample was run on 3-4 gels and an average value for each animal was calculated. The amount of target protein in brain samples of treated rats was compared to an internal control sample in the same gel composed of prefrontal cortices from 3 control rats that had not received any treatment. This internal control was run 3 times on each gel and an average was calculated for comparison with other samples on that gel. Finally, percent changes in immunoreactivity with respect to internal controls (100%) were calculated for each sample in each of the various gels. In all the experiments the content of b-actin, a cytoskeletal protein not altered in brain by cocaine treatments (Imam et al., 2005), was quantified as a loading control.

Statistical Analyses

Cocaine Self-Administration. The number of infusions for the first hour of each session and for the entirety of the session was determined for each day of self-administration. Two-way repeated measures ANOVAs were performed with escalation session (1-16) as the repeated variable, drug group (ShA, LgA) as the independent variable and first hour or total infusions as the dependent variable.

When significant main effects or interactions were revealed, Fisher's post-hoc comparisons were conducted. For all analyses, alpha was set at 0.05.

Sustained Attention. For each sustained attention session, the number of hits, misses, correct rejections, false alarms, and omissions was calculated. To examine each animal's accuracy on the task, the relative number of hits [hits/(hits + misses)] for each signal length, and the relative number of correct rejections [correct rejections/(correct rejections + false alarms)] were calculated. In addition the vigilance index (VI), an overall measure of sustained attention performance, was calculated using the relative number of hits (h) and false alarms (fa) with the following formula: $VI= (h-fa) / [2(h+fa)-(h+fa)^2]$. This index is similar to the sensitivity index (Frey and Colliver, 1973) except that omitted trials are excluded from the calculation. The VI provides a sensitive measure of overall performance, taking into account both trial types; VI varies between 1 and -1, with 1 indicating that all responses were hits and correct rejections, 0 that responses were randomly distributed over the four response types (hits, misses, false alarms, correct rejections), and -1 that all responses were misses and false alarms. Performance on each of the trial types (signal vs. non-signal) was also examined to reveal the specific responses contributing to the overall deficits. Two-way ANOVAs were performed with withdrawal day (1 or 30) and drug group (ND, ShA, LgA) as the independent variables and VI or the relative number of hits or correct rejections as the dependent variables. When significant main effects or

interactions were revealed, individual group comparisons were made using either additional ANOVAs, or Fisher's post-hoc tests.

Gene Expression. The levels of mRNA in the experimental groups were expressed as a percent of the mean value for the control group. Group differences in gene expression were assessed using two-way ANOVAs with withdrawal time (4 or 33 days) and drug group (ND, ShA, LgA) as the independent variables and percent of control mRNA levels as the dependent variable. Fisher's post-hoc comparisons were conducted when main effects or interactions were present.

Western Blot Analysis. The levels of D2 receptor protein were expressed as a percent of internal control values for each individual gel (see above). Group differences in protein expression were assessed using a two-way ANOVA with withdrawal time (3 or 30 days) and drug group (ND, ShA, LgA) as the independent variables and percent of control protein levels as the dependent variable.

Results

Experiment I: Within-Subjects Study

Cocaine Self-administration. Following the initial 6-day training period ShA animals continued with 1-hour self-administration sessions and LgA animals were given 6 hr daily sessions. Figure 2.2A shows the number of infusions taken during the first hour of each of these sessions, and Figure 2.2B the number of infusions over the entire session. The ShA group showed a small but statistically significant increase in cocaine intake over time. However, the LgA group showed a much greater increase in both their total intake and their intake during the first hour of each session, as indicated by significant group by time interactions (Figure 2.2).

Sustained Attention Performance. Animals were trained on the sustained attention task for approximately 8 weeks prior to drug self-administration and animals assigned to the LgA, ShA and no-drug groups were matched based on training performance (Figure 2.3 a & d). Twenty-four hours following the final self-administration session animals in the LgA group were markedly impaired on the sustained attention task, as illustrated by the decrease in VI relative to no-drug controls. In contrast, the ShA group did not differ from control (Figure 2.3b). The performance of animals in the LgA group was impaired with respect to their ability to detect signals as well as to respond correctly to non-signal events

Figure 2.2. A. Mean (+/- SEM) number of cocaine infusions during the first hour of each self-administration session in Experiment I. Both the ShA (One-way ANOVA, day, $F(15,240)=1.86$, $p=.03$) and the LgA groups (One-way ANOVA, day, $F(15,224)=4.89$, $p<.0001$) increased their intake over time, but this effect was much greater in the LgA group than the ShA group, as indicated by a significant group by time interaction [main effect of group, $F(1,15)=16.49$, $p=0.0003$, main effect of day, $F(15,435)=12.87$, $p<0.0001$, group by day interaction, $F(15,435)=2.90$, $p=0.0002$]. By the 7th session, animals allowed extended access to cocaine (long access, 6 hr session; LgA) took significantly more infusions than animals allowed only limited access (short access, 1 hr sessions; ShA) B. The mean (+/- SEM) number of cocaine infusions over the entire session for LgA (6 hr sessions) and ShA (1 hr sessions) groups. (Note that similar data were obtained in Experiments II and III, but in these two experiments there was no significant change in drug intake over time in the ShA groups. The self-administration data for Experiments II and III are not shown because the graphs look very similar to the data presented here.

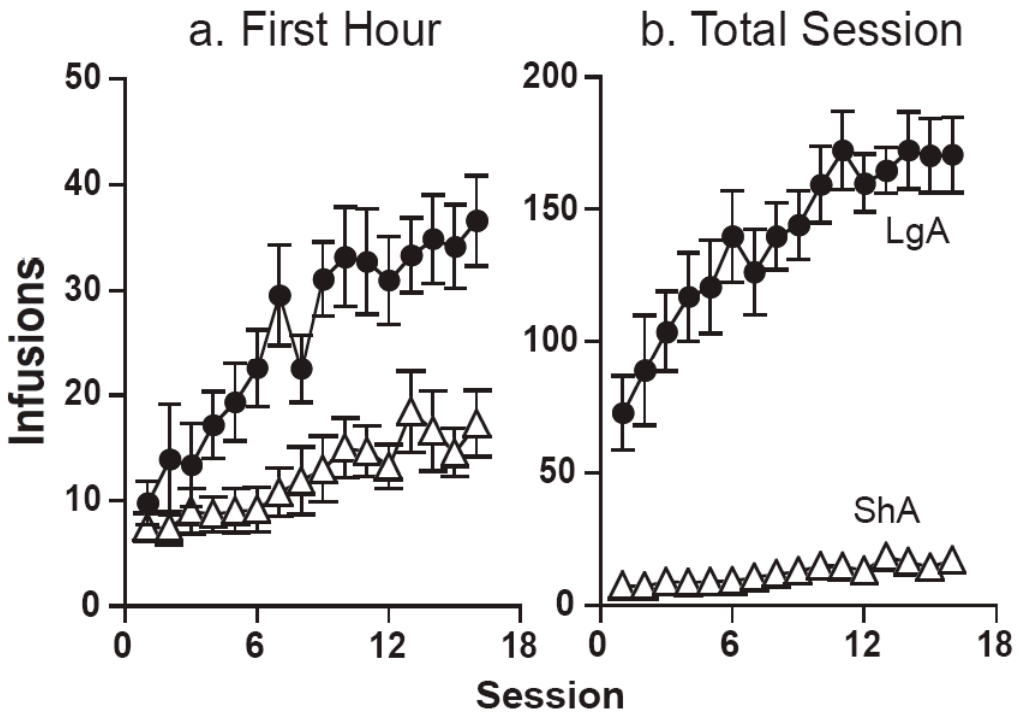
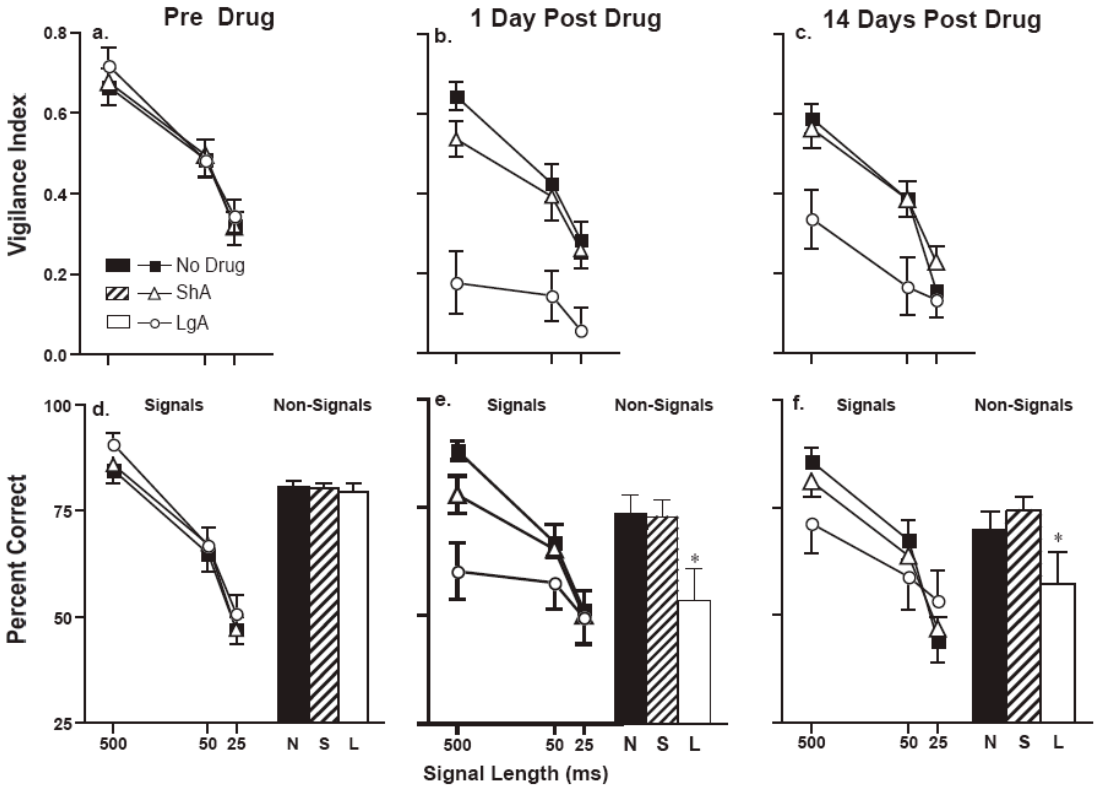

a. First Hour
b. Total Session
Infusions
Session
LgA
ShA
0
10
20
30
40
50
0
50
100
150
200
0
6
12
18

Figure 2.3. The effect of extended vs. limited access to self-administered cocaine on performance on the sustained attention task as a function of time following the last self-administration session. The top three panels show the data expressed as a vigilance index (VI), which provides an overall index of performance incorporating both signal and non-signal trials (see Methods). The bottom three panels show the percent correct responses on both signal trials (as a function of signal duration; line graph) and non-signal trials (bar graph). The panels on the left (a, d) show performance on the last day of training, prior to any cocaine self-administration experience, in the animals that will eventually be given limited (ShA) or extended (LgA) access to cocaine, or no treatment (No Drug). The groups were balanced to equate performance at the end of training, so there are no group differences on any measure at this point in time. The panels in the middle (b, e) show performance one day following the final self-administration session. Panel b shows that at this time there was a significant decrease in VI in the LgA group relative to both the No Drug and ShA groups, which did not differ from one another (Overall ANOVA, main effect of group, comparing all groups, $F(2,80)= 11.49$, $p=0.0001$, group X signal length interaction, $F(4,80)= 5.68$, $p=.0004$. Individual group comparisons: LgA vs. ND, group, $F(1,50)= 17.13$, $p=0.0003$, interaction, $F(2,50)=11.59$, $p<.0001$; LgA vs. ShA, group, $F(1,58)=12.56$, $p=0.0014$, interaction, $F(2,58)=4.36$, $p=0.0172$; ShA vs. No Drug, group, $F(1,52)=.77$, $p=0.3867$, interaction, $F(2,52)=1.64$, $p=0.2$). Panel e shows that the LgA group performed more poorly than either the ShA or No Drug groups on both signal and non-signal trials, and the ShA and No Drug groups did not differ from one another on either trial type [Signal Trials Overall ANOVA, interaction, $F(4,90)= 6.92$, $p<.0001$. Individual group comparisons: LgA vs. ND, interaction, $F(2,50)=13.57$, $p<.0001$; LgA vs. ShA, interaction, $F(2,58)=5.37$, $p=0.007$; ND vs. ShA, interaction $F(2,52)=2.2$, $p=0.12$. Non-signal Trials ANOVA, $F(2,40)=4.28$, $p=0.02$; *, differs from ShA and No Drug groups as determined by Fisher's Tests. The panels on the right (c, f) show performance when the same animals were retested 14 days following the final self-administration session. Panel c shows that at this time there was still a significant decrease in VI in the LgA group, relative to both the No Drug and ShA groups [Overall ANOVA, group, $F(2,80)= 4.78$, $p=.01$, interaction, $F(4,80)= 4.28$, $p=0.004$. Individual group comparisons: LgA vs. ND, group, $F(1,50)=5.48$, $p=.028$, interaction, $F(2,50)=6.09$, $p=0.004$; LgA vs. ShA, group, $F(1,58)=5.69$, $p=0.016$, interaction, $F(2,58)=3.31$, $p=.04$; ND vs. ShA, group, $F(1,52)=0.077$, $p=0.78$, interaction, $F(2,52)=2.24$, $p=0.12$). However, by this time the deficit was primarily a function of impaired performance on non-signal trials [see Panel f, group, $F(2,40)=2.9$, $p=0.06$; *, differs from ShA and No Drug groups as determined by Fisher's Tests].


Pre Drug
1 Day Post Drug
14 Days Post Drug
a.
b.
c.
Vigilance Index
0.8
0.6
0.4
0.2
0.0
No Drug
ShA
LgA
d.
e.
f.
Signals
Non-Signals
Percent Correct
100
75
50
25
500
50
25
N
S
L
*
Signal Length (ms)

(Figure 2.3e). Fourteen days following the last self-administration session the LgA animals were still impaired relative to the control group, as indicated by a significant decrease in VI (Figure 2.3c). However, by 14 days of withdrawal the deficit was due primarily to an increase in the number of false alarms ("claims" for signals on non-signal trials), as there was no longer a significant effect of prior self-administration on signal trial performance (Figure 2.3f). Following the test on day 14, animals were tested daily for an additional 7 days. Throughout this retraining period (Figure 2.4), there were no group differences on signal trials, i.e., in the relative number of hits (Figure 2.4a). However, the LgA group continued to show a significant deficit on non-signal trials relative to the control and ShA groups throughout this period (Figure 2.4b). It is important to note that there were no group differences in either response latencies or the number of omissions at any point in time, indicating that all animals were equally motivated to perform the task (Figure 2.5).

Experiment II. Between-subjects study

Cocaine Self-administration. As in Experiment I, ShA and LgA groups were balanced to match drug intake during the initial 6-day self-administration training period. Animals then allowed extended access to cocaine showed a progressive increase in both their total intake and their intake during the first hour of each session [main effect of drug condition, $F(1,768)=19.92$, $p<0.0001$, main effect of day, $F(16,768)=6.05$, $p<0.0001$, interaction, $F(16,768)=3.11$, $p<0.0001$, pair-wise

Figure 2.4. The effect of one week of “retraining” on the sustained attention task beginning 14 days after the last self-administration session. Data are shown as percent correct responses (a) on signal trials in which the signal duration was 500 msec, and (b) on non-signal trials. There were no group differences on signal trials over this period of time (Overall ANOVA, group, $F(2,240)=1.41$, $p=0.25$). However, on non-signal trials the LgA group showed significantly fewer correct responses than either of the other two groups [Overall ANOVA, group, $F(2,240)= 3.80$, $p=0.03$. Individual group comparisons: LgA vs. ND, group, $F(1,150)=4.15$, $p=0.05$, interaction, non-significant (NS); LgA vs. ShA, group, $F(1,174)=4.1$, $p=0.05$, interaction, NS; ND vs. ShA, group, $F(1,156)=.18$, $p=0.68$, interaction, NS].

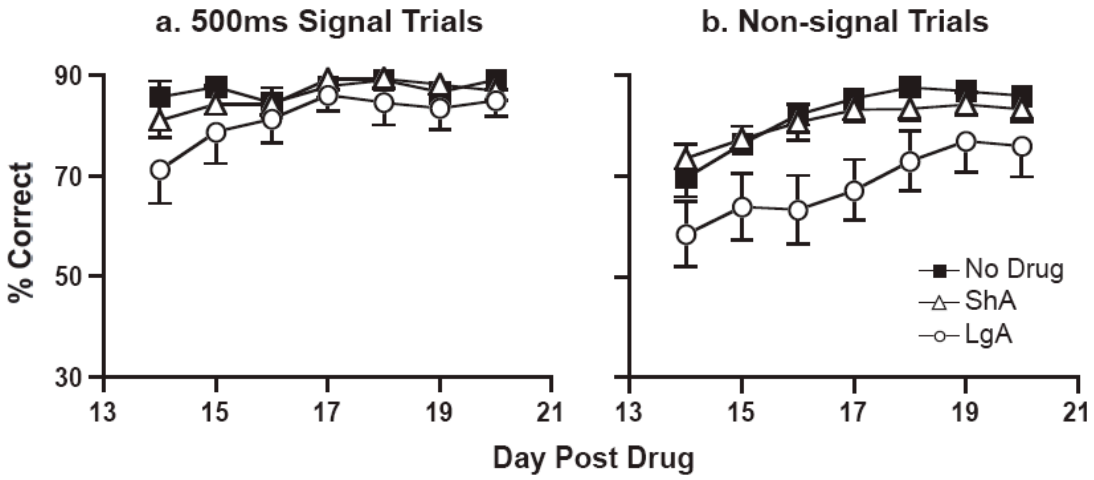
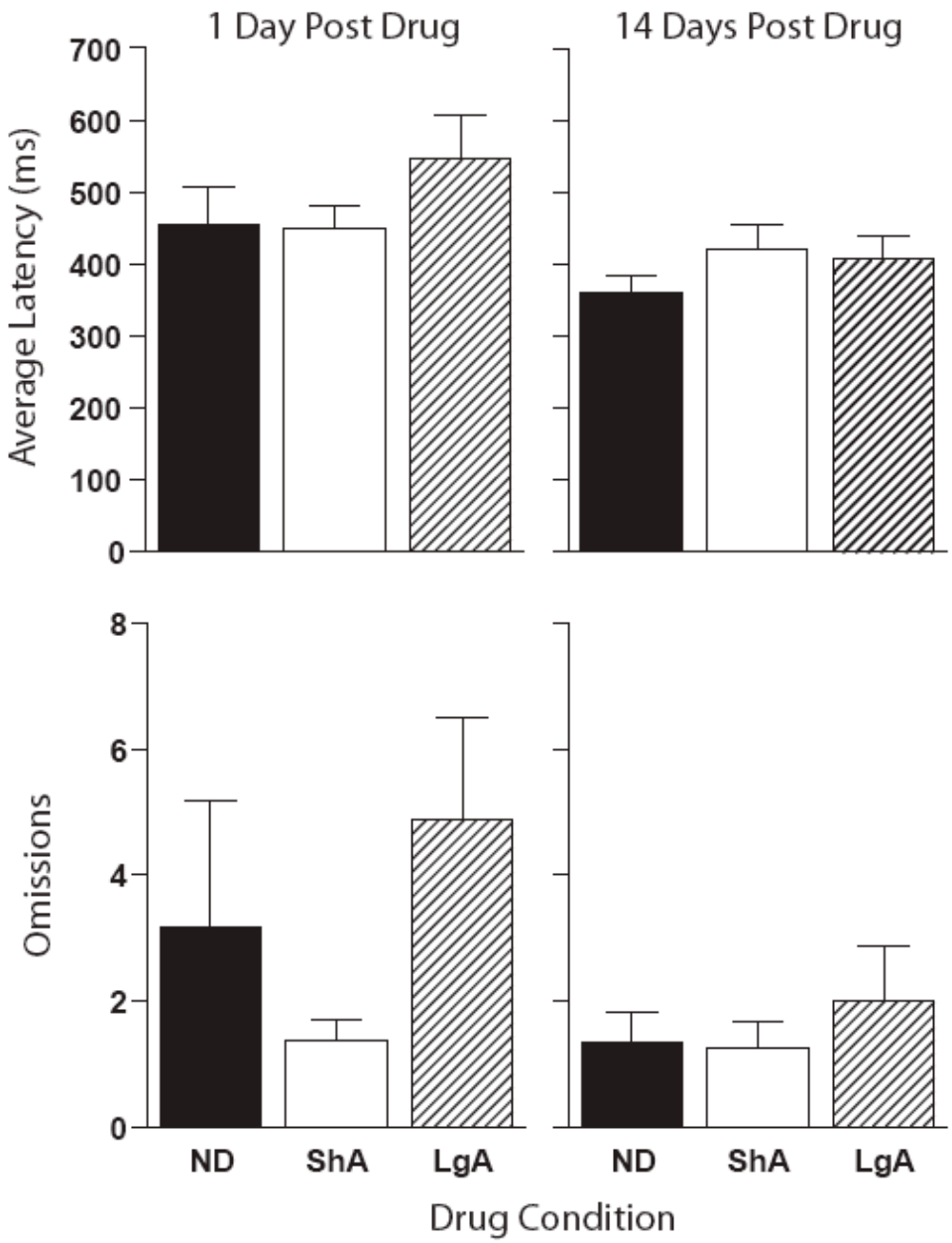

a. 500ms Signal Trials
b. Non-signal Trials
% Correct
90
70
50
30
13
15
17
19
21
Day Post Drug
No Drug
ShA
LgA

Figure 2.5. The effect of extended vs. limited access to self-administered cocaine on response latency and omission rate (Mean ± SEM) as a function of time following the last self-administration session in Experiment I. No differences were found between the drug groups in either response latencies (Overall ANOVA, group, $F(2,24)=3.01$, $p=.41$, withdrawal time, $F(1,24)=13.09$, $p=.001$, interaction, $F(2,24)=1.95$, $p=.17$) or omission rates (Overall ANOVA, group, $F(2,40)=2.12$, $p=.13$, withdrawal time, $F(1,40)=3.11$, $p=.09$, interaction, $F(2,40)=.84$, $p=.44$).

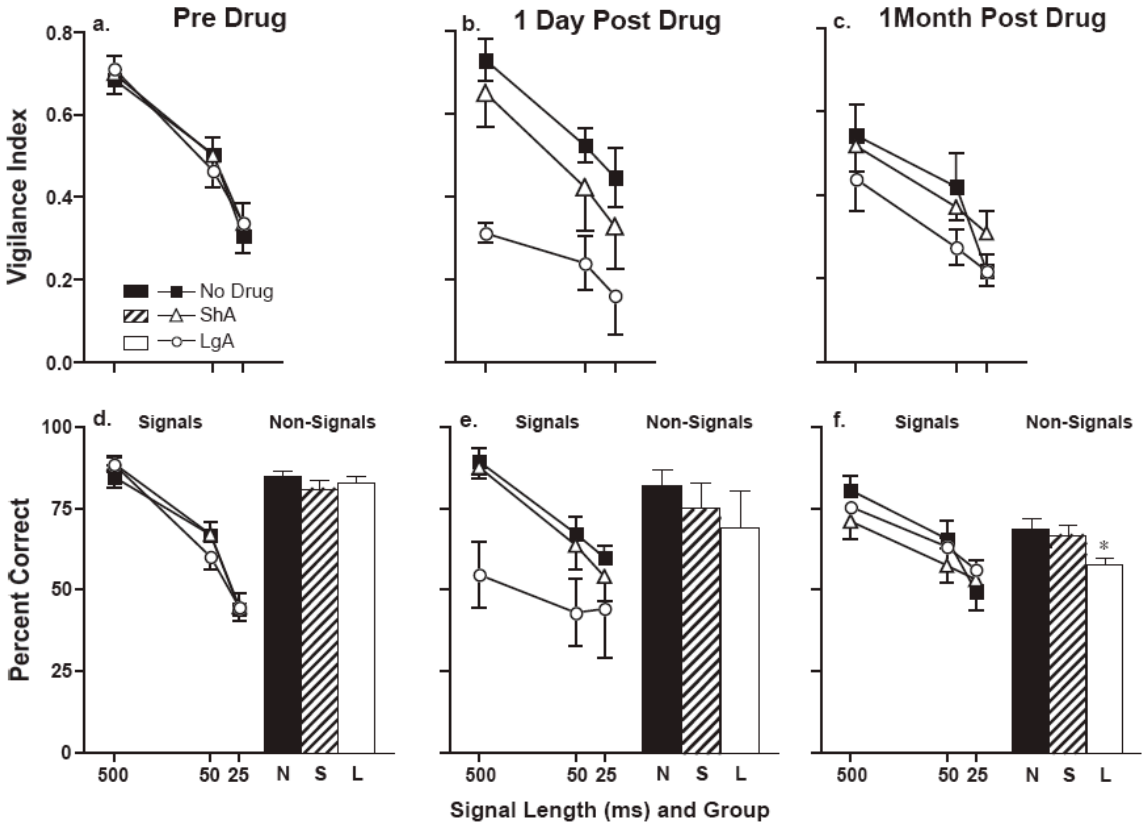

1 Day Post Drug
14 Days Post Drug
Average Latency (ms)
700
600
500
400
300
200
100
0
Omissions
8
6
4
2
0
ND
ShA
LgA
ND
ShA
LgA
Drug Condition

comparisons, $p<0.05$ (LgA vs. ShA, days 8-17)]. In this experiment there was no significant change in drug intake in the ShA group over days of testing (One-way ANOVA, day, $F(16,340)=.64$, $p=.85$). These data are very similar to those described in Experiment 1 (Figure 2.2) and are therefore not shown.

Sustained Attention Performance. All groups were balanced based on their attention training performance (Figure 2.6a & d). As in Experiment 1, when tested 24 hours after the final self-administration session animals in the LgA group were markedly impaired on the sustained attention task, as indicated by a significant decrease in VI (Figure 2.6b). This was due to a decrease in performance on both signal and non-signal trials, although only the effect on signal trials was statistically significant (Figure 2.6e). The absence of a significant effect on non-signal trials, as seen in Experiment I, was probably due to a lack of power because a relatively small number of animals were tested at this time point. A larger independent group of LgA animals was tested 30 days after the last self-administration session, and these animals were significantly impaired on non-signal, but not signal trials (Figure 2.6f). There were no differences between the ShA and control groups at either point in time. Also, as in Exp. I, the LgA group did not differ from controls in either response latencies or rate of omissions at either time point examined (data not shown).

In Experiment I and II the LgA group spent 6 hours per day actively engaged in an operant task during the 4 week self-administration period, whereas the ShA

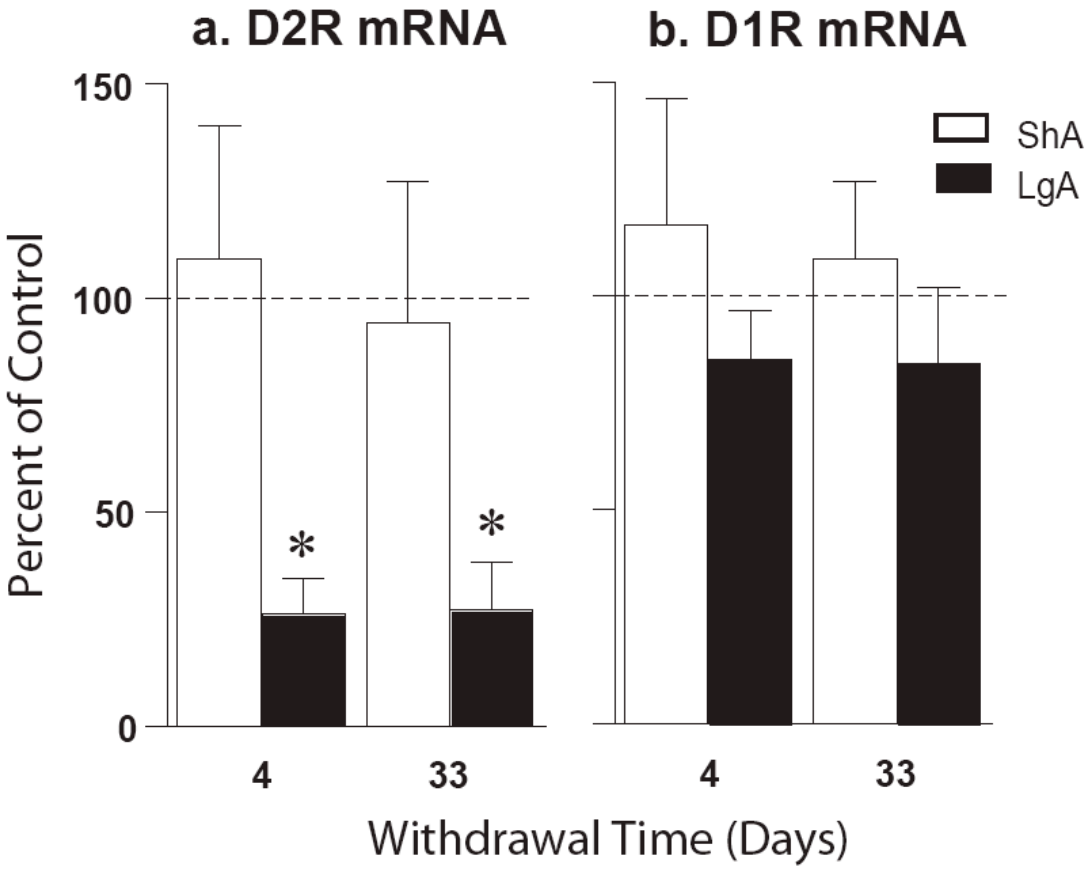
Figure 2.6. The effect of extended vs. limited access to self-administered cocaine on performance on the sustained attention task as a function of time following the last self-administration session in Experiment 2. The data are presented as described for Figure 2.3. As in Experiment I, there was a significant decrease in VI in the LgA group relative to both the No Drug and ShA groups when animals were tested one day after the last self-administration session, and the latter two groups did not differ from one another [Panel b; Overall ANOVA, group, $F(2,26)=6.06$, $p=0.01$. Individual group comparisons: LgA vs. ND, group, $F(1,16)=32.49$, $p=0.0005$; LgA vs. ShA, group, $F(1,18)=4.1$, $p=0.05$; ND vs. ShA, group, $F(1,18)=.85$, $p=0.38$]. Panel e shows that the LgA group made significantly fewer correct responses on signal trials [Overall ANOVA, group, $F(2,39)= 5.6$, $p=0.007$. Individual group comparisons: LgA vs. ND, group, $F(1,24)=7.7$, $p=0.01$; LgA vs. ShA, group, $F(1,27)=5.4$, $p=0.028$; ND vs. ShA, group, $F(1,27)=.65$, $p=0.45$), but the effect on non-signal trials was not statistically significant [($F(2,16)=.52$, $p=0.35$], presumably because a relatively small number of animals were tested on this day. The panels on the right (c, f) show the performance of an independent group of animals tested for the first time 30 days following the final self-administration session. At this time there were no significant group differences on VI [Overall ANOVA, group, $F(2,38)=0.53$, $p=0.56$, interaction, $F(4,38)=1.88$, $p=0.1$]. Panel f shows that again, as in Experiment 1, when tested long after the last self-administration session the LgA group was not impaired in their performance on signal trials [Overall ANOVA, main effect of group, $F(2,58)=0.37$, $p=0.69$] but was impaired on non-signal trials [Overall ANOVA, $F(2,29)= 3.37$, $p =0.04$, ; *, differs from ShA and No Drug groups as determined by Fisher's Tests].


a.
Pre Drug
b.
1 Day Post Drug
c.
1Month Post Drug
Vigilance Index
0.8
0.6
0.4
0.2
0.0
No Drug
ShA
LgA
d.
Signals
Non-Signals
e.
Signals
Non-Signals
f.
Signals
Non-Signals
Percent Correct
100
75
50
25
0
500
50 25
N
S
L
Signal Length (ms) and Group
*

group only spent 1 hour per day and the ND animals none. It is possible, therefore, that group differences in time spent performing this instrumental response could in some way have contributed to group differences in performance when the animals were retested on the sustained attention task task. To address this issue we conducted a separate control experiment in which rats were given extended experience (3 weeks) making a similar instrumental response (nose-poking for a food reward), and then 24 hrs later retested on the sustained attention task. There was no effect of 3 weeks of training on a food-rewarded instrumental task (relative to controls that did not work for food) when animals were re-exposed to the sustained attention task 24 hrs following the last session of instrumental training (data not shown). We conclude, therefore, that group differences in the time spent simply making an instrumental response during the self-administration phase of Experiments I and II were not responsible for the subsequent group differences in performance on the sustained attention task.

Dopamine Receptor mRNA Expression in the Prefrontal Cortex. Brains were obtained for *in situ* hybridization histochemistry 3 days after the final test on the sustained attention task, which for one group was 4 days and for the other group 33 days following the last self-administration session. There were no differences between the control and ShA groups in D2 mRNA expression in the prefrontal cortex at either time point (Figure 2.7a). In contrast, relative to the control group, the LgA animals showed a significant (approximately 70%) decrease in D2

Figure 2.7. Prefrontal dopamine D2 receptor (a) and D1 receptor (b) mRNA expression in rats allowed extended (LgA) or limited (SgA) access to self-administered cocaine, and then examined either 4 or 33 days after the last self-administration session. The data represent the mean (+ SEM) levels of mRNA in the LgA and SgA groups expressed as a percent of the average amount of mRNA in the No Drug control group (represented by the horizontal dashed lines). The LgA group showed a significant decrease in D2 receptor mRNA expression, relative to both the No Drug control group and the ShA group, which did not differ from one another [Overall ANOVA, group, $F(2,24)=3.87$, $p=0.03$, *, differs from ShA and No Drug groups as determined by Fisher's Tests, time, NS, interaction, NS]. There were no group differences in D1 mRNA expression in the prefrontal cortex (Overall ANOVA, group, NS, time, NS).


a. D2R mRNA
b. D1R mRNA
150
100
50
0
Percent of Control
ShA
LgA
*
*
4
33
4
33
Withdrawal Time (Days)

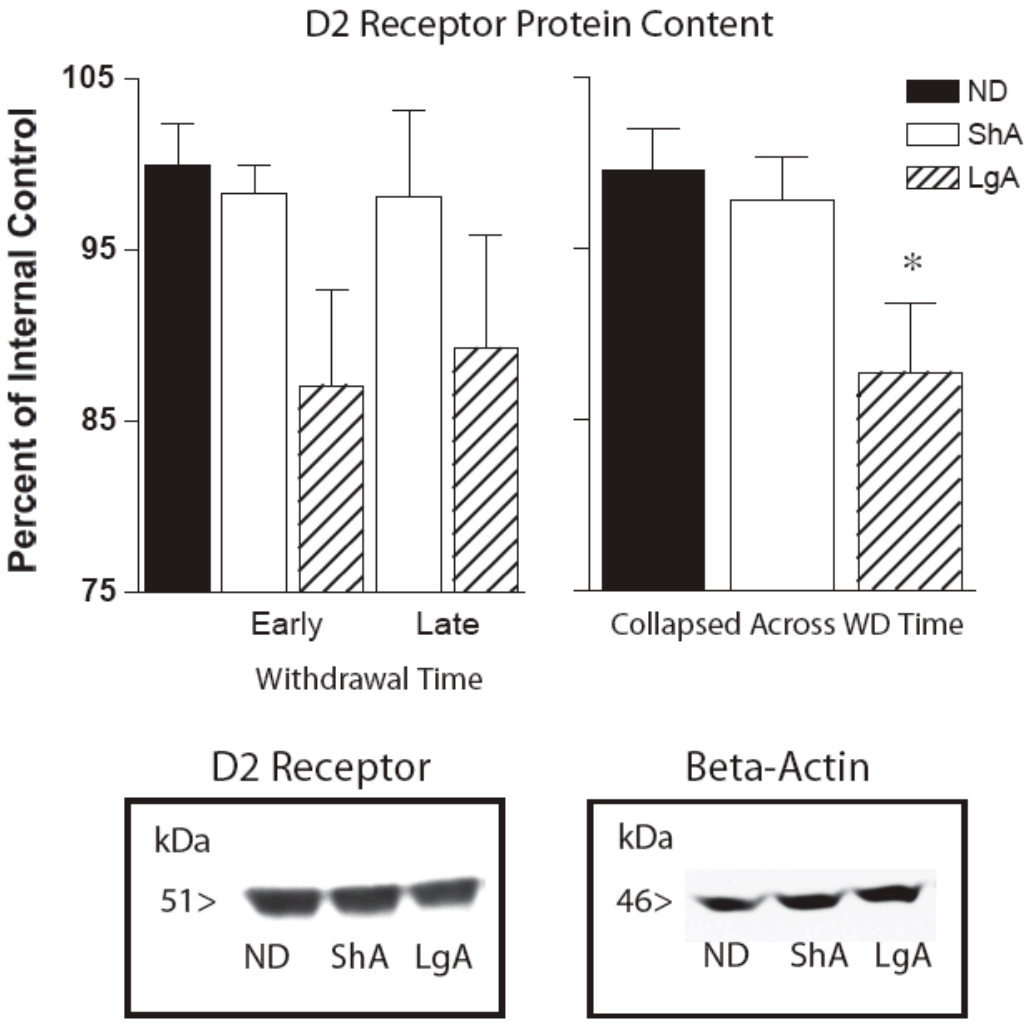
mRNA expression in the prefrontal cortex, which was evident at both 4 and 33 days after the last self-administration session (Figure 2.7a). There were no group differences in levels of D1 receptor mRNA in prefrontal cortex (Figure 2.7b)

Experiment III. D2 Receptor Protein in Prefrontal Cortex

Cocaine Self-administration. As in Experiment I & II, ShA and LgA groups were balanced to match drug intake during the initial 6-day self-administration training period. Animals allowed extended access to cocaine showed a progressive increase in both their total intake and their intake during the first hour of each session [main effect of drug condition, $F(1,476)= 64.24$, $p<0.0001$, main effect of day, $F(17,476)=6.61$, $p<0.0001$, interaction, $F(17,476)= 6.97$, $p<0.0001$, pair-wise comparisons, $p<0.05$ (LgA vs. ShA, escalation days 7-18)]. As in Exp. II, the ShA group did not significantly change drug intake over time (One-way ANOVA, day, $F(17,270)=1.57$, $p=.07$). These data are similar to those described in Experiment 1 (Figure 2.2) and are therefore not shown.

Western Blot Analysis. There were no significant differences between the control and ShA groups in D2 receptor protein levels in the prefrontal cortex at either withdrawal time point (Figure 2.8). In contrast, relative to the control group, the LgA animals showed a significant (about 10%) decrease in D2 receptor protein, as indicated by a significant main effect of group but no effect of time or a group by time interaction (Figure 2.8).

Figure 2.8. Prefrontal dopamine D2 receptor protein levels in rats allowed extended (LgA), limited (ShA) or no (ND) access to self-administered cocaine examined either 3 or 30 days after the last self-administration session. The data represent the mean (+SEM) of D2 receptor protein levels relative to an internal control. The LgA group showed a significant decrease in D2 receptor protein levels, relative to both the No Drug control group and the ShA group, which did not differ from one another (Overall ANOVA, group, $F(2,42)=4.08$, $p=0.02$, withdrawal time, $F(1,42)=.04$, $p=.83$, interaction, $F(2,42)=.28$, $p=.76$). As there is no effect of withdrawal time and no interaction, the data were collapsed across the two time points to demonstrate that the group effect is due to a decrease in LgA group (panel b; *, differs from ShA and No Drug groups as determined by Fisher's Tests). No group differences were seen in a beta-actin control.


D2 Receptor Protein Content
Percent of Internal Control
105
95
85
75
ND
ShA
LgA
*
Early
Late
Withdrawal Time
Collapsed Across WD Time
D2 Receptor
kDa
51>
ND
ShA
LgA
Beta-Actin
kDa
46>
ND
ShA
LgA

Discussion

Effects of past experience with self-administered cocaine on cognitive function

The day after the discontinuation of cocaine self-administration, during acute withdrawal, animals that had extended access to self-administered cocaine (LgA) had a profound deficit on the sustained attention task, as indicated by a large decrease in the vigilance index (VI), and by impairments on both signal and non-signal trials. In contrast, animals that had limited access to cocaine (SgA) showed no deficits. With time (within 14 days) performance on signal trials recovered in the LgA group. However, the deficit on non-signal trials persisted for at least 30 days. Experimenter-administered cocaine has been reported to produce persistent cognitive deficits in both rats (Schoenbaum et al., 2004; Burke et al., 2006) and monkeys (Jentsch et al., 2002), but to our knowledge this is the first report of a persistent cognitive deficit in non-human animals following cocaine self-administration experience.

It is not clear why persistent deficits in cognitive function have not been found in previous self-administration studies. However, most previous studies involved limited access procedures and it is possible that these do not produce some of the changes in brain and behavior characteristic of addiction. Indeed, accumulating evidence suggests that extended access to cocaine has a greater likelihood of producing a number of symptoms of addiction, relative to limited

access, one of which is the tendency to greatly escalate drug intake (Ahmed and Koob, 1998; Paterson and Markou, 2003; Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Ferrario et al., 2005). However, in one previous study, rats were allowed to self-administer a maximum of 150 injections of cocaine over 8 hours, and these animals achieved a level of intake comparable to the LgA group here, but did not show persistent deficits on a 5-choice serial reaction time task (2005a). There are at least two possible reasons for this. One, the self-administration procedure used by Dalley et al. (2005a) may not have resulted in an escalation of drug intake or other changes in brain and behavior characteristic of addiction. However, a more likely reason is that in the 5-choice serial reaction time task all trials are signal trials, in that animals are required to report the location of a brief visual stimulus. We also saw only a transient deficit on signal trials, and in this sense the results reported here are consistent with those reported by Dalley and his colleagues (2005a; 2005b).

In the present study there was a persistent deficit only on non-signal trials, and therefore, it is important to consider how the cognitive demands of the task used here differ as a function of trial type, and what this might tell us about the nature of the deficit. First, it is important to emphasize that there were no group differences in omissions or response latencies, suggesting that the animals were equally motivated to perform and did not suffer from obvious sensorimotor deficits. In contrast, in studies using the 5-choice serial reaction time task drug treated animals did show a significant increase in omissions and response

latencies, complicating interpretation of the nature of their deficit (2005a; 2005b). In addition, after the initial withdrawal period LgA animals' signal trial performance was normal suggesting that the deficits are not attributable to a failure to retrieve the rules governing the responses in the two types of trials. Nevertheless, on non-signal trials the LgA animals generated an abnormally high number of false alarms, reporting a signal had occurred when it had not. Although more work is required to determine the exact nature of this deficit, the behavioral and cognitive operations underlying the performance during non-signal trials certainly differ from those governing signal trials, and/or tasks that involve exclusively signal trials. On signal trials the correct response is prompted by the presence of a visual stimulus and animals typically select the correct lever side and await lever insertion to respond. On non-signal trials, however, animals must first probe their memory as to whether or not a signal occurred before initiating an action. This may not only be more demanding in terms of taxing memory, but it also represents a more ambiguous situation, as the decision that a signal did not occur would be expected to be associated with high levels of uncertainty and therefore a reduced response criterion. Finally, performance on this task also taxes cognitive flexibility, which is required to shift constantly between the processing of the conflicting response rules governing the unpredictable occurrence of trials with or without signal events. Therefore, we hypothesize that prior cocaine use may have produced a persistent impairment in the cognitive flexibility required to respond correctly in trials involving high levels

of uncertainty about signal status (see also Yu & Dayan 2005), while leaving the ability to respond correctly to a signal intact.

Effects of past experience with self-administered cocaine on brain function

Perhaps the most striking finding in the present study was that extended (but not limited) access to self-administered cocaine decreased dopamine D2 (but not D1) receptor mRNA expression in prefrontal cortex by approximately 70%, and this effect persisted for at least 34 days after the last self-administration session. Furthermore, there was a significant decrease (about 10%) in levels of D2 receptor protein in the prefrontal cortex of LgA but not ShA animals, which also persisted for at least 30 days after the last exposure to cocaine. There has been relatively little research on the long-term effects of cocaine on D2 receptors in the frontal cortex, but Nogueira et al. (2006) recently reported that experimenter-administered cocaine produces a persistent (2-4 weeks) decrease in, "D2-mediated regulation of cortical excitability" (p. 12308). The alterations in D2 receptor mRNA and protein may be related to the well-documented "hypofrontality" seen in cocaine addicts (Bolla et al., 2003; Bolla et al., 2004), and in animals treated repeatedly with cocaine (Sun and Rebec, 2005; Beveridge et al., 2006; Sun and Rebec, 2006).

These data suggest, therefore, that self-administered cocaine is capable of producing a marked and persistent dysregulation of dopamine function in the

prefrontal cortex, which may represent another manifestation of marked frontal cortical dysfunction associated with cocaine addiction. Given that this effect was only seen in animals that received extended access to cocaine it is possible that dysregulation of dopamine D2 receptor signaling contributes to the cognitive deficit, which was also only seen in the LgA group. The integrity of D2 receptor function is closely associated with the ability to manipulate acutely relevant information in prefrontal regions (Wang et al. 2004) and inhibition of D2 receptor activation in both humans and animals, either by antagonist administration or genetic manipulation, results in a decrease in the acquisition of working memory task rules (Glickstein et al., 2005), attentional set-shifting (Tost et al., 2006), and spatial working memory (Von Huben et al., 2006; Rinaldi et al., 2007). This is consistent with the idea that cocaine impaired cognitive flexibility primarily by disrupting prefrontal D2 receptor function. Finally, we found no effect of cocaine self-administration experience on D1 mRNA expression in the prefrontal cortex, consistent with the recent report that experimenter-administered cocaine has no effect on D1 receptor sensitivity in this region (Nogueira et al., 2006).

Although the decrease in mRNA was large (~70%), the decrease in protein was much more modest (~10%). Discrepancies between mRNA and protein levels are not uncommon (Mansour et al., 1990; Mansour et al., 1991) and may be due to a number of different factors. As D2 receptor mRNA is localized primarily in the cell bodies of prefrontal neurons and D2 receptor protein is present in both presynaptic and postsynaptic terminals, the population of neurons sampled by

the two measures may be quite different. More research is needed to determine if the decreases seen in the current study are primarily due to changes in a specific cell type. It is also possible that rather than reflecting a cell type specificity, the dissociation between mRNA and protein is due to alterations in the translation of mRNA into protein. Nevertheless, despite the difference in the magnitude of the effect of cocaine on mRNA versus protein, both were decreased, indicating a potential persistent dysregulation of D2 function in the prefrontal cortex of animals that had extended access to self-administered Chcocaine.

Conclusions

Extended, but not limited, access to self-administered cocaine produced a persistent deficit on a sustained attention task indicative of reduced cognitive flexibility, and this was associated with a persistent decrease in both dopamine D2 receptor mRNA expression and D2 protein levels in the prefrontal cortex. These findings are consistent with the hypothesis that similar deficits in brain and cognitive function seen in addicts may be a consequence of drug use, rather than just reflecting a premorbid condition. Furthermore, the fact that these changes in brain and behavior were only evident in animals allowed extended assess to self-administered cocaine suggests that this procedure may be especially valuable in studying the neurobiology of addiction.

CHAPTER III

IMPAIRED OBJECT RECOGNITION MEMORY FOLLOWING EXTENDED ACCESS COCAINE SELF-AMINISTRATION

Summary

Chronic cocaine abusers exhibit memory deficits that persist following prolonged abstinence and understanding these deficits may help develop more effective treatment strategies for addicts. To this date, very little work has been done in animal models to determine whether these deficits are a result of drug use rather than a pre-existing vulnerability. Work out of our lab has recently demonstrated that extended access cocaine self-administration provides us with an excellent animal model for examining persistent cognitive deficits. This work in conjunction with work out of numerous other labs suggest that extended, but not limited, access to cocaine produces a number of symptoms characteristic of addiction. Thus, the present experiment utilized an extended access cocaine self-administration protocol found to induce escalation of drug intake and symptoms associated with drug dependence, to examine recognition memory performance following chronic cocaine exposure. Animals were divided into drug-naïve, short

(ShA) and long (LgA) access groups and underwent four weeks of cocaine self-administration. Following a two-week withdrawal period, all animals underwent object familiarization training and testing. When recognition memory was assessed either 24 hours or 7 days after training, LgA animals exhibit poorer recognition memory performance than controls. There is some evidence that the ShA animals may exhibit slight memory impairments, however this was only seen in one of the two replications during the 7-day test. Given the potential involvement in cognitive deficits in relapse, characterizing the memory deficits resulting from cocaine abuse may be important for developing better treatments for drug addiction.

Introduction

Cocaine addicts exhibit impaired memory function, including recognition and visuospatial memory, and these deficits are evident even long after the discontinuation of drug use (Miller, 1985; Hoff et al., 1996; Rosselli and Ardila, 1996; Bolla et al., 1999). Cocaine addicts also show alterations in the functional activation of brain regions important for normal memory function, including the hippocampus (Kilts et al., 2001; Fishbein et al., 2005). It is not clear, however, whether these behavioral and neurobiological deficits precede drug use, and thus contribute to the propensity for addiction, or are a consequence of drug use. It is difficult to address this issue in humans, but studies with non-human animals

allow one to determine whether exposure to drugs of abuse are at least capable of producing changes in brain and behavior comparable to those seen in addicts.

Studies with non-human animals examining the effects of psychostimulant administration on memory function have focused on the effects of methamphetamine. Marshall and colleagues have demonstrated that experimenter administered methamphetamine (mAMPH), but not d-amphetamine, produces deficits in short-term (90-min) recognition memory performance following one week of withdrawal (Schroder et al., 2003; Belcher et al., 2005b; Belcher et al., 2006). These deficits in recognition memory are seen following both neurotoxic (Schroder *et al.*, 2003) and nontoxic (Belcher *et al.*, 2006) methamphetamine regimens.

While these studies have demonstrated that experimenter-administered methamphetamine produces memory deficits, studies with cocaine have not been able to demonstrate persistent memory deficits (Santucci et al., 2004). However, previous work has utilized experimenter-administered drug rather that self-administration procedures that aim to model human use patterns. Work from our lab and numerous others suggests that extended access to cocaine self-administration produces a number of symptoms characteristic of addiction that are not seen following more limited drug access (Ahmed and Koob, 1998; Paterson and Markou, 2003; Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Ferrario et al., 2005; Briand et al., 2006). Therefore, the purpose of

the current study was to determine the ability of an extended access cocaine self-administration procedure to produce persistent deficits in recognition memory performance.

Methods

Subjects. Eighty-seven male Wistar rats (Harlan, Indianapolis, IL) weighing 200-225g at the start of the experiment were individually housed in square plastic hanging cages (8 x 9 x 8 cm). The animals were housed in a temperature and humidity controlled room with a 14:10 light/dark cycle, with water available ad libitum. Animals were food restricted throughout the experiment to maintain at least 90% of their free feeding body weight. All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA).

Apparatus. Drug administration took place in 16 operant chambers measuring 22 x 18 x 13 cm (Med Associates, St. Albans, VT, USA) located inside larger sound-attenuating chambers. For the drug self-administration procedure, each operant chamber had two nose-poke holes equipped with cue lights. A tone (2900 Hz) was present inside of the chamber. The floor of the chamber consisted of 19 stainless steel rods (4 mm in diameter) spaced 1.5 cm apart (center-to-center). Med-PC for Windows software (v. 1.1, Med Associates) controlled all drug delivery, tone presentation and data collection in each system via a Pentium PC. Object recognition familiarization and testing took place in a black triangular shaped open field (92cm along each side, 46cm high).

Surgical Procedures. Animals were anaesthetized with ketamine and xylazine anesthesia (77:1.5 mg/ml, intraperitoneal [IP], at 0.1 mL/100 gm of body weight) and a silicone catheter (Plastics One, Roanoke, VA) was inserted into the right jugular vein and passed subcutaneously to exit from the animals' back (see (Caine et al., 1993). Animals were allowed to recover from surgery for a minimum of 3 days prior to drug administration. Catheters were flushed daily with 0.1mL of gentamicin (50 mg/kg, in 0.9% sterile bacteriostatic saline).

Cocaine Self-Administration. Catheterized rats were transported from their home cage to an operant chamber 6 days a week for four weeks, where they were allowed to nose-poke for cocaine (0.4 mg/kg/infusion in 50 µL of saline administered over 1.6 s) on a continuous reinforcement schedule (FR1) with a time-out of 20 s. A training session commenced with the illumination of the "active" nose-poke hole stimulus light. Responding in this hole resulted in drug delivery. Responding in the other nose-poke hole, designated inactive, had no consequences. Rats were initially trained during daily one-hour sessions for a total of six days. Animals that did not acquire stable self-administration behavior (at least 5 infusions each day for 3 consecutive days) were removed from the study. After the initial 6-day training period animals were divided into two groups: a long access group (LgA) (n=16) and a short access group (ShA) (n=13). These groups were balanced according to both the amount of drug they administered during the first week of training. Animals in the ShA group

continued to receive one-hour test sessions, whereas animals in the LgA group received 6-hour test sessions for an additional 16 sessions. A third group of rats (no-drug control group) received sham surgery and were transported each day to a novel test room where they were placed into Plexiglas chambers, similar to the operant chambers.

Experiment I.

Object Recognition. Fourteen days following four weeks of drug self-administration (or transport in the no-drug control groups), animals were tested on a novelty preference task of object recognition. During the training phase, each animal was placed into the open field and given 5 min to explore two identical objects (positioned 10cm from the walls). Animals were tested 3 hours, 24 hours, and 1 week after training to determine short- and long-term memory retention. During each successive test animals were presented with the familiar object and a novel object and allowed to explore the open field for 5 min. The novel objects were changed for each test and randomized across animals. Object placement was counterbalanced so that half of the animals in each treatment group saw the novel object on the left side of the animals' starting position and half on the right. Objects were made from colorful plastic children's toys and were chosen after determining, in preliminary experiments with other animals, that they were equally preferred. Between each trial both the open field and the objects were washed with a 70% ethanol solution.

Experiment II.

As the previous experiment utilized the same familiar object for all three of the tests, we replicated the experiment with separate familiar objects for each test to eliminate the confound of further learning on test days.

Object Recognition. Training and testing were identical to Experiment I with the following exceptions. During the training phase, each animal was placed into the open field and given two 5 min exploration sessions (in which they were able to explore two identical objects; positioned 10cm from the walls) for each of two objects. Animals were then tested after 24 hours, and 1 week to determine memory retention. Separate familiar and novel objects were used for each test.

Statistical Analyses

Cocaine Self-Administration. The number of infusions for the first hour of each session and for the entirety of the session was determined for each day of self-administration. A two-way ANOVA was performed with test session (1-16) and drug group (ShA, LgA) as the independent variables and first hour or total infusions as the dependent variable. When significant main effects or interactions were revealed, Fisher's post-hoc comparisons were conducted. For all analyses, alpha was set at 0.05.

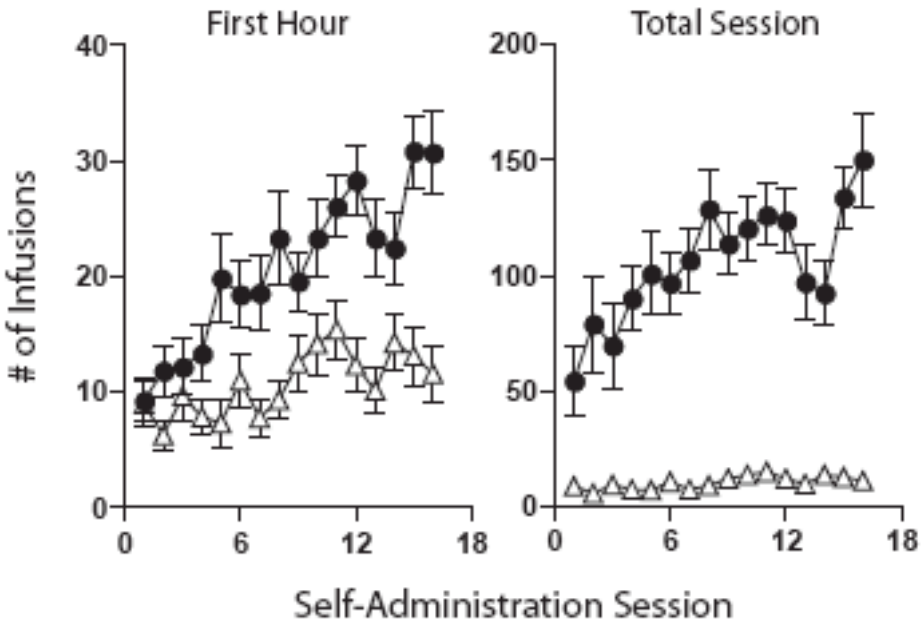
Object Recognition. Observers who were blind to the group assignments recorded the exploration time for each object. Exploration was defined as sniffing or touching the object with the nose; sitting on the object was not considered exploration. The proportion of the total exploration time that the animal spent investigating the novel object was the index of recognition memory [time spent exploring novel object/(time spent exploring novel object + time spent exploring familiar object)]. One-way ANOVAs were performed for each memory test (3hr, 24hr, 7d) with drug group as the independent variable and percent time exploring the novel object as the dependent variable. When significant main effects were revealed, Fisher's post-hoc comparisons were conducted. For all analyses alpha was set at 0.05.

Results

Experiment I.

Cocaine Self-administration. Following the initial 6-day training period ShA animals continued with 1-hour self-administration sessions and maintained stable drug intake over the next 16 test sessions. LgA animals were given 6 hours of daily access to cocaine and showed a progressive increase in both their total intake and their intake during the first hour of each session over the remaining 17 sessions (see Figure 3.1 for statistics).

Figure 3.1. A. Mean (+/- SEM) number of cocaine infusions during the first hour of each self-administration session. By the 5th session, animals allowed extended access to cocaine (long access, 6 hr session; LgA) took significantly more infusions than animals allowed only limited access (short access, 1 hr sessions; ShA) [main effect of group, $F(1,405)=18.30$, $p=0.0002$, main effect of day, $F(15,405)=9.36$, $p<0.0001$, group by day interaction, $F(15,435)=2.61$, $p=.0009$]. B. The mean (+/- SEM) number of cocaine infusions over the entire session for LgA (6 hr sessions) and ShA (1 hr sessions) groups.

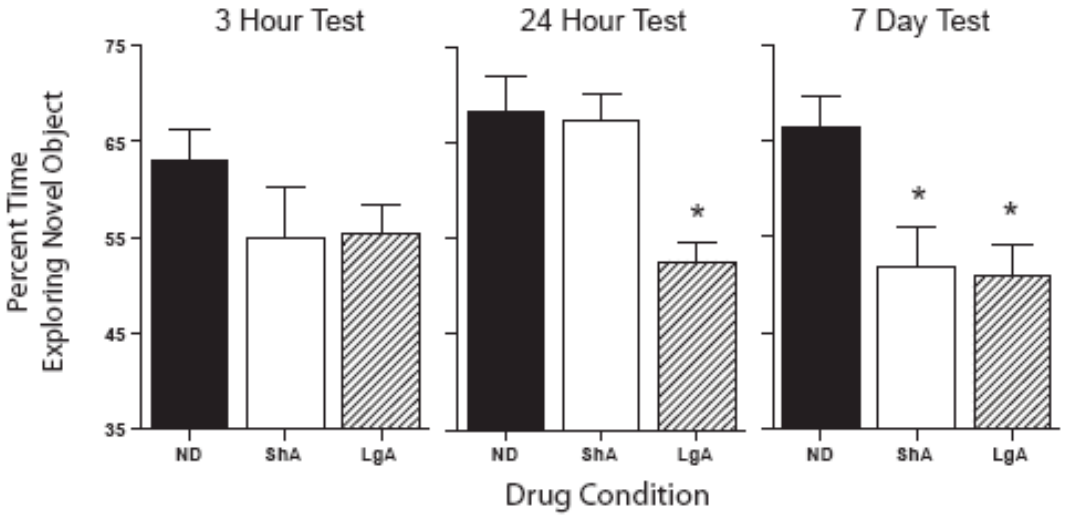

First Hour
Total Session
# of Infusions
40
30
20
10
0
200
150
100
50
0
0
6
12
18
Self-Administration Session

Object Recognition. No group differences were seen in the total time exploring the two objects during the training sessions (ND=61±3.3s, ShA=63±4.9s, LgA=66±4.4s; data not shown). No differences were seen between the three groups at the 3-hour short-term memory test (see Figure 3.2a). Twenty-four hours after training, animals in the LgA group showed a decrease in novel object exploration compared to both no drug and ShA animals, and these two groups did not differ from each other (Figure 3.2b). At the 1-week test, both LgA and ShA animals displayed a decrease in novel object exploration compared to the no drug controls (see Figure 3.2c). No differences were seen between the groups in total exploration time at any of the test time points (Figure 3.3).

Experiment II.

Cocaine Self-administration. Following the initial 6-day training period ShA animals continued with 1-hour self-administration sessions and maintained stable drug intake over the next 16 test sessions (Mean ± SEM infusions, first test session (following 6 training sessions), 6.18 ± 1.11, last test session, 5.09 ± 1.53; data not shown). LgA animals were given 6 hours of daily access to cocaine and showed a progressive increase in both their total intake and their intake during the first hour of each session over the remaining 17 sessions (Mean ± SEM infusions, First hour, first escalation session, 7.6 ± .96, last escalation session, 14.8 ± 4.3; Total session, first escalation session, 18.3 ± 2.3, last escalation session, 91.9 ± 23.0; data not shown).

Figure 3.2. The effect of extended vs. limited access to self-administered cocaine on performance on the novel object recognition memory task following a 14-day withdrawal period. The left hand panel (a) shows the percent object exploration time (as a function of the total open field time) during the familiarization phase. There were no differences in object exploration time between the groups (ANOVA, main effect of group, $F(2,33)=0.09$, $p=.92$). The three panels show the percent novel object exploration time (as a function of the total object exploration time) at the three retention intervals. Panel a shows that there were no differences between the three drug conditions 3 hours after the familiarization training (ANOVA, group, $F(2,42)=1.33$, $p=.28$). However, after 24 hours (panel b), there was a significant decrease in recognition memory performance in LgA animals relative to both the No Drug and ShA groups, which did not differ from one another (ANOVA, group, $F(2,42)=10.89$, $p=.0002$, *, differs from ShA and No Drug groups as determined by Fisher's Tests). One week following the familiarization training (panel c), both drug groups (LgA, ShA) exhibit impaired recognition performance relative to the No Drug control group (ANOVA, group, $F(2,42)=5.98$, $p=.005$, *, differs from the No Drug groups as determined by Fisher's Tests).


3 Hour Test
24 Hour Test
7 Day Test
Percent Time
Exploring Novel Object
75
65
55
45
35
ND
ShA
LgA
*
Drug Condition

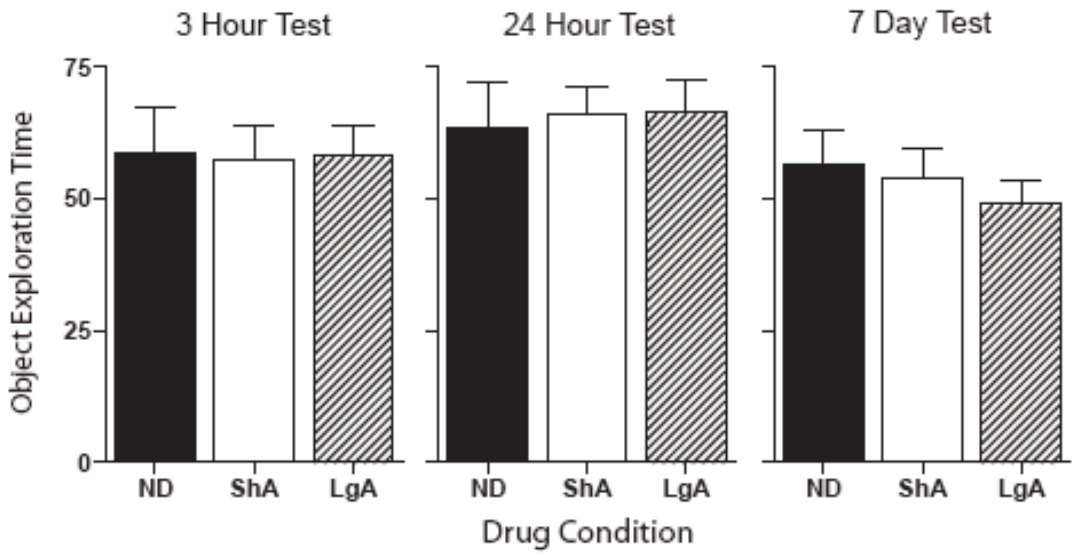


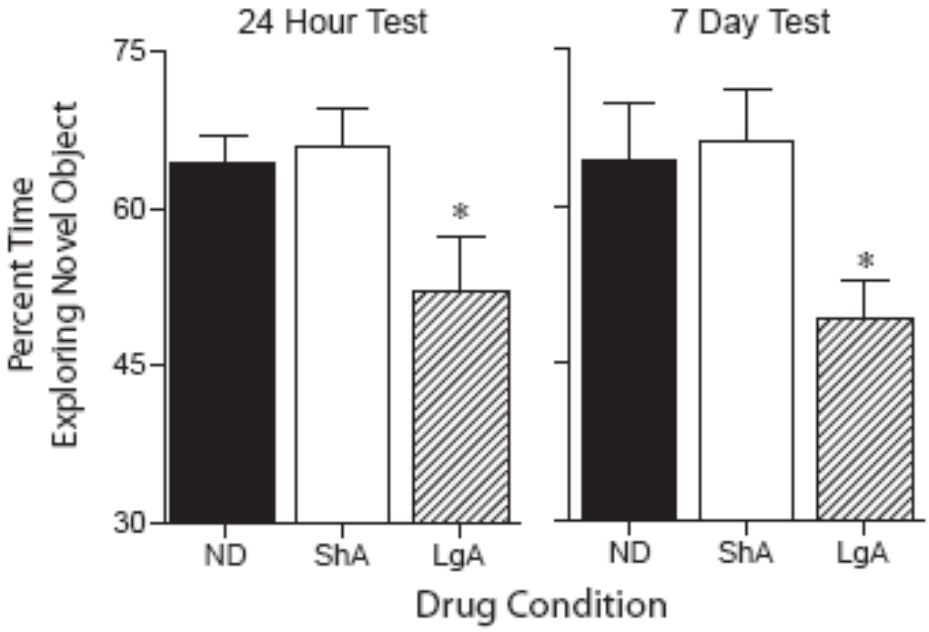
Figure 3.3. Total object exploration times from Experiment I. No differences were seen between the drug groups in the total object exploration time at the 3 hour test [ANOVA, group, $F(2,42)=0.007$, $p=.99$], 24 hour test [ANOVA, group, $F(2,42)=0.05$, $p=.95$], or the 7 day test [ANOVA, group, $F(2,42)=0.54$, $p=.59$].

Object Recognition. No group differences were seen in object exploration time during the training sessions [ND=186 ± 24s, ShA=182 ± 18s, LgA=155 ± 20s; ANOVA, group, $F(2,31)=0.55$, $p=0.58$] Twenty-four hours after training, animals in the LgA group showed decreased novel object exploration compared to both no drug and ShA animals, and these two groups did not differ from each other (Figure 3.4a). At the 1-week test, both LgA and ShA animals displayed an impaired recognition memory compared to the no drug controls (see Figure 3.4b). No differences were seen between the groups in total exploration time at any of the test time points (Figure 3.5).

Discussion

The results of the current experiments indicate that extended access cocaine self-administration produced a persistent decrease in novel object recognition performance. We propose that this indicates a persistent memory deficit in the extended access animals. While there is a possibility that similar results could be seen if extended access cocaine lead to performance deficits, the lack of differences in either object exploration time during training or total object exploration during testing indicates otherwise. While we do not have locomotor activity counts, these measures suggest indicate that the extended access animals were actively engaged in exploring the objects but they did not recognize the previously trained object.

Figure 3.4. Replication. Extended access cocaine self-administration produced deficits in recognition memory performance. The two panels show the percent novel object exploration time (as a function of the total object exploration time) at the two retention intervals. At both the 24-hour (a) and 7-day retention intervals, the LgA animals exhibit a significant decrease in recognition memory performance relative to both the No Drug and ShA groups, which did not differ from one another [(a), ANOVA, group, $F(2,31)=3.38$, $p=.048$; (b), ANOVA, group, $F(2,31)=3.36$, $p=.049$; *, differs from ShA and No Drug groups as determined by Fisher's Tests).


24 Hour Test
7 Day Test
Percent Time Exploring Novel Object
75
60
45
30
*
*
ND
ShA
LgA
ND
ShA
LgA
Drug Condition

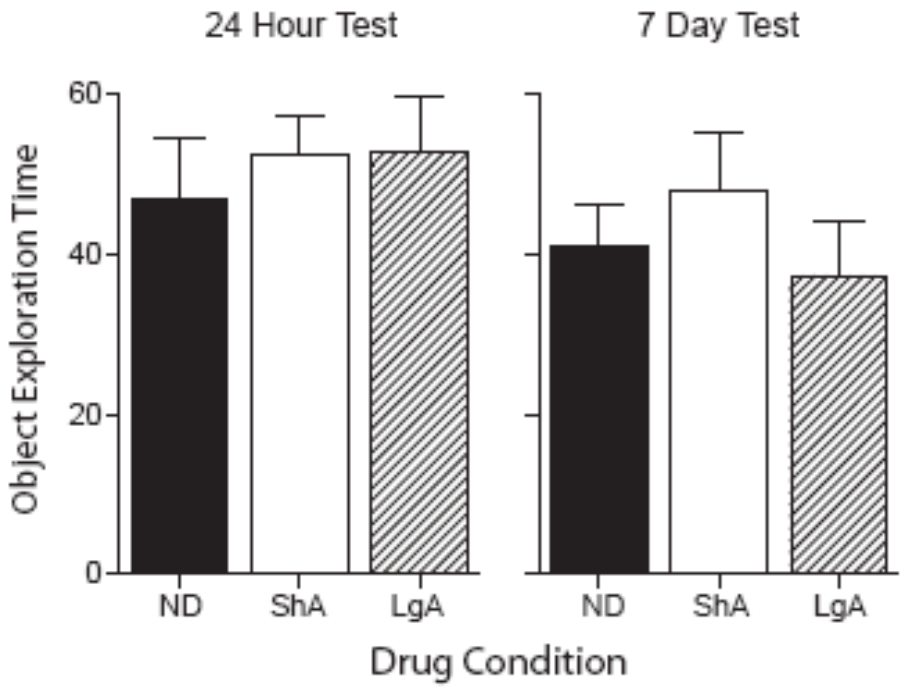


Figure 3.5. Total object exploration times from Experiment II. No differences were seen between the drug groups in the total object exploration time at the 24 hour test [ANOVA, group, $F(2,31)=0.25$, $p=.78$] or the 7 day test [ANOVA, group, $F(2,31)=0.65$, $p=.53$].

Although short access animals exhibited a deficit at the long retention interval in Experiment I, this was not replicated in Experiment II. This indicates that while short access cocaine may lead to mild deficits, they are not evident in all situations and are clearly less severe than the deficits seen following extended access cocaine.

Although we believe that the results of the current study indicate that extended access cocaine self-administration leads to a memory deficit, the recognition memory task does not allow us to parse apart the exact nature of the deficit. Further work will need to be done to determine whether cocaine leads to specific deficits in memory acquisition and/or memory retrieval. As memory deficits in human addicts seem to be in the acquisition phase (Mittenberg and Motta, 1993), it is hypothesized that the deficits seen here may be similar to those seen in clinical populations.

The results of the present study are consistent with those seen following both neurotoxic and nontoxic methamphetamine administration (Schroder et al., 2003; Belcher et al., 2005a, 2006). However, not all studies examining memory performance following drug administration have demonstrated deficits. Clark and colleagues (2007) found that an escalating dose binge regimen of methamphetamine administration did not impair recognition memory. Del Olmo and colleagues (2006) examined the effects of cocaine self-administration on spatial memory and did not find deficits. While there is one study that

demonstrated spatial memory deficits following cocaine administration in adolescent animals, these deficits did not persist in adulthood (Santucci et al., 2004).

As the total intake in the above studies were more consistent intake seen in our ShA animals, it is possible that a more aggressive regimen would have lead to more consistent persistent memory deficits. The extended access self-administration protocol used in this study has been shown to produce a number of symptoms characteristic of addiction that are not seen following more limited drug access (Ahmed and Koob, 1998; Paterson and Markou, 2003; Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Ferrario et al., 2005; Briand et al., 2006). The current study suggests that extended access self-administration procedures may also be more able to produce cognitive deficits seen in human addicts as well, further validating the model as a means to study the effects of drug addiction on brain function.

The results of the current study provide support for the contention that deficits in cognitive function seen in human addicts may be at least in part a consequence of drug use, rather than just reflecting a “premorbid condition”, and that these changes may be more readily apparent when animals are allowed extended access to self-administered drugs.

CHAPTER IV

COCAINE SELF-ADMINISTRATION PRODUCES A PERSISTENT INCREASE IN DOPAMINE $D2^{HIGH}$ RECEPTORS

Summary

Cocaine addicts are reported to have decreased numbers of striatal dopamine D2 receptors. However, in rodents repeated cocaine administration consistently produces hypersensitivity to the psychomotor activating effects of both indirect DA agonists, such as cocaine itself, and importantly, to direct-acting D2 receptor agonists. The current study reports a possible resolution to this long-standing paradox. The dopamine D2 receptor exists in both a low and a high affinity state, and dopamine exerts its effects via the more functionally relevant high-affinity D2 receptor ($D2^{High}$). We report here that cocaine self-administration experience produces a large (approximately 150%) increase in the proportion of $D2^{High}$ receptors in the striatum with no change in the total number of D2 receptors, and this effect is evident both 3 and 30 days after the discontinuation of cocaine self-administration. Changes in $D2^{High}$ receptors would not be evident with the probes used in human (and non-human primate) imaging studies. We suggest,

therefore, that cocaine addicts and animals previously treated with cocaine may be hyper-responsive to dopaminergic drugs in part because an increase in $D2^{High}$ receptors results in dopamine supersensitivity. This may also help explain why stimuli that increase dopamine neurotransmission, including drugs themselves, are so effective in producing relapse in individuals with a history of exposure to cocaine.

Introduction

A consistent finding from imaging studies is that cocaine addicts show a very persistent decrease in striatal dopamine D2 receptors (Volkow et al., 1990; Martinez et al., 2004; Volkow et al., 2004), and studies in non-human primates report similar decreases following cocaine self-administration, suggesting that this may be a consequence of cocaine use (Nader and Czoty, 2005; Nader et al., 2006). However, a long-standing puzzle in this area is that preclinical studies have consistently found that rats pretreated with amphetamine or cocaine, and then withdrawn, are hypersensitive ("sensitized") to the psychomotor activating and incentive motivational effects of these indirect agonists (Robinson and Berridge, 1993), and even more importantly, to the psychomotor effects of direct D2 agonists (Ujike et al., 1990; De Vries et al., 2002; Edwards et al., 2007). Studies examining the effects of cocaine treatment on D2 receptor binding in rodents are mixed, with reports of increases, decreases or no change (Ujike et al., 1990). Obviously, the puzzle is: why are cocaine-treated rats functionally

hypersensitive to D2 agonists if cocaine treatment decreases D2 receptor binding?

There are number of possible explanations for this discrepancy, but an especially interesting explanation is raised by the finding that the dopamine D2 receptor exists in two interconvertible affinity states for the agonist dopamine, a high-affinity state and a low-affinity state. The transmitter dopamine binds primarily to the high-affinity state of the D2 receptor ($D2^{High}$), making this the most functionally relevant state (Seeman et al., 2005). Furthermore, many different treatments that produce a functional supersensitivity to dopamine (including repeated amphetamine administration) produce a large increase in the number of $D2^{High}$ receptors, even if they produce no change or even a decrease in the total number of D2 receptors (Seeman et al., 2004; Seeman et al., 2005; Seeman et al., 2007). Unfortunately, the ligands used in the human imaging and non-human primate studies do not discriminate between the low- and high-affinity states of the D2 receptor and are, therefore, insensitive to changes in the number of $D2^{High}$ receptors.

Although it has been reported that experimenter-administered amphetamine increases the number of $D2^{High}$ receptors, no studies have examined the effects of self-administered cocaine on $D2^{High}$ receptors, which is more directly relevant to human imaging studies. Therefore, in the present study we asked whether cocaine self-administration experience increases the proportion of $D2^{High}$

receptors in the striatum, which could account for the well-documented behavioral hypersensitivity to D2 agonists seen in animals with a history of exposure to cocaine. In addition, we studied rats that had either limited or extended access to self-administered cocaine because the latter procedure is thought to be especially effective in producing a number of symptoms of addiction (Ahmed and Koob, 1998; Vanderschuren and Everitt, 2004; Ferrario et al., 2005).

Methods

Subjects. Forty-eight male Wistar rats (Harlan, Indianapolis, IL) weighing 200-225g at the start of the experiment were individually housed in square plastic hanging cages (8 x 9 x 8 cm). The animals were housed in a temperature and humidity controlled room with a 14:10 light/dark cycle, with water available ad libitum. Animals were food restricted throughout the experiment to maintain at least 90% of their free feeding body weight. All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA).

Apparatus. Drug administration took place in 16 operant chambers measuring 22 x 18 x 13 cm (Med Associates, St. Albans, VT, USA) located inside larger sound-attenuating chambers. For the drug self-administration procedure, each operant chamber had two nose-poke holes equipped with cue lights. A tone (2900 Hz) was also available inside of the chamber. The floor of the chamber consisted of

19 stainless steel rods (4 mm in diameter) spaced 1.5 cm apart (center-to-center). Med-PC for Windows software (v. 1.1, Med Associates) controlled all drug delivery, tone presentation and data collection in each system via a Pentium PC.

Surgical Procedures. Animals were anaesthetized with ketamine and xylazine anesthesia (77:1.5 mg/ml, intraperitoneal [IP], at 0.1 mL/100 gm of body weight) and a silicone catheter (Plastics One, Roanoke, VA) was inserted into the right jugular vein and passed subcutaneously to exit from the animals' back (see Caine et al., 1993). Animals were allowed to recover from surgery for a minimum of 3 days prior to drug administration. Catheters were flushed daily with 0.1mL of gentamicin (50 mg/kg, in 0.9% sterile bacteriostatic saline). Catheter patency was verified by intravenous infusion of sodium pentothal (2 mg/infusion).

Cocaine Self-Administration. Rats were transported from their home cage to an operant chamber 6 days a week for 4 weeks, where they were allowed to nose-poke for cocaine (0.4 mg/kg/infusion in 50 µL of saline administered over 1.6 s) on a continuous reinforcement schedule (FR1) with a time-out of 20 s. A training session commenced with the illumination of the "active" nose-poke hole stimulus light. Responding in this hole resulted in drug delivery. Responding in the other nose-poke hole, designated inactive, had no consequences. Rats were initially trained during daily one-hour sessions for a total of 6 days. Animals that did not acquire stable self-administration behavior (at least 5 infusions each day for 3

consecutive days) were removed from the study. After the initial 6-day training period animals were divided into two groups: a long access group (LgA) and a short access group (ShA). These groups were balanced according to the amount of drug they administered during the first week of training. Animals in the ShA group continued to receive one-hour test sessions, whereas animals in the LgA group received 6-hour test sessions for an additional 18 sessions. A third group of rats (no-drug control group) received sham surgery and were transported each day to a novel test room where they were placed into Plexiglas chambers, similar to the operant chambers.

Tissue Collection. Either 3 or 30 days following their final self-administration session all animals were decapitated and their brains removed. The striata were dissected and stored at -70°C until used. The striata were homogenized in buffer (4 mg frozen tissue per ml buffer), using a Teflon-glass homogenizer with the piston rotating at 500 rpm and 10 up and down strokes of the glass container. The buffer contained 50 mM Tris-HC1 (pH 7.4 at 208C), 1 mM EDTA, 5 mM KCl, 1.5 mM CaCl2, 4 mM MgCl2, and 120 mM NaCl. The homogenate was washed three times by centrifugation at 10,000 rpm at 4 ˚C and resuspending the pellet in 15 ml of buffer, although it is known that some of the D2 receptors can be lost by this procedure (Seeman et al., 1984).

[3H]domperidone/DA competition. [^{3}H]Domperidone was custom synthesized as [phenyl-^{3}H(N)]domperidone (42-68 Ci/mmol) by PerkinElmer Life Sciences Inc.,

Boston, MA, and used at a final concentration of 2 nM. Because the Kd of [^{3}H]domperidone is 0.47 nM at D2 for rat striatum, the final concentration of 2 nM occupied 81% of the D2 receptors, using the equation f = C/(C + Kd), where f is the fraction of receptors occupied by [^{3}H]domperidone, and C is 2 nM (Seeman et al., 2003).

The competition between dopamine and [^{3}H]domperidone for binding at the receptors was done as follows. Each incubation tube (12 x 75 mm, glass) received, in the following order, 0.5 ml buffer (containing dopamine at various concentrations, and with or without a final concentration of 10 μM S-sulpiride to define nonspecific binding to the dopamine D2 receptors), 0.25 ml [^{3}H]domperidone and 0.25 ml of tissue homogenate. The tubes, containing a total volume of 1 ml, were incubated for 2 h at room temperature (20°C), after which the incubates were filtered, using a 12-well cell harvester (Titertek, Skatron, Lier, Norway) and buffer-presoaked glass fiber filter mats (Whatman GF/C). After filtering the incubate, the filter mat was rinsed with buffer for 15 s (7.5 ml buffer). The filters were pushed out and placed in scintillation polystyrene minivials (7 ml, 16 x 54 mm; Valley Container Inc., Bridgeport, Conn.). The minivials received 4 ml each of scintillant (Research Products International Corp., Mount Prospect, IL), and were monitored 6 h later for tritium in a Beckman LS5000TA scintillation spectrometer at 55% efficiency. The specific binding of [^{3}H]domperidone was defined as total binding minus that in the presence of 10 μM S-sulpiride.

The proportion of $D2^{High}$ receptors in the striata was measured by the competition of dopamine with [^{3}H]domperidone, as previously described (Seeman et al., 2003). Dopamine inhibited the binding of [^{3}H]domperidone in two phases. At low concentrations, corresponding to the high-affinity state of the dopamine D2 receptor ($D2^{High}$) dopamine inhibited the binding of [^{3}H]domperidone between 1 nM and 100 nM dopamine. A second phase of inhibition occurred above 100 nM dopamine. The demarcation between the two phases were sharp and unambiguous, clearly and readily permitting the measurement of the $D2^{High}$ component as a percent of the total amount of specific [^{3}H]domperidone binding, as defined by the presence of 10 μM S-sulpiride. No computer-assisted analysis was needed.

Statistical Analyses

Cocaine Self-Administration. The number of infusions for the first hour of each session and for the entirety of the session was determined for each day of self-administration. Repeated measures two-way ANOVAs were performed with session (1-16) as the repeated variable, drug group (ShA, LgA) as the independent variable and the number of infusions (i.e. during the first hour or entire session) as the dependent variable. When significant main effects or interactions were revealed, Bonferroni corrected t-tests were conducted. For all analyses, alpha was set at 0.05.

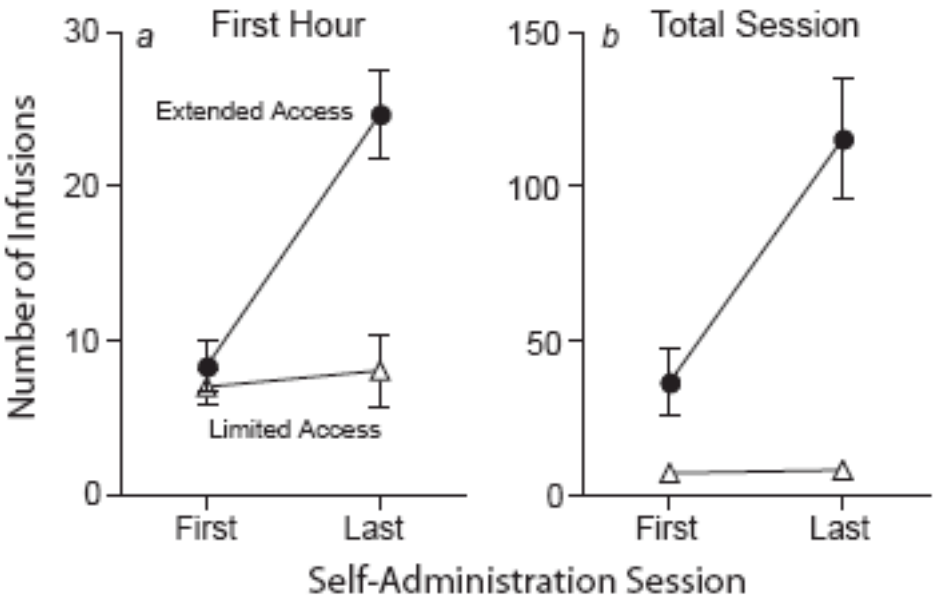
D2 High Affinity Receptor Density. As a relative measure of D2 receptor density the total number of receptors occupied by [^{3}H]domperidone was calculated (reflecting 81% of the total number of D2 receptors). A two-way ANOVA was performed with drug group (ND, ShA, LgA) and withdrawal time (3 or 30 days) as the independent variables and disintegrations per minute (DPM), a measure of [^{3}H]domperidone binding, as the dependent variable. For the proportion of $D2^{High}$ receptors, a two-way ANOVA was performed with drug group and withdrawal time as the independent variables and the percentage of total binding representing the $D2^{High}$ receptors as the dependent variable. When significant main effects or interactions were revealed, Bonferroni corrected t-tests were conducted.

Results

Cocaine Self-administration. Figure 4.1 shows that limited access (1 hr/day) to self-administered cocaine produced a stable level of intake over time (approximately 2.8 mg/kg/session), but as expected, extended access to cocaine (6 hr/day) resulted in a pronounced escalation of intake between the first and last test session (by the last test session these animals were taking an average of 45 mg/kg/day).

D2 Receptors. There were no group differences in the total number of receptors occupied by [^{3}H]domperidone at either withdrawal time (Figure 4.2a). However,

Figure 4.1. A. Mean (+/- SEM) number of cocaine infusions during the first hour of each self-administration session. By the 7th escalation session, animals allowed extended access to cocaine (long access, 6 hr session; LgA) took significantly more infusions than animals allowed only limited access (short access, 1 hr sessions; ShA) [main effect of group, $F(1,476)=64.24$, $p<.0001$, main effect of day, $F(17,476)=6.61$, $p<0.0001$, group by day interaction, $F(17,476)=6.97$, $p<.0001$]. B. The mean (+/- SEM) number of cocaine infusions over the entire session for LgA (6 hr sessions) and ShA (1 hr sessions) groups.

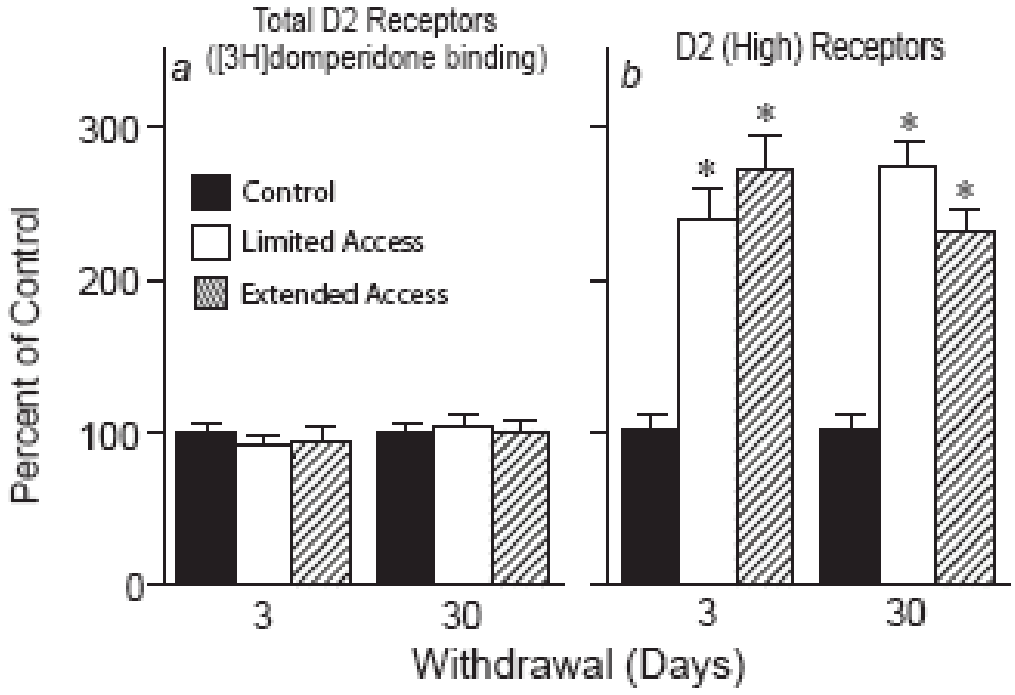

a
First Hour
Extended Access
Limited Access
30
20
10
0
First
Last
b
Total Session
150
100
50
0
First
Last
Number of Infusions
Self-Administration Session

there was a large (approximately 150%) increase in the proportion of striatal $D2^{High}$ receptors in both the ShA and LgA groups, relative to the no drug control group (Figure 4.2b), and this was evident both 3 and 30 days after the discontinuation of cocaine self-administration.

Discussion

Cocaine self-administration experience had a profound effect on the proportion of $D2^{High}$ receptors in the striatum. Relative to drug-naïve controls, rats that had 4 weeks of either limited (1 hr/day) or extended (6 hr/day) access to cocaine had approximately a 150% increase in striatal $D2^{High}$ receptors, and this effect was evident both 3 and 30 days after the discontinuation of cocaine self-administration. It is interesting that there was no difference in the magnitude of the increase in $D2^{High}$ receptors in animals that had limited vs. extended access to cocaine, as these two procedures result in a number of different outcomes (Ahmed and Koob, 1998; Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Ferrario et al., 2005). It is not clear, therefore, which of the many behavioral or psychological consequences of cocaine self-administration are due to changes in $D2^{High}$ receptors. Seeman and his colleagues (2002; 2007) have reported that sensitization to amphetamine is associated with an increase in $D2^{High}$ receptors, and both experimenter-administered cocaine and cocaine self-administration experience have been reported to produce behavioral supersensitivity to a challenge injection with a direct D2 receptor agonist

Figure 4.2. Dopamine D2 and $D2^{High}$ receptors in the striatum of no-drug control animals and animals given limited or extended access to self-administered cocaine, either 3 or 30 days after the last self-administration session. Panel a shows the total number of D2 receptors ([^{3}H]domperidone binding) in the cocaine-treated groups expressed as a percent of the control group. There were no group differences in the total number of D2 receptors (Overall ANOVA, group, $F(2,40)=.03$, $p=.97$). Panel b shows the proportion of $D2^{High}$ receptors, again with the cocaine-treated groups expressed as a percent of the control group. Cocaine self-administration experience greatly increased the proportion of $D2^{High}$ receptors at both 3 and 30 days of withdrawal [main effect of group, $F(2,42)=68.89$, $p<.0001$, main effect of withdrawal time, $F(1,42)=.65$, $p=.43$, group by time interaction, $F(2,32)=2.8$, $p=.07$, *, differs from the no drug group as determined by Bonferroni's corrected t-tests].


Total D2 Receptors
([3H]domperidone binding)
a
D2 (High) Receptors
b
Control
Limited Access
Extended Access
Percent of Control
300
200
100
0
3
30
3
30
Withdrawal (Days)
*
*
*
*

(De Vries et al., 2002; Edwards et al., 2007). It has also been reported that both limited and extended access to self-administered cocaine produces psychomotor sensitization (Ferrario et al., 2005). In this latter study, however, animals given extended access showed more robust sensitization than those given only limited access. Therefore, the absence of any difference between the LgA and ShA groups in $D2^{High}$ receptors may reflect a ceiling effect. Although the LgA animals took significantly more cocaine than the ShA animals, even the ShA group took considerably more cocaine (nearly 3mg/kg/day i.v. for 24 days) than used in a typical sensitization study. Thus, to determine the nature of the relationship between the increase in $D2^{High}$ receptors and behavioral sensitization will require examination of a greater range of cocaine doses, treatment regimens and conditions.

Nevertheless, it is important to contrast the *increase* in striatal $D2^{High}$ receptors produced by cocaine self-administration experience reported here with reports that there is a *decrease* in striatal D2 receptors in cocaine addicts (Volkow et al., 1990; 1993; Martinez et al., 2004) and in non-human primates allowed to self-administer cocaine (Volkow et al., 1990; Volkow et al., 1993; Martinez et al., 2004; Nader and Czoty, 2005; Nader et al., 2006). Although it might be argued this could be a result of species differences, the amount of cocaine used, etc., it is important to consider another possibility. The ligands used in the human imaging and non-human primate studies do not discriminate between the low- and high-affinity states of the D2 receptor and would be, therefore, insensitive to

changes in the number of $D2^{High}$ receptors (Seeman et al., 2003; 2005). Furthermore, there are many examples where an increase in $D2^{High}$ receptors has been associated with dopamine supersensitivity, even when there was no change or even a decrease in total D2 receptors (Seeman et al., 2005). Thus, it will be important to conduct imaging studies in addicts using ligands that allow quantification of the $D2^{High}$ receptor (Graff-Guerrero et al., 2007) to determine if the reported decrease in D2 receptors in addicts is more apparent than real.

The results reported here are also of some clinical importance because they suggest that rather than being in a hypo-dopaminergic state, addicts may actually be supersensitive to dopamine and dopaminergic drugs after the discontinuation of drug use, and may remain so for very long periods of time. This may help explain why stimuli that activate dopaminergic systems, which include addictive drugs themselves, direct D2 agonists, stress, and cues associated with drugs, are so effective in reinstating self-administration behavior (producing relapse) after extinction of the behavior (Shaham et al., 2003). In thinking about the development of therapies for cocaine addiction it will obviously be critical to determine whether a hypo- or hyper-dopaminergic state best represents the situation in addiction, and under what conditions.

CHAPTER V

PERSISTENT ALTERATIONS IN REGIONAL CEREBRAL BLOOD FLOW FOLLOWING EXTENDED (BUT NOT LIMITED) ACCESS TO SELF-ADMINISTERED COCAINE IN RATS

Summary

Functional imaging studies in human cocaine addicts have identified brain regions that exhibit alterations in functional activity following both acute and prolonged abstinence. Although these studies provide insight into alterations in brain function in human addicts, it has not been determined whether these changes reflect consequences of drug use or a pre-existing condition that may increase vulnerability to addiction. To determine if self-administration of cocaine in rats is capable of producing long-lasting changes in brain activation similar to those seen in human addicts, an extended access self-administration procedure capable of producing symptoms of human addiction was used. Rats were allowed access to cocaine for either 1 hr/day (short access; ShA) or 6 hrs/day (long access; LgA) for 3 weeks. Regional cerebral blood flow (rCBF) was examined under basal conditions at both 3 and 30 days after the last self-

administration session. Contrary to the findings in human addicts, we did not observe any differences in the frontal cortex following drug administration. In the striatum, however, animals in the LgA group exhibited significantly higher activation in the caudate-putamen at the early withdrawal time point (3 days). In contrast, at the late withdrawal time point (30 days), the LgA animals exhibited a decrease in both dorsal and ventral striatal rCBF compared to the ShA and drug-naïve controls. This shift in activation patterns during withdrawal suggests that between the acute withdrawal time point (3 days post-drug) and the prolonged withdrawal time point (30 days post-drug) suggests that alterations in the striatum occur throughout withdrawal. Further work is needed to determine the neurotransmitter systems responsible for this functional alteration and how this change may contribute to relapse.

Introduction

Repeated exposure to psychostimulant drugs, such as cocaine, has been shown to alter functional activation patterns, as defined by changes in regional cerebral blood flow (rCBF), glucose utilization and functional magnetic resonance imaging (fMRI) bold signaling, in numerous brain regions (Lyons et al., 1996; Bolla et al., 2003; Macey et al., 2004; Febo et al., 2005; Beveridge et al., 2006). For example, long-term cocaine abusers exhibit lower rCBF, glucose utilization, and fMRI bold signaling in regions of the frontal cortex relative to controls (Volkow et al., 1988; Volkow et al., 1991; Volkow et al., 1992) and these alterations persist

following long periods of abstinence (Volkow et al., 1992). Although the focus of most functional activation studies has been on alterations in regions of the frontal cortex, other subcortical regions have been examined as well. Volkow and colleagues (1991) demonstrated that cocaine addicts exhibit an increase in glucose utilization in the basal ganglia during acute withdrawal that decreases with increasing periods of abstinence. It is not clear however, whether these differences in functional activation in the brains of human addicts are a consequence of drug use or precede drug use.

Studies with non-human animals allow one to determine whether exposure to a drug of abuse is capable of producing changes in functional activation comparable to those seen in addicts. Work by Febo and colleagues (2004; 2005) examined the effects of acute cocaine or a cocaine challenge following repeated treatment on functional activation patterns; however none have examined changes in basal activity following drug exposure, as in addicts. In addition, these previous animal imaging studies have utilized experimenter-administered cocaine, while extended access contingent drug administration procedures have been more readily able to lead to symptoms characteristic of addiction (Ahmed and Koob, 1998; Paterson and Markou, 2003; Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004; Kippin et al., 2006). Therefore, in the current study we utilized an extended access cocaine self-administration procedure to examine basal changes in rCBF following chronic cocaine. We hypothesized that extended, but not limited, access to self-administered cocaine may produce

persistent changes in rCBF similar to those seen in human addicts. Thus, in the current experiment, we examined basal regional cerebral blood flow in animals given extended, limited, and no access to cocaine self-administration following both a 3-day acute withdrawal and a 30-day prolonged withdrawal.

Methods

Subjects. Sixty male Wistar rats (Harlan, Indianapolis, IL) weighing 200-225g at the start of the experiment were individually housed a temperature and humidity controlled room with a 14:10 light/dark cycle, with water available ad libitum. The animals were housed in square plastic hanging cages (8 x 9 x 8 cm). Animals were food restricted throughout the experiment to maintain at least 90% of their free feeding body weight. All procedures were approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA).

Cocaine Self-administration Procedures

Apparatus. Drug administration took place in 16 operant chambers measuring 22 x 18 x 13 cm (Med Associates, St. Albans, VT, USA) located inside larger sound-attenuating chambers. For the drug self-administration procedure, each operant chamber had a tone (2900 Hz) and two nose poke holes equipped with cue lights. The floor of the chamber consisted of 19 stainless steel rods (4 mm in diameter) spaced 1.5 cm apart (center-to-center). Med-PC for Windows software

(v. 1.1, Med Associates) controlled all drug delivery, tone presentation and data collection via a Pentium PC.

Jugular catheterization. Following anaesthesia with ketamine and xylazine (77:1.5 mg/ml, intraperitoneal [IP], at 0.1 mL/100 gm of body weight), animals were catheterized by insertion of a silicone catheter (Plastics One, Roanoke, VA) into the right jugular vein that passed subcutaneously to exit from the animals' back (see Caine et al., 1993). Catheters were flushed daily with 0.1mL of gentamicin (50 mg/kg, in 0.9% sterile bacteriostatic saline) and catheter patency was verified by intravenous infusion of the short-activing barbiturate sodium pentothal (2 mg/infusion). Animals were allowed to recover from surgery for a minimum of 3 days prior to the start of drug administration.

Self-Administration Paradigm. Catheterized rats were transported from their home cage to an operant chamber 6 days a week for 4 weeks, where they were allowed to nose-poke for cocaine (0.4 mg/kg/infusion in 50 µL of saline administered over 1.6 s) on a continuous reinforcement schedule (FR1) with a time-out of 20 s. An acquisition session commenced with the illumination of the "active" nose-poke hole stimulus light and drug delivery followed responses in this hole. Responding in the other nose-poke hole, designated inactive, was recorded, but had no consequence. Rats were initially trained during daily one-hour sessions for a total of 6 days. Animals that did not acquire at least 5 infusions each day for 3 consecutive days were removed from the study. Animals

were divided into two groups after the initial 6-day training period: a long access group (LgA) (n=21) and a short access group (ShA) (n=22), balanced according to the amount of drug they administered during the first week of training. Animals in the ShA group continued to receive one-hour test sessions, whereas animals in the LgA group received six-hour test sessions for an additional 16 sessions. A third group of rats (no-drug control group) received sham surgery and were transported each day to a novel test room where they were placed into Plexiglas chambers, similar to the operant chambers.

Regional cerebral blood flow measurements.

Tail catheterization. Animals were transported to the testing facility for 2 days prior to the testing day to allow for acclimation. Either 3 or 30 days following the final self-administration session animals underwent the rCBF procedure. The day of rCBF measurement, each rat was placed in a towel restraint and a flexible 24 gauge intravenous catheter was inserted in the tail vein. A rubber capped injection port was attached to the catheter and the catheter was immediately flushed with 0.25 ml of saline. Each animal was then removed from the towel restraint and placed in a Plexiglas box (similar to the animals' home cage) for 30-40 min to recover from the stress induced by the catheter implantation and to habituate to the novel environment.

Radiotracer injection and tissue collection. Eight to 10mCi of technetium ($^{[99m]}$Tc) exametazime in a 0.5-0.6 ml total volume was injected through the tail vein catheter over 10-15 sec and followed with 0.5 ml of saline (0.9% NaCl). Five minutes following the tracer injection, the rat was overdosed with chloral hydrate (300mg/kg, i.v.) and decapitated.

Brain sectioning and image exposure. The brain was removed and prepared for sectioning by rapid freezing with isopentane. Standard 10μm coronal sections were cut (4 consecutive sections cut at fixed intervals, see Table 1 for more detail) on a cryostat at -18°C and thaw mounted on Superfrost/Plus Gold slides (Fisher Scientific, Pittsburgh, PA). The slides were rapidly desiccated on a hotplate at 40°C and arranged in an imaging cassette to be exposed to a Fuji film imaging plate (Fujifilm Life Sciences, Stamford, CA). Exposure time (ranging from 20-45 minutes) was calculated based upon the radioactive content of the brain prior to slicing and the time since injection. Following the appropriate exposure time, the imaging plates were placed in the Fuji Bio-imaging analyzer system (Bas) 5000 to be read and converted into digital autoradiograms. For more details on the imaging procedure, see Morrow et al.(1998).

Quantification of images. Densitometric analysis of autoradiograms was performed utilizing MCID Elite imaging analysis and quantification software (Interfocus, Ltd., Linton, England). Anatomic location of selected regions of interest (ROIs) was determined using a method designed to ensure accuracy and

consistency of structural identification. For each coronal brain section, a transparent stereotaxic template of the same level (i.e. distance from bregma) (Paxinos and Watson, 1998) was overlaid on the digitized brain images displayed on the computer monitor and the two images were aligned using prominent landmarks. An index of activation from individual ROIs was calculated as a percent of the average total activity of all brain sections. Quantified optical densities were converted to radioactivity concentration of the tissue (nCi/mg) by MCID using the optical densities of [^{14}C] standards also imaged on each plate. The total brain activity for a given animal was estimated by sampling all pixels in each brain section and averaging the activity across all sections (approximately 160 sections per animal spanning the anterior posterior gradient, see Table 1). Activation index (AI) values for each sampled ROI were calculated as a percent difference from the average total activity of the entire brain using the following formula:

AI = (Sampled ROI activity – Total Brain Activity) / (Total Brain Activity) × 100%

Table 5.1 lists all the ROIs and levels sampled in the current study. We sampled structures known to participate in reward signaling and areas that have been previously shown to be altered by chronic cocaine (Kleven et al., 1990; Volkow et al., 1990; Macedo et al., 2004; Nasif et al., 2005; Beveridge et al., 2006).

Level (AP relative to Bregma)	Regions Sampled	Abbreviation
Level 4.2	Medial Orbital Cortex	MO
	Ventral Orbital Cortex	VO
	Lateral Orbital Cortex	LO
Level 3.7	Ventral Orbital Cortex	VO
	Lateral Orbital Cortex	LO
	Cingulate Cortex	Cg1
	Prelimbic Cortex	PrL
Level 2.7	Cingulate Cortex	Cg1
	Prelimbic Cortex	PrL
	Infralimbic Cortex	IL
Level 2.2	Nucleus Accumbens	NAc
	Nucleus Accumbens Core	NAc core
	Nucleus Accumbens Shell	NAc shell
Level 1.7	Nucleus Accumbens	NAc
	Nucleus Accumbens Core	NAc core
	Nucleus Accumbens Shell	NAc shell
	Caudate Putamen	Cpu
	Dorsomedial Caudate	dmCPu
	Ventromedial Caudate	vmCPu
	Dorsolateral Caudate	dlCPu
	Ventrolateral Caudate	vlCPu
Level 1.6	Caudate Putamen	Cpu
	Dorsomedial Caudate	dmCPu
	Ventromedial Caudate	vmCPu
	Dorsolateral Caudate	dlCPu
	Ventrolateral Caudate	vlCPu
Level 1.2	Same as Level 1.7	
Level 0.7	Same as Level 1.6	

Table 5.1. Brain Regions Quantified via rCBF technique.

Statistical Analyses

Cocaine Self-Administration. The number of infusions for the first hour of each session and for the entirety of the session was determined for each day of self-administration. Repeated measures two-way ANOVAs were performed with escalation session (1-16) as the repeated variable, drug group (ShA, LgA) as the independent variable and the number of infusions (first hour or total infusions) as the dependent variable. When significant main effects or interactions were revealed, Student-Newman-Keuls post-hoc comparisons were conducted. For all analyses, alpha was set at 0.05.

Regional Cerebral Blood Flow. Each brain structure was sampled bilaterally and an AI (see methods above) was calculated for each hemisphere. Hemispheric differences in rCBF in each brain region were assessed using a t-test. If no overall differences were found between the two hemispheres, the data was combined for analysis of group differences. If hemispheric differences were found separate group difference analyses were conducted for each hemisphere. Group differences in rCBF for both withdrawal time points were assessed using repeated measures two-away ANOVAs with drug group (ND, ShA, LgA) as a factor, distance from bregma as the repeated variable and the index of activation as the dependent variable. Separate repeated measures ANOVAs were calculated for each brain region. When significant main effects or interactions

were revealed, Student-Newman-Keuls post-hoc comparisons were conducted. For all analyses, alpha was set at 0.05.

Results

Cocaine Self-Administration. Following the initial 6-day training period animals were divided into ShA and LgA groups balanced for drug intake during acquisition. ShA animals maintained stable drug intake over the next 16 test sessions. LgA animals showed a progressive increase in both their total intake and their intake during the first hour of each session (Figure 5.1).

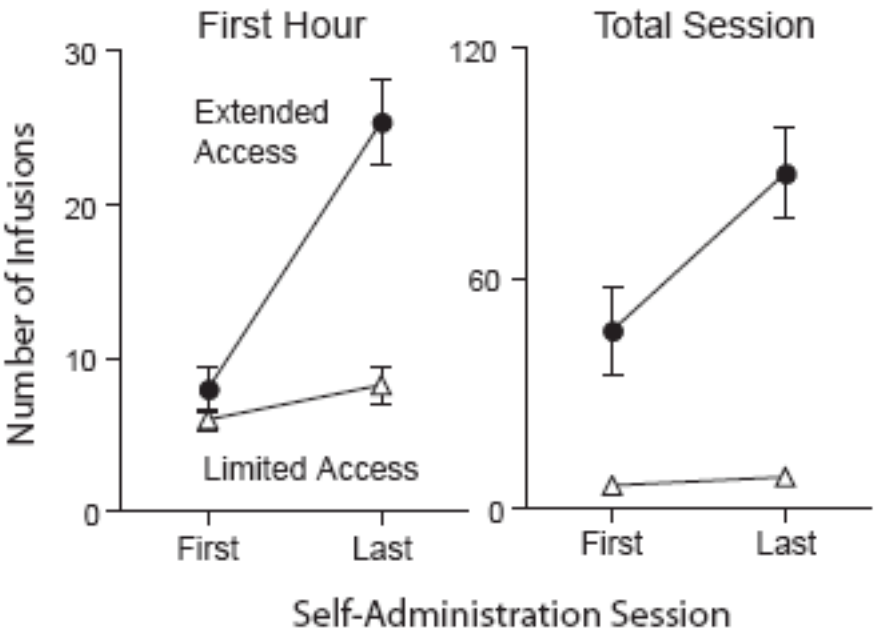
Regional Cerebral Blood Flow.

Total Brain. There were no group differences in total brain rCBF at either the 3 or 30 day withdrawal time point (Figure 5.2), indicating that the regional differences described below were not due to general alterations in cerebral perfusion.

Frontal Regions. No significant differences were seen between the groups in any of the cortical regions at either withdrawal time point (see Table 5.2).

Nucleus Accumbens. At the early withdrawal time point there were no differences in nucleus accumbens activity between the LgA and drug naïve groups, however a decrease in rCBF was seen in the ShA animals compared to both groups [NAc

Figure 5.1. A. Mean (+/- SEM) number of cocaine infusions during the first hour of each self-administration session. By the 11th session, animals allowed extended access to cocaine (long access, 6-hr session; LgA) took significantly more infusions than animals allowed only limited access (short access, 1-hr sessions; ShA) [main effect of group, $F(1,690)=28.57$, $p<.0001$, main effect of day, $F(15,690)=6.30$, $p<0.0001$, group by day interaction, $F(15,690)=3.13$, $p<.0001$]. B. The mean (+/- SEM) number of cocaine infusions over the entire session for LgA (6-hr sessions) and ShA (1-hr sessions) groups.


First Hour
Total Session
Number of Infusions
30
20
10
0
120
60
0
Extended
Access
Limited Access
First
Last
First
Last
Self-Administration Session

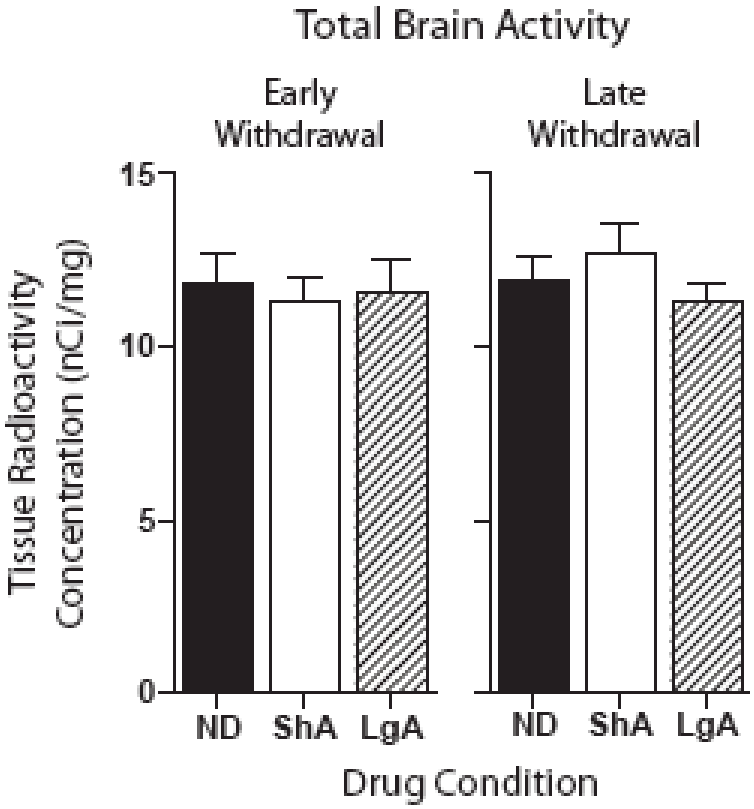


Figure 5.2. Mean (+/- SEM) total brain rCBF. The lack of differences between the drug groups at either time point indicates that there were no general alterations in cerebral perfusion [Early withdrawal: ANOVA, group, $F(2,34)=.09$, $p=.91$; Late withdrawal: ANOVA, group, $F(2,29)=.92$, $p=41$]

Table 5.2. Mean regional cerebral blood flow in the prefrontal cortex and striatum at the early (3) and late (30) withdrawal time points. The data represent the mean activation index [AI= (Sampled ROI activity – Total Brain Activity) / (Total Brain Activity) × 100%]. * Significantly lower activation than ShA and ND controls ($p<.05$). ** Significantly higher activation than ShA and ND controls ($p<.05$). # Significantly lower than LgA and ND animals ($p<.05$).

Brain Region	AP Level Relative to Bregma	Mean rCBF (% Change from Total Brain)					
		ND		ShA		LgA	
		3	30	3	30	3	30
Medial Orbital Cortex	4.2	34.3	34.9	50.8	40.5	41.0	33.3
Ventral Orbital Cortex	4.2	48.6	43.1	46.4	49.8	46.8	35.1
	3.7	50.7	46.3	50.0	42.0	50.1	41.0
Lateral Orbital Cortex (LO)	4.2	33.2	38.6	33.1	29.6	29.3	29.3
Right LO	3.7	49.8	23.6	51.2	27.4	48.4	30.0
Left LO		30.9	-0.1	33.4	-0.7	27.8	7.8
Cingulate Cortex	3.7	31.0	28.3	30.9	28.8	30.0	23.4
	2.7	30.5	30.6	30.2	24.7	29.0	33.7
Prelimbic Cortex	3.7	28.1	27.5	23.8	25.1	28.6	21.7
	2.7	29.3	26.6	24.9	26.3	28.7	26.5
Infralimbic Cortex	2.7	17.0	13.7	15.6	10.2	17.9	12.9
Nucleus Accumbens							
	2.2	10.5	9.5	5.6	4.2	8.9	9.8
	1.7	9.2	10.9	1.5#	7.4	9.6	1.0*
	1.2	-2.0	2.0	-1.5	-0.2	0.6	0.8
Core	2.2	13.2	17.3	11.7	10.5	16.2	14.1
	1.7	13.5	18.3	10.3	11.0	15.8	2.6*
	1.2	6.4	4.3	4.8	5.5	2.9	2.6
Shell	2.2	11.2	12.7	3.4#	9.7	9.9	12.0
	1.7	11.7	14.3	5.4	11.2	13.5	4.5*
	1.2	5.7	0.1	-3.6	1.7	-1.8	3.2
Caudate Putamen	1.7	6.3	11.6	1.5	6.5	12.1**	1.9
	1.2	6.1	9.7	5.8	5.9	9.6**	7.7
	1.6	5.3	7.4	5.8	7.1	7.5**	8.8
	0.7	5.3	9.3	2.0	1.7	8.4**	9.9

Whole, Overall ANOVA, group, F(2,114)=3.80, p=.028, level, F(2,114)=26.29, p<.0001, interaction, ns; individual group comparisons: ShA vs. LgA, group, F(1,76)=4.2, p=.05; ShA vs. ND group, F(1,76)=6.34, p=.02; LgA vs. ND, group, ns]. This effect seems to be primarily driven by a decrease in rCBF in the shell subregion of the ShA animals rather than the core [NAc Shell: Overall ANOVA, group, F(2,114)=3.87, p=.027, level, F(2,114)=21.33, p<.0001, interaction, ns; individual group comparisons: ShA vs. ND, group, F(1,76)=6.7, p=.01; ShA vs. LgA, group, ns; LgA vs. ND, group, ns; NAc Core: Overall ANOVA, group, ns, level, F(2,114)=12.77, p<.0001, interaction, ns]. In contrast, at the late withdrawal time point, LgA animals exhibited a decrease in rCBF, specifically at level 1.7 relative to bregma, relative to both the ShA and drug naïve controls [Overall ANOVA, group, ns, level, F(2,106)=23.04, p<.0001, interaction, F(4,106)=5.77, p=.0003; see Figure 5.3]. This effect was similar in both the core and the shell of the NAc (see Figure 5.3). No differences were seen between the ShA and ND animals at the late withdrawal time point (see Table 5.2).

Caudate Putamen. Regional CBF levels in the ShA and ND groups did not differ in the caudate putamen at either withdrawal time. At the early withdrawal time point, however, animals in the LgA group exhibited a significant bilateral increase in rCBF in the caudate relative to both the ND and ShA groups and this effect was greatest in the rostral caudate [Overall ANOVA, group, F(2,165)=5.90, p=.005, level, ns, interaction, F(4,165)=3.35, p=.004;See Figure 5.4]. At the late withdrawal time point, animals in the LgA group exhibited a significant bilateral

Figure 5.3. Decreased regional cerebral blood flow in the nucleus accumbens of LgA animals at anterior posterior level 1.7 (Paxinos and Watson, 1998) following prolonged withdrawal. The data represent the mean (± SEM) activation index [AI= (Sampled ROI activity – Total Brain Activity) / (Total Brain Activity) × 100%]. Following prolonged withdrawal, the LgA animals exhibited a specific decrease in activation at 1.7mm from bregma in the NAc [Overall ANOVA, group, $F(2,59)=5.26$, $p=.008$, *, differs from ShA and ND groups as determined by Student Newman Keuls Tests], the NAc core [Overall ANOVA, group, $F(2,59)=7.26$, $p=.002$, *, differs from ShA and ND groups as determined by Student Newman Keuls Tests], and the NAc shell [Overall ANOVA, group, $F(2,59)=4.71$, $p=.01$, *, differs from ShA and ND groups as determined by Student Newman Keuls Tests]. No differences were seen between the ShA and ND animals at this time point.

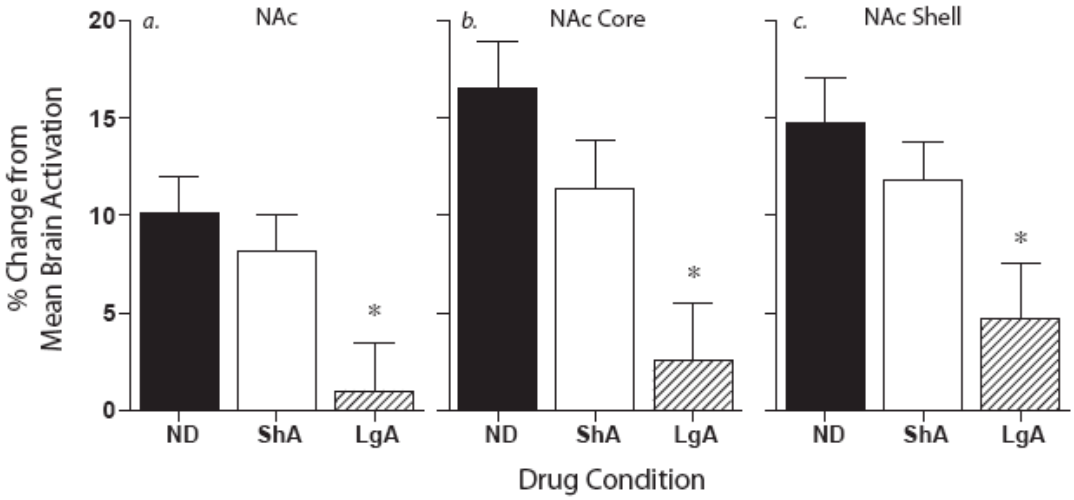
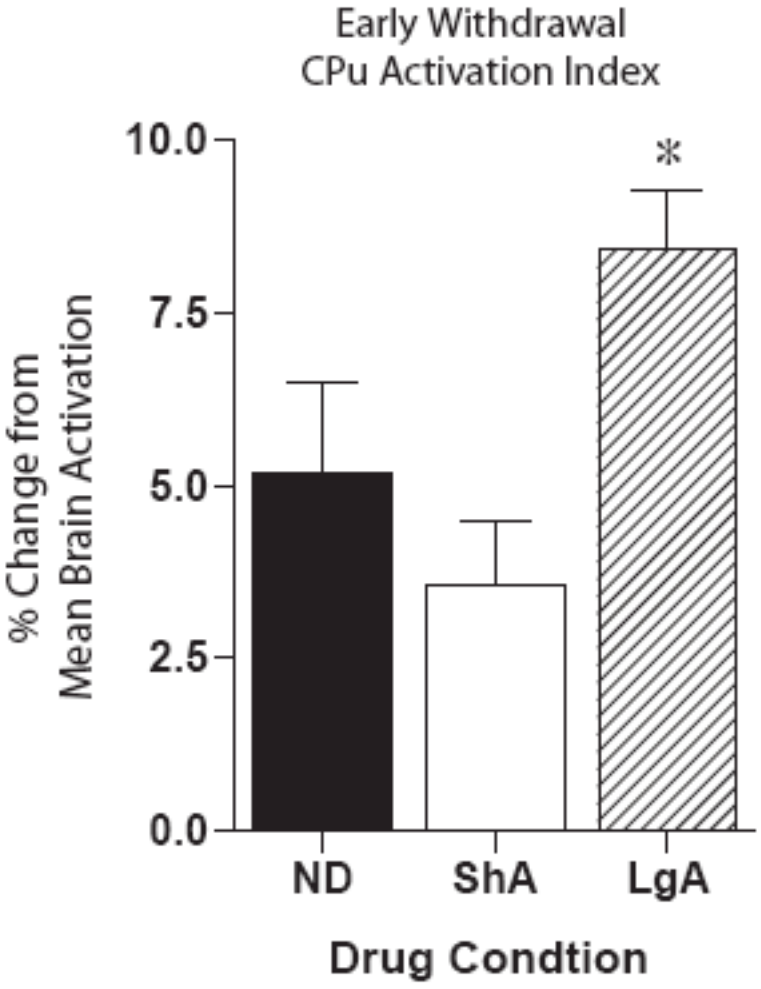

a.
NAc
b.
NAc Core
c.
NAc Shell
20
15
10
5
0
% Change from
Mean Brain Activation
*
*
*
ND
ShA
LgA
ND
ShA
LgA
ND
ShA
LgA
Drug Condition

Figure 5.4. Increased regional cerebral blood flow in the caudate putamen (CPu) of the LgA animals at the early withdrawal time point. The data represent the mean (± SEM) activation index [AI= (Sampled ROI activity – Total Brain Activity) / (Total Brain Activity) × 100%]. During early withdrawal, the LgA animals exhibited an increase in activation in the entire CPu [Overall ANOVA, group, $F(2,68)=5.23$, $p=.007$, *, differs from ShA and ND groups as determined by Student Newman Keuls Tests]. No differences were seen between the ShA and ND animals.


Early Withdrawal
CPu Activation Index
% Change from
Mean Brain Activation
10.0
7.5
5.0
2.5
0.0
*
ND
ShA
LgA
Drug Condtion

decrease in rCBF in the rostral caudate relative to both the ND and ShA group [Overall ANOVA, group, ns, level, ns, interaction, $F(4,171)=5.89$, $p<.0001$; see Figure 5.5; Table 5.2]. At both withdrawal time points, the pattern of changes was consistent throughout all dorsolateral and ventromedial subregions of the Cpu (data not shown).

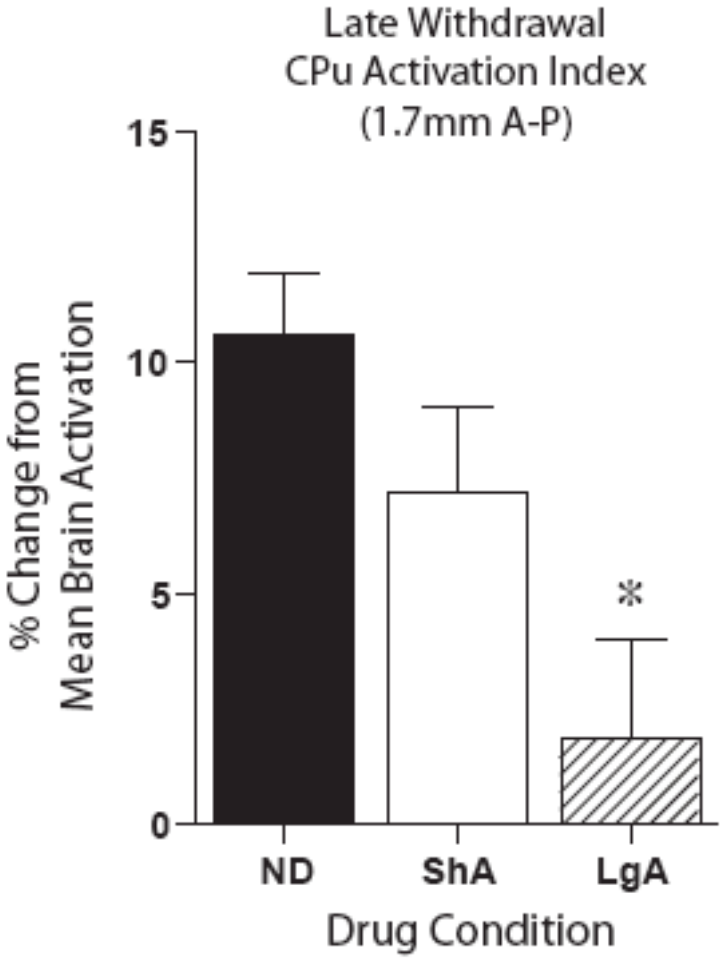
Discussion

Effects of past experience with self-administered cocaine on rCBF in the frontal cortex

Contrary to our hypothesis, we observed no effect of extended access cocaine self-administration on rCBF in the frontal cortex. These results differ from studies in the clinical literature demonstrating that human cocaine addicts exhibit a basal decrease in frontal rCBF (Volkow et al., 1988) and glucose utilization (Volkow et al., 1992; Hammer et al., 1993). While the inability of cocaine to alter frontal rCBF under baseline conditions may support the contention that the hypofrontality seen in human addicts is not a result of drug use, there are a number of factors that may have influenced our ability to detect basal changes in rCBF.

Numerous studies in both human addicts and non-human primates have examined responses to cocaine cue presentation and acute cocaine challenge.

Figure 5.5. Decreased regional cerebral blood flow in the caudate putamen of LgA animals at anterior posterior Level 1.7 (Paxinos and Watson, 1998) following prolonged withdrawal. The data represent the mean (± SEM) activation index [AI= (Sampled ROI activity – Total Brain Activity) / (Total Brain Activity) × 100%]. Following prolonged withdrawal, the LgA animals exhibited a specific decrease in activation at 1.7mm from bregma in the CPu [Overall ANOVA, group, $F(2,59)=5.98$, $p=.004$, *, differs from ShA and ND groups as determined by Student Newman Keuls Tests]. No differences were seen between the ShA and ND animals.


Late Withdrawal
CPu Activation Index
(1.7mm A-P)
% Change from
Mean Brain Activation
15
10
5
0
*
ND
ShA
LgA
Drug Condition

Both cue presentation and acute cocaine administration result in an increase in glucose utilization and rCBF in frontal brain regions in chronic cocaine users (London et al., 1990; Pearlson et al., 1993; Wang et al., 1999; Bonson et al., 2002) and monkeys (Lyons et al., 1996; Beveridge et al., 2006). In the present experiment, despite the fact that we did not explicitly present the animal with cocaine cues, the presence of the experimenters may have served as a cue. Since rats were only removed from their home cages for self-administration sessions, the experimenter or the change of environment may have been enough to elicit cue-induced increases in rCBF that could mask basal decreases.

Stress has also been shown to elevate rCBF at the whole brain level (Wik et al., 1997) and specifically in the frontal cortex (Ohata et al., 1982). Although we attempted to minimize the effect of stress by allowing the rats to habituate prior to injection, we cannot dismiss the possibility that an increased responsivity to stress in the LgA animals masked basal differences in rCBF. This possibility is supported by evidence that chronic cocaine administration leads to an increased response to stress (Erb et al., 2003; Erb et al., 2004; Erb et al., 2006). Without further assessment of corticosterone levels during the habituation period or during the radiotracer infusion, we are unable to eliminate the possibility that a stress-induced increase in rCBF is masking any basal decreases resulting from chronic cocaine administration.

While these methodological issues are important to consider, it is also a possibility that the lack of alterations in rCBF following chronic cocaine self-administration indicate that the changes seen in human addicts may preexist drug addiction rather than being a consequence of drug taking. Future studies will have to directly address the aforementioned methodological issues in order to determine whether alterations in rCBF can been seen following chronic cocaine under different experimental conditions.

Effects of past experience with self-administered cocaine on rCBF in the striatum

This is the first study to demonstrate long-term changes in basal mesolimbic activation following extended access cocaine self-administration in rats. The results indicate a biphasic rCBF response in LgA animals relative to both ShA and drug naïve controls: increased striatal rCBF was seen during early withdrawal and decreased striatal rCBF was present following prolonged withdrawal.

Although most human rCBF studies have focused on cortical brain regions, Volkow and colleages (1991) examined another measure of functional activity, glucose metabolism, and found that cocaine addicts that were abstinent for less than one week exhibited a 10% increase in glucose metabolism in the basal ganglia as compared to drug naïve control subjects. Similar to the increase seen

in the present study, this increase in functional activation was transient and was no longer present in subjects tested after 2-4 weeks of abstinence.

As alterations in regional cerebral blood flow are believed to reflect changes in synaptic activity (Sharkey et al., 1991; Mathiesen et al., 1998; Mathiesen et al., 2000), the increase in rCBF seen in the current study indicates that extended access cocaine self-administration leads to an increase in synaptic activity in the dorsal striatum. However, as the dorsal striatum contains at the very least dopaminergic, glutamatergic, and gabaergic synapses, it is not clear which neurotransmitter system or systems might be responsible for the changes in functional activation. Although there is ample evidence that repeated cocaine increases evoked dopamine release in the dorsal striatum (Akimoto et al., 1989; Pettit et al., 1990), there is no evidence that there is an increase in basal dopamine transmission. However, repeated cocaine administration has been shown to decrease both dorsal striatal GABA release as well as $GABA_A$ receptor density, indicating a decrease in inhibitory neurotransmission in the dorsal striatum following cocaine (Pecins-Thompson and Peris, 1993; Peris, 1996; Jung et al., 1999). This decrease in inhibitory neurotransmission is believed to lead to an increase in dorsal striatal output. Therefore, it is possible that alterations in GABAergic function are responsible for the changes in functional activation that we see during early withdrawal.

Although the neurotransmitter system underlying the upregulation in dorsal striatal activation during early withdrawal is not entirely clear, the downregulation in striatal activation seen in the current study is consistent with numerous reports suggesting that repeated psychostimulant administration leads to a downregulation in striatal dopamine transmission. Basal decreases in both dorsal and ventral extracellular dopamine have been reported during prolonged withdrawal from chronic cocaine (Rossetti et al., 1992; Clay et al., 1998). Along with this basal decrease in dopamine release, there is evidence for postsynaptic dopamine alterations as well. Przewlocka and Larson (1995) found that binge cocaine administration lead to downregulation in proenkephalin during prolonged withdrawal. As proenkephlin is colocalized with D2 receptors, this study would suggest that during prolonged withdrawal from chronic cocaine there is a downrgulation in dopamine D2 receptors. This is supported by numerous studies indicating a decrease in D2 receptor density in human addicts following long periods of abstinence (Volkow et al., 1993; Martinez et al., 2004). As rCBF is a marker of synaptic activity (Sokoloff, 1978; Sharkey et al., 1991; Mathiesen et al., 1998), this decrease seen in the current study is consistent with the idea that chronic cocaine leads to a downregulation in striatal dopamine transmission (Zhang et al., 2003; Volkow et al., 2004).

Conclusions

Extended, but not limited, access to self-administered cocaine resulted differential alterations in rCBF during early vs. late withdrawal. Regional cerebral blood flow in the dorsal striatum of the LgA animals underwent a shift from increased rCBF (compared to controls) during early withdrawal to a decrease following prolonged withdrawal. As changes in rCBF are thought to reflect changes in synaptic activity, our results may indicate differential alterations during early withdrawal vs. late withdrawal in striatal neurotransmitter systems. Further work need to be done to determine the underlying neurobiology of these functional changes.

CHAPTER VI

GENERAL DISCUSSION

The focus of the present dissertation was to determine whether chronic cocaine self-administration could lead to persistent deficits in cognitive function and changes in dopamine systems underlying cognition. The preceding chapters addressed the following questions: (1) How does differential access to cocaine self-administration alter sustained attention performance both during early and late withdrawal? (2) How does differential access to cocaine self-administration alter recognition memory performance following prolonged withdrawal? (3) Does cocaine self-administration lead to changes in prefrontal dopamine receptors? (4) Does cocaine self-administration lead to changes in striatal dopamine receptors? (5) Are there changes in the functional activation in mesolimbic and/or mesocortical dopamine systems following cocaine self-administration? In the following discussion I will summarize the results of these experiments and present a cumulative discussion of the findings.

The ability of extended access, but not limited access, cocaine self-administration to lead to persistent changes in cognitive function is addressed in both Chapters II and III. Chapter II demonstrates that following extended access

cocaine self-administration, rats exhibit a decrement in sustained attention performance both during acute and prolonged withdrawal. Three days after their final self-administration session, extended access animals exhibited a generalized deficit on the task, encompassing both signal and non-signal trials, when compared to both limited access animals and drug naïve animals. Furthermore, after up to 30 days of withdrawal, a specific non-signal trial deficit was seen in the extended access animals while no deficits were seen in either of the other two groups. Chapter III demonstrates that following extended access cocaine self-administration, rats exhibit a decrement in novel object recognition performance following 14 days of withdrawal compared with both limited access and drug naïve animals. Slight recognition memory deficits were also present in the limited access animals in the first experiment however these were not replicated in the second experiment.

The second and third experiments described in Chapter II show that extended access cocaine self-administration leads to a decrease in both prefrontal dopamine D2 receptor mRNA and protein levels both during acute and prolonged withdrawal. No decrease in either mRNA or protein was seen following limited access cocaine self-administration at either withdrawal time point. No changes were seen in D1 receptor mRNA following cocaine self-administration. Chapter IV demonstrates a 150% increase in the proportion of striatal high-affinity dopamine D2 receptors following both extended and limited access self-

administration. However, this increase in high-affinity receptors was not accompanied by a change in total D2 receptor density.

As demonstrated in Chapter V, extended access cocaine self-administration led to an increase in the functional activity, as measured by rCBF, of the caudate-putamen during acute withdrawal, compared to both limited access and drug naïve controls. However, after prolonged withdrawal there was a decrease in rCBF in both the caudate-putamen and the nucleus accumbens in the extended access animals. No changes were seen in the functional activity of the frontal cortex following chronic cocaine.

Cause vs. Consequence

One of the main goals of this dissertation was to determine whether the cognitive deficits, as well as alterations in functional activation, seen in human addicts could be a consequence of drug use rather than solely a cause. The results of Chapter II clearly demonstrate that chronic cocaine, when self-administered on an extended access schedule, produces deficits in cognitive flexibility that are similar to the executive functioning deficits seen in human addicts (1991; Rosselli and Ardila, 1996; Beason-Held et al., 1999; Bolla et al., 1999; Grant et al., 2000; Ornstein et al., 2000).

As these cognitive deficits were seen well past the period of acute withdrawal and up to 30 days after the final drug self-administration session, they are the first evidence that chronic cocaine administration can lead to persistent deficits in cognitive function in animals. As previous preclinical studies have not demonstrated persistent cognitive deficits (Dalley et al., 2005a; Dalley et al., 2005b), our results may be due in part to the extended access cocaine self-administration procedure as well as the specific testing parameters. Our results are particularly striking due to the lack of performance deficits, i.e. lack of increased omissions and lack of a decrease in total exploration time, which show that the animals truly demonstrated deficits in specific arenas of cognitive function rather than exhibiting a generalized deficit. This is consistent with the human literature as well, as addicts show deficits in some cognitive functions while remaining proficient in others (Ardila et al., 1991; Berry et al., 1993; Hoff et al., 1996; Rosselli and Ardila, 1996; Bolla et al., 1999).

The persistent deficits seen in this study were specific to the non-signal trials, and performance on these trials involves holding more information in mind than signal trials due to the lack of stimulus signaling the correct response. Therefore, we believe that extended access cocaine self-administration leads to a deficit in cognitive flexibility, or the ability to switch between two different response modes. Human addicts display increased preservative errors on the WCST and other tasks that are also aimed at measuring the ability to switch between response strategies (Beatty et al., 1995; Kubler et al., 2005; Verdejo-Garcia et al., 2006).

Performance on this task has also shown to be negatively correlated with duration of cocaine use and total dose consumed (Bolla et al., 1999). Therefore our results support the contention that cocaine abuse leads to deficits in these cognitive tasks.

Studies have shown that activation of the prefrontal cortex occurs when healthy volunteers perform the WCST as well as other cognitive tasks that involve task-switching (Konishi et al., 1999; Wagner, 2001; Loose et al., 2006). Animal studies have also shown that prefrontal cortex lesions lead to deficits on a modified version of the WCST (Birrell and Brown, 2000). As performance on task-switching paradigms requires an intact prefrontal cortex and both addicts and the animals in the current study exhibit deficits on these tasks it is hypothesized that human addicts exhibit a "hypofrontality". This is supported by evidence that cocaine addicts exhibit decreased prefrontal activation in fMRI studies and decreased prefrontal glucose metabolism when performing working memory tasks (Goldstein et al., 2004; Hester and Garavan, 2004; Tomasi et al., 2007). In further support of the idea that cocaine abuse leads to a decrease in prefrontal function, the results of Chapter II indicate that extended access cocaine led to a decrease in D2 receptor mRNA and protein suggesting a downregulation in dopamine D2 receptor transmission following chronic cocaine.

While the current dissertation does not determine whether these prefrontal D2 receptor changes are responsible for the cognitive deficits presented by the

extended access animals, other work suggest that this downregulation may play a role. Systemic administration of D2 receptor antagonists in both animals and humans suggest that D2 receptors play a critical role in cognitive flexibility (Lanser et al., 2001; Mehta et al., 2004; Tost et al., 2006). More specifically, Floresco and collegues (2006) have shown that antagonizing prefrontal D2 receptors disrupts rats ability to change response strategies. In addtion, prefrontal D2 receptors have also been shown to impair performance on other prefrontal dependent tasks, such as spatial working memory performance (Rinaldi et al., 2007). Together, these studies, along with the results of Chapter II, support the idea that a disruption in prefrontal D2 receptor function caused by cocaine abuse could lead to deficits in behavioral flexibility.

It should be noted that the results of Chapter V may seem to support the idea that the hypofrontality seen in imaging studies of human addicts does pre-exist drug abuse rather than result from it. However, taken together with the results of both the cognitive experiments as well as the ability of chronic cocaine to decrease dopamine receptor levels in the prefrontal cortex, a potential mechanism for this “hypofrontality”, we believe that our inability to see basal decreases in this study were a result of technical limitations.

Along with these deficits in prefrontal cognitive function, in Chapter III we found that chronic cocaine can also produce deficits in recognition memory in animals that are similar to those seen in human addicts (O'Malley et al., 1992; Mittenberg

and Motta, 1993). Although there is some controversy in the field (Baxter and Murray, 2001b, a), work in monkeys has clearly demonstrated that hippocampal lesions lead to a decrement in recognition memory performance (Zola-Morgan et al., 1992; Alvarez et al., 1995; Zola et al., 2000; Zola and Squire, 2001). In further support of the role of the hippocampus in recognition memory, human patients with lesions primarily limited to bilateral hippocampal damage exhibit deficits on three separate recognition memory tasks (Manns et al., 2003). Furthermore, damage to the hippocampus seems to affect both the recall and familiarity components of recognition memory performance (Manns et al., 2003; Wais et al., 2006). Similarly, object recognition memory performance in rats is impaired following dorsal hippocampal lesions, however the extent of the lesion must be relatively large (75-100% of hippocampal volume) (Broadbent et al., 2004). The dorsal hippocampus may be particularly involved in the consolidation of object recognition memory as temporary inactivation of this region only impairs performance during the first 3 hours after training (de Lima et al., 2006).

This would suggest that the results of Chapter III may indicate that extended access cocaine leads to alterations in hippocampal function. While the studies in the current dissertation did not examine alterations in the hippocampus, others have examined the effects of chronic cocaine on this brain region. Repeated cocaine administration leads to a decrease in adult hippocampal neurogenesis (Yamaguchi et al., 2004), specifically leading to a decrease in progenitor cell proliferation rather than altering cell survival or maturation (Dominguez-Escriba et

al., 2006). Although there is no conclusive evidence that decreased neurogenesis leads to impaired hippocampal-dependent memory, there is a large body of correlative evidence that this may be the case (Gould et al., 1999b; Gould and Tanapat, 1999; Gould et al., 1999a; van Praag et al., 1999; Shors et al., 2001; Drapeau et al., 2003; Lu et al., 2005; Ueda et al., 2005; van Praag et al., 2005; Leuner et al., 2006). Therefore, the decrease in neurogenesis that is caused by repeated cocaine administration may provide us with a mechanism for the reduction in recognition memory seen in Chapter III.

Along with providing us with insight into alterations in underlying brain structures, the ability of drugs of abuse to lead to deficits in cognitive function is of particular import when considering treatment options. Numerous studies have documented that addicts exhibiting cognitive deficits are less likely to remain in treatment and less likely to succeed in remaining abstinent (Moeller et al., 2001; Aharonovich et al., 2003; Aharonovich et al., 2006). As many of the more effective treatment programs involve cognitively based therapies (Carroll et al., 1991b; Carroll et al., 1991a; Carroll et al., 1994a; Rawson et al., 2002; Epstein et al., 2003; Rawson et al., 2006), gaining a full understanding of how drugs of abuse can alter cognitive function could help us to determine how we can potentially use pharmacological treatments in conjunction with more behavioral approaches to maximize treatment success.

Alterations in the Ascending Dopamine Systems Following Chronic Cocaine

As hypothesized, we were able to produce alterations in the both the mesocortical and mesolimbic dopamine systems as a result of chronic cocaine self-administration. In Chapter II we found a decrease in dopamine D2 receptor levels in the prefrontal cortex, suggesting a decrease in dopaminergic transmission following extended access cocaine. As these decreases were not seen following limited access self-administration schedules, it would suggest that the hyposensitivity in the prefrontal cortex would only occur following chronic cocaine abuse or addiction rather than after more casual cocaine use. As human functional imaging studies are aimed at measuring indicators of synaptic function (for evidence on the coupling of rCBF and/or metabolic activity to synaptic activity, see Sharkey et al., 1991; Buxton and Frank, 1997; Mathiesen et al., 1998), a decrease in dopaminergic transmission is consistent with decreases in rCBF (Volkow et al., 1988; Bell et al., 1994; Ernst et al., 2000) and glucose metabolism (Volkow et al., 1991) and provides us with a potential mechanistic explanation for these imaging results.

The effect of dopamine at D2 receptors in the PFC is somewhat complex. Slice electrophysiology studies have shown that stimulation of D2 receptors with high concentrations of dopamine leads to a decrease in inhibitory post-synaptic potentials (IPSPs) (Trantham-Davidson et al., 2004). When any step of the D2

receptor cascade is inhibited dopamine acts upon D1 receptors to increase IPSPs. This would suggest that a decrease in D2 receptor activity would lead to an increase in IPSPs and therefore a decrease in the overall activation of the PFC.

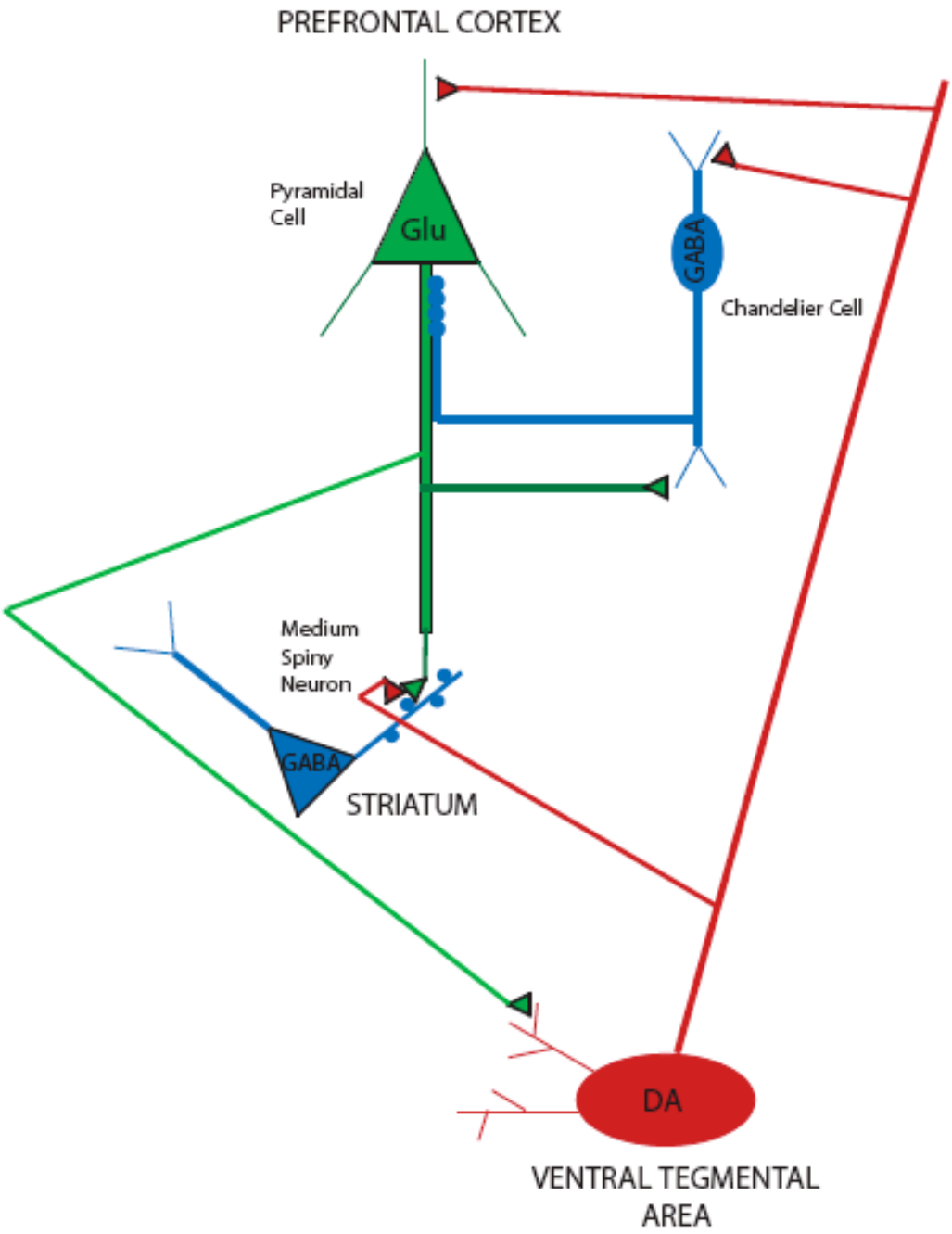
However, Tseng & O'Donnell (Tseng and O'Donnell, 2007) have shown that D2 receptor agonists lead to a decrease in pyramidal cell excitatory post-synaptic potentials (EPSPs) in slice preparations. This decrease in EPSPs is at least partially mediated by GABA as application of a $GABA_A$ antagonist blocks the lasting component of this effect. This is consistent with work demonstrating that activation of D2 receptors in the PFC in both rats (Del Arco and Mora, 2002, 2005) and monkeys (Wang and Goldman-Rakic, 2004) leads to a facilitation of glutamate-evoked GABA release in the PFC. The net result of this seems to a decrease in the activity of glutamatergic descending projections to the NAc (Brady and O'Donnell, 2004) and a corresponding decrease in NAc dopamine release (Del Arco and Mora, 2005). Therefore, a reciprocal relationship exists between dopamine transmission in the PFC and the NAc, with high levels in one region corresponding with low levels in the other.

As the results of Chapter II suggest that extended access cocaine leads to a decrease in prefrontal D2 receptor activity, I would hypothesize that this is accompanied by a decrease in prefrontal glutamate-evoked GABA release and therefore an increase in cortico-accumbal glutamate release. While this is

contrary to hypotheses based on the work of Trantham-Davidson and colleagues (2004), there is overwhelming evidence suggesting a reciprocal relationship between prefrontal and striatal dopamine transmission. For example, lesions of the PFC lead to an increase in striatal D2 receptor expression (Baca et al., 1998). In addition, stimulation of the prefrontal cortex at physiologically relevant frequencies leads to a decrease in NAc dopamine release (Jackson et al., 2001) which is consistent with the idea that glutamate in the PFC (specifically activation of AMPA receptors) exerts a tonic inhibitory control on dopamine release (Takahata and Moghaddam, 1998). While this may seem contrary to work demonstrating that inhibition of prefrontal $GABA_A$ leads to an increase in striatal dopamine release, when you consider the entire circuit it becomes more clear (see Figure 6.1). The prefrontal cortex contains not only descending glutamateric projections that excite striatal dopamine release but also glutamatergic neurons that synapse on GABAergic interneurons that in turn inhibit the descending projections.

As prefrontal glutamatergic efferents also project to the dorsal striatum, the decrease in D2 receptor function in the PFC should also lead to a corresponding increase in glutamatergic transmission here as well as in the NAc. As glutamate in the dorsal striatum leads to an increase in dopamine transmission, this increase in glutamatergic transmission should lead to a dopaminergic increase. Although we did not measure the effects of cocaine self-administration on dorsal striatal dopamine release, the results of the experiment in Chapter IV indicate

Figure 6.1. Interactions between ascending dopaminergic projections, intracortical interneurons, and decending glutmatatergic projections. Dopaminergic projections from the ventral tegmental area (VTA; in red) synapse both in the striatum (NAc) and the prefrontal cortex. The projections to the cortex synapse both onto GABAergic interneurons (in blue) and glutamatergic pyramidal cells (in green). In turn, the pyramidal cells synapse both onto the GABAergic interneurons, thus potentiating GABA release, and onto both ventral and dorsal striatal medium spiny neurons (in blue), thus potentiating dopamine striatal dopamine transmission. In addition, glutamatergic projections exist from the PFC to the VTA. Note that the chandelier cell is only one type of GABAergic interneuron that may be playing a role in this circuit and it was included for illustrative purposes.


PREFRONTAL CORTEX
Pyramidal Cell
Glu
GABA
Chandelier Cell
Medium Spiny Neuron
GABA
STRIATUM
DA
VENTRAL TEGMENTAL AREA

that there is an increase in dopamine D2 receptor transmission due to an increase in proportion of the high-affinity state of the receptor. The results of Chapter V also indicate that chronic cocaine, at least during acute withdrawal, can lead to an increase in functional activity in the dorsal striatum. Although there was a decrease seen after prolonged withdrawal, this was only seen in one portion of the striatum and it is unclear exactly how this specific alteration in functional activity may effect dopamine transmission.

Despite the fact that studies of human addicts have reported decreases in dorsal striatal functional activation during working memory task performance (Tomasi et al., 2007) as well as decreased basal dopamine receptor availability in both the dorsal and ventral striatum (Volkow et al., 1990; Volkow et al., 1993; Martinez et al., 2004), an increase in dopamine transmission in the ventral and dorsal striatum is consistent with a large body of literature indicating that chronic cocaine leads to hypersensitivity or sensitization of the reward system (Robinson and Berridge, 1993; Robinson and Berridge, 2000; Berridge, 2007). For example, both repeated amphetamine and cocaine lead to an increase in evoked dopamine release in the ventral and dorsal striatum (Robinson and Becker, 1982; Wilcox et al., 1986; Castaneda et al., 1988; Robinson et al., 1988; Akimoto et al., 1989; Akimoto et al., 1990; Kalivas and Duffy, 1990; Pettit et al., 1990; Ng et al., 1991; Clay et al., 1998). Chronic cocaine also has been shown to increase cocaine-induced dopamine overflow (Heidbreder et al., 1996), drug-paired cue-induced dopamine release (Kiyatkin and Stein, 1995), and the behavioral effects

of direct dopaminergic agonists (Kiyatkin, 1994; De Vries et al., 2002; Edwards et al., 2007). While clearly the story is not as simple as an overall increase in dopamine transmission throughout the striatum, as the heterogeneity of these structures alone suggests otherwise (Cadoni et al., 2000), the results of the current dissertation in conjunction with a multitude of other studies suggest that the mesolimbic and nigrostriatal dopamine systems are rendered hypersensitive following chronic cocaine.

Although the above hypothesis would suggest that it is hypoactivity in the PFC that leads to hyperactivity in the striatum, it is also possible that alterations in dopamine transmission in the striatum lead to changes in PFC functioning. Kellendonk and colleagues (2006) demonstrated that overexpression of D2 receptors in the striatum leads to both deficits in performance on prefrontally-mediated cognitive tasks but also an increase in dopamine turnover, and a decrease in prefrontal D1 receptor activity. However, as the results of this study suggest that the effects of D2 overexpression are primarily due to changes during development, it is more likely that changes in the PFC lead to striatal changes than vice versa. On the other hand, as alterations in striatal D2 receptors were seen in following limited access self-administration and no changes were seen in the PFC following this drug administration protocol, there may also be independent changes in the striatum that do not depend on hypoactivity in the PFC.

The Role of Cocaine-Induced Alterations in Mesocortical and Mesolimbic Dopamine Transmission in Relapse

These discussed alterations in both prefrontal and striatal dopamine functioning could be involved in reinstatement of drug-seeking and relapse. For example, cocaine primed reinstatement leads to an increase in NAc glutamate and blocking prefrontal descending glutamatergic projections prevents reinstatement (Cornish and Kalivas, 2000; McFarland et al., 2003). Furthermore, the injection of GABA agonists into the PFC prevents cocaine reinstatement (McFarland and Kalivas, 2001). In addition, the activation of D1 or D2 receptors in the nucleus accumbens leads to reinstatement of drug-seeking (Bachtell et al., 2005; Schmidt et al., 2006; Schmidt and Pierce, 2006). These studies support the hypothesis that chronic cocaine leads to decrease prefrontal GABA transmission thereby increasing glutamatergic input to the NAc, which in turn increases dopamine transmission thereby mediating reinstatement. As the ability of D2 agonist infusion into the PFC to reinstate drug-seeking behavior has not yet been examined, future work will have to confirm or reject the idea that it is a hypoactive D2 receptor system that leads to these GABAergic changes.

While the NAc seems to be primarily involved in cocaine-primed reinstatment, the dorsal striatum has been demonstrated to be central to the processing of drug-paired cues and for cue-controlled drug seeking. Vanderschuren and colleagues (2005) have shown that either administration of glutamtate or dopamine

antagonists into the dorsal striatum decreased cue-controlled drug seeking. In addition, inactivation of the prefrontal cortex also prevents cue-induced reinstatement, in further support of the role of corticostriatal glutamate projections in reinstatment (Di Pietro et al., 2006). Together, these studies provide evidence that an upregulation in cortical glutamate projections (perhaps due to dopaminergic alterations) leads to an increased sensitivity to both cocaine primed and cue-induced reinstatement.

Concluding Remarks

The experiments presented in the current dissertation provide evidence that both cognitive function and neurobiological systems underlying cognition can be altered as a consequence of cocaine administration. This work has important implications for the development of both pharmacological and behavioral addiction treatment strategies. The current findings provide us with a model to examine these drug-induced changes and hopefully gain a better understanding of how these changes underlie alterations in behavior. As a next step toward examining potential therapies, it will be necessary to assess the effect of dopamine D2 receptor agents, both systemically and locally administered into the PFC and the striatum, on cocaine-induced decrements in cognitive function as well as their ability to alter reinstatement responding.

REFERENCES

Adams S, Kesner RP, Ragozzino ME (2001) Role of the medial and lateral caudate-putamen in mediating an auditory conditional response association. Neurobiol Learn Mem 76:106-116.

Adcock RA, Constable RT, Gore JC, Goldman-Rakic PS (2000) Functional neuroanatomy of executive processes involved in dual-task performance. Proc Natl Acad Sci U S A 97:3567-3572.

Aharonovich E, Nunes E, Hasin D (2003) Cognitive impairment, retention and abstinence among cocaine abusers in cognitive-behavioral treatment. Drug Alcohol Depend 71:207-211.

Aharonovich E, Hasin DS, Brooks AC, Liu X, Bisaga A, Nunes EV (2006) Cognitive deficits predict low treatment retention in cocaine dependent patients. Drug Alcohol Depend 81:313-322.

Ahmed SH, Koob GF (1998) Transition from moderate to excessive drug intake: change in hedonic set point. Science 282:298-300.

Ahmed SH, Koob GF (1999) Long-lasting increase in the set point for cocaine self-administration after escalation in rats. Psychopharmacology (Berl) 146:303-312.

Ahmed SH, Koob GF (2004) Changes in response to a dopamine receptor antagonist in rats with escalating cocaine intake. Psychopharmacology (Berl) 172:450-454.

Ahmed SH, Walker JR, Koob GF (2000) Persistent increase in the motivation to take heroin in rats with a history of drug escalation. Neuropsychopharmacology 22:413-421.

Ahmed SH, Kenny PJ, Koob GF, Markou A (2002) Neurobiological evidence for hedonic allostasis associated with escalating cocaine use. Nat Neurosci 5:625-626.

Ainge JA, Heron-Maxwell C, Theofilas P, Wright P, de Hoz L, Wood ER (2006) The role of the hippocampus in object recognition in rats: examination of

the influence of task parameters and lesion size. Behav Brain Res 167:183-195.

Akimoto K, Hamamura T, Otsuki S (1989) Subchronic cocaine treatment enhances cocaine-induced dopamine efflux, studied by in vivo intracerebral dialysis. Brain Res 490:339-344.

Akimoto K, Hamamura T, Kazahaya Y, Akiyama K, Otsuki S (1990) Enhanced extracellular dopamine level may be the fundamental neuropharmacological basis of cross-behavioral sensitization between methamphetamine and cocaine--an in vivo dialysis study in freely moving rats. Brain Res 507:344-346.

Alburges ME, Narang N, Wamsley JK (1993) Alterations in the dopaminergic receptor system after chronic administration of cocaine. Synapse 14:314-323.

Alderson HL, Parkinson JA, Robbins TW, Everitt BJ (2001) The effects of excitotoxic lesions of the nucleus accumbens core or shell regions on intravenous heroin self-administration in rats. Psychopharmacology (Berl) 153:455-463.

Allen JD, Davison CS (1973) Effects of caudate lesions on signaled and nonsignaled Sidman avoidance in the rat. Behav Biol 8:239-250.

Alvarez P, Zola-Morgan S, Squire LR (1995) Damage limited to the hippocampal region produces long-lasting memory impairment in monkeys. J Neurosci 15:3796-3807.

Annett LE, McGregor A, Robbins TW (1989) The effects of ibotenic acid lesions of the nucleus accumbens on spatial learning and extinction in the rat. Behav Brain Res 31:231-242.

Ardila A, Rosselli M, Strumwasser S (1991) Neuropsychological deficits in chronic cocaine abusers. IntJNeurosci 57:73-79.

Aytaclar S, Tarter RE, Kirisci L, Lu S (1999) Association between hyperactivity and executive cognitive functioning in childhood and substance use in early adolescence. J Am Acad Child Adolesc Psychiatry 38:172-178.

Baca SM, Lipska BK, Egan MF, Bachus SE, Ferguson JN, Hyde TM (1998) Effects of prefrontal cortical lesions on neuropeptide and dopamine receptor gene expression in the striatum-accumbens complex. Brain Res 797:55-64.

Bachtell RK, Whisler K, Karanian D, Self DW (2005) Effects of intra-nucleus accumbens shell administration of dopamine agonists and antagonists on cocaine-taking and cocaine-seeking behaviors in the rat. Psychopharmacology (Berl) 183:41-53.

Balster RL, Kilbey MM, Ellinwood EH, Jr. (1976) Methamphetamine self-administration in the cat. Psychopharmacologia 46:229-233.

Bartzokis G, Beckson M, Lu PH, Edwards N, Rapoport R, Wiseman E, Bridge P (2000) Age-related brain volume reductions in amphetamine and cocaine addicts and normal controls: implications for addiction research. Psychiatry Res 98:93-102.

Battig K, Rosvold HE, Mishkin M (1960) Comparison of the effects of frontal and caudate lesions on delayed response and alternation in monkeys. J Comp Physiol Psychol 53:400-404.

Baxter MG, Murray EA (2001a) Opposite relationship of hippocampal and rhinal cortex damage to delayed nonmatching-to-sample deficits in monkeys. Hippocampus 11:61-71.

Baxter MG, Murray EA (2001b) Effects of hippocampal lesions on delayed nonmatching-to-sample in monkeys: a reply to Zola and Squire (2001). Hippocampus 11:201-203.

Beason-Held LL, Rosene DL, Killiany RJ, Moss MB (1999) Hippocampal formation lesions produce memory impairment in the rhesus monkey. Hippocampus 9:562-574.

Beatty WW, Katzung VM, Moreland VJ, Nixon SJ (1995) Neuropsychological performance of recently abstinent alcoholics and cocaine abusers. Drug Alcohol Depend 37:247-253.

Bechara A, Dolan S, Denburg N, Hindes A, Anderson SW, Nathan PE (2001) Decision-making deficits, linked to a dysfunctional ventromedial prefrontal

cortex, revealed in alcohol and stimulant abusers. Neuropsychologia 39:376-389.

Belcher AM, O'Dell SJ, Marshall JF (2005a) Impaired object recognition memory following methamphetamine, but not p-chloroamphetamine- or d-amphetamine-induced neurotoxicity. Neuropsychopharmacology 30:2026-2034.

Belcher AM, O'Dell SJ, Marshall JF (2005b) Impaired object recognition memory following methamphetamine, but not p-chloroamphetamine or d-amphetamine-induced neurotoxicity. Neuropsychopharmacology 30:2026-2034.

Belcher AM, O'Dell SJ, Marshall JF (2006) A sensitizing regimen of methamphetamine causes impairments in a novelty preference task of object recognition. Behav Brain Res 170:167-172.

Bell KM, Milne N, Lyons KP (1994) Regional cerebral blood flow and cocaine abuse. West J Med 161:412-413.

Ben-Shahar O, Moscarello JM, Ettenberg A (2006) One hour, but not six hours, of daily access to self-administered cocaine results in elevated levels of the dopamine transporter. Brain Res 1095:148-153.

Ben-Shahar O, Keeley P, Cook M, Brake W, Joyce M, Nyffeler M, Heston R, Ettenberg A (2007) Changes in levels of D1, D2, or NMDA receptors during withdrawal from brief or extended daily access to IV cocaine. Brain Res 1131:220-228.

Bernal B, Ardila A, Bateman JR (1994) Cognitive impairments in adolescent drug-abusers. Int J Neurosci 75:203-212.

Berridge KC (2007) The debate over dopamine's role in reward: the case for incentive salience. Psychopharmacology (Berl) 191:391-431.

Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Brain Res Rev 28:309-369.

Berridge KC, Venier IL, Robinson TE (1989) Taste reactivity analysis of 6-hydroxydopamine-induced aphagia: implications for arousal and anhedonia hypotheses of dopamine function. Behav Neurosci 103:36-45.

Berry J, van Gorp WG, Herzberg DS, Hinkin C, Boone K, Steinman L, Wilkins JN (1993) Neuropsychological deficits in abstinent cocaine abusers: preliminary findings after two weeks of abstinence. Drug Alcohol Depend 32:231-237.

Beveridge TJ, Smith HR, Daunais JB, Nader MA, Porrino LJ (2006) Chronic cocaine self-administration is associated with altered functional activity in the temporal lobes of non human primates. Eur J Neurosci 23:3109-3118.

Birrell JM, Brown VJ (2000) Medial frontal cortex mediates perceptual attentional set shifting in the rat. J Neurosci 20:4320-4324.

Bisaga A, Aharonovich E, Garawi F, Levin FR, Rubin E, Raby WN, Nunes EV (2006) A randomized placebo-controlled trial of gabapentin for cocaine dependence. Drug Alcohol Depend 81:267-274.

Blackson TC, Butler T, Belsky J, Ammerman RT, Shaw DS, Tarter RE (1999) Individual traits and family contexts predict sons' externalizing behavior and preliminary relative risk ratios for conduct disorder and substance use disorder outcomes. Drug Alcohol Depend 56:115-131.

Bolla K, Ernst M, Kiehl K, Mouratidis M, Eldreth D, Contoreggi C, Matochik J, Kurian V, Cadet J, Kimes A, Funderburk F, London E (2004) Prefrontal cortical dysfunction in abstinent cocaine abusers. Journal of Neuropsychiatry & Clinical Neurosciences 16:456-464.

Bolla KI, Cadet JL, London ED (1998) The neuropsychiatry of chronic cocaine abuse. J Neuropsychiatry Clin Neurosci 10:280-289.

Bolla KI, Rothman R, Cadet JL (1999) Dose-related neurobehavioral effects of chronic cocaine use. JNeuropsychiatry ClinNeurosci 11:361-369.

Bolla KI, Funderburk FR, Cadet JL (2000) Differential effects of cocaine and cocaine alcohol on neurocognitive performance. Neurology 54:2285-2292.

Bolla KI, Eldreth DA, London ED, Kiehl KA, Mouratidis M, Contoreggi C, Matochik JA, Kurian V, Cadet JL, Kimes AS, Funderburk FR, Ernst M (2003) Orbitofrontal cortex dysfunction in abstinent cocaine abusers performing a decision-making task. Neuroimage 19:1085-1094.

Bonson KR, Grant SJ, Contoreggi CS, Links JM, Metcalfe J, Weyl HL, Kurian V, Ernst M, London ED (2002) Neural systems and cue-induced cocaine craving. Neuropsychopharmacology 26:376-386.

Bozarth MA, Wise RA (1985) Toxicity associated with long-term intravenous heroin and cocaine self-administration in the rat. Jama 254:81-83.

Bradberry CW, Barrett-Larimore RL, Jatlow P, Rubino SR (2000) Impact of self-administered cocaine and cocaine cues on extracellular dopamine in mesolimbic and sensorimotor striatum in rhesus monkeys. J Neurosci 20:3874-3883.

Brady AM, O'Donnell P (2004) Dopaminergic modulation of prefrontal cortical input to nucleus accumbens neurons in vivo. J Neurosci 24:1040-1049.

Briand LA, Flagel SB, Watson SJ, Akil H, Sarter M, Robinson TE (2006) Long Access Cocaine Self-Administration Leads to Persistent Cognitive Deficits. In: Society for Neuroscience Annual Meeting. Atlanta, GA.

Broadbear JH, Winger G, Cicero TJ, Woods JH (1999) Effects of response contingent and noncontingent cocaine injection on hypothalamic-pituitary-adrenal activity in rhesus monkeys. J Pharmacol Exp Ther 290:393-402.

Broadbent NJ, Squire LR, Clark RE (2004) Spatial memory, recognition memory, and the hippocampus. Proc Natl Acad Sci U S A 101:14515-14520.

Broderick PA (1986) Rat striatal dopamine release mechanisms of cocaine. NIDA Res Monogr 75:367-370.

Brook JS, Whiteman MM, Finch S (1992) Childhood aggression, adolescent delinquency, and drug use: a longitudinal study. J Genet Psychol 153:369-383.

Brook JS, Whiteman M, Finch S, Cohen P (1995) Aggression, intrapsychic distress, and drug use: antecedent and intervening processes. J Am Acad Child Adolesc Psychiatry 34:1076-1084.

Brook JS, Finch SJ, Whiteman M, Brook DW (2002) Drug use and neurobehavioral, respiratory, and cognitive problems: precursors and mediators. J Adolesc Health 30:433-441.

Browndyke JN, Tucker KA, Woods SP, Beauvals J, Cohen RA, Gottschalk PC, Kosten TR (2004) Examining the effect of cerebral perfusion abnormality magnitude on cognitive performance in recently abstinent chronic cocaine abusers. JNeuroimaging 14:162-169.

Brozoski TJ, Brown RM, Rosvold HE, Goldman PS (1979) Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. Science 205:929-932.

Bubser M, Koch M (1994) Prepulse inhibition of the acoustic startle response of rats is reduced by 6-hydroxydopamine lesions of the medial prefrontal cortex. Psychopharmacology (Berl) 113:487-492.

Burke KA, Franz TM, Gugsa N, Schoenbaum G (2006) Prior cocaine exposure disrupts extinction of fear conditioning. Learn Mem 13:416-421.

Buxton RB, Frank LR (1997) A model for the coupling between cerebral blood flow and oxygen metabolism during neural stimulation. J Cereb Blood Flow Metab 17:64-72.

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

Cagniard B, Balsam PD, Brunner D, Zhuang X (2006a) Mice with chronically elevated dopamine exhibit enhanced motivation, but not learning, for a food reward. Neuropsychopharmacology 31:1362-1370.

Cagniard B, Beeler JA, Britt JP, McGehee DS, Marinelli M, Zhuang X (2006b) Dopamine scales performance in the absence of new learning. Neuron 51:541-547.

Caine SB, Lintz R, Koob GF (1993) Intravenous drug self-administration techniques in animals. In: Behavioral Neuroscience, a practical approach (Sahgal A, ed), pp 117-143. New York: Oxford University Press.

Caine SB, Negus SS, Mello NK (2000) Effects of dopamine D(1-like) and D(2-like) agonists on cocaine self-administration in rhesus monkeys: rapid assessment of cocaine dose-effect functions. Psychopharmacology (Berl) 148:41-51.

Caine SB, Heinrichs SC, Coffin VL, Koob GF (1995) Effects of the dopamine D-1 antagonist SCH 23390 microinjected into the accumbens, amygdala or striatum on cocaine self-administration in the rat. Brain Res 692:47-56.

Caine SB, Negus SS, Mello NK, Bergman J (1999) Effects of dopamine D(1-like) and D(2-like) agonists in rats that self-administer cocaine. J Pharmacol Exp Ther 291:353-360.

Carli M, Jones GH, Robbins TW (1989) Effects of unilateral dorsal and ventral striatal dopamine depletion on visual neglect in the rat: a neural and behavioural analysis. Neuroscience 29:309-327.

Carroll KM, Rounsaville BJ, Keller DS (1991a) Relapse prevention strategies for the treatment of cocaine abuse. Am J Drug Alcohol Abuse 17:249-265.

Carroll KM, Rounsaville BJ, Gawin FH (1991b) A comparative trial of psychotherapies for ambulatory cocaine abusers: relapse prevention and interpersonal psychotherapy. Am J Drug Alcohol Abuse 17:229-247.

Carroll KM, Rounsaville BJ, Nich C, Gordon LT, Wirtz PW, Gawin F (1994a) One-year follow-up of psychotherapy and pharmacotherapy for cocaine dependence. Delayed emergence of psychotherapy effects. Arch Gen Psychiatry 51:989-997.

Carroll KM, Rounsaville BJ, Gordon LT, Nich C, Jatlow P, Bisighini RM, Gawin FH (1994b) Psychotherapy and pharmacotherapy for ambulatory cocaine abusers. Arch Gen Psychiatry 51:177-187.

Carroll ME, France CP, Meisch RA (1981) Intravenous self-administration of etonitazene, cocaine and phencyclidine in rats during food deprivation and satiation. J Pharmacol Exp Ther 217:241-247.

Castaneda E, Becker JB, Robinson TE (1988) The long-term effects of repeated amphetamine treatment in vivo on amphetamine, KCl and electrical stimulation evoked striatal dopamine release in vitro. Life Sci 42:2447-2456.

Christakou A, Robbins TW, Everitt BJ (2004) Prefrontal cortical-ventral striatal interactions involved in affective modulation of attentional performance: implications for corticostriatal circuit function. J Neurosci 24:773-780.

Clark DB, Kirisci L, Moss HB (1998) Early adolescent gateway drug use in sons of fathers with substance use disorders. Addict Behav 23:561-566.

Clark DB, Cornelius JR, Kirisci L, Tarter RE (2005) Childhood risk categories for adolescent substance involvement: a general liability typology. Drug Alcohol Depend 77:13-21.

Clark RE, Kuczenski R, Segal DS (2007) Escalating dose, multiple binge methamphetamine regimen does not impair recognition memory in rats. Synapse 61:515-522.

Clay LH, Mazzio EA, Kolta MG, Soliman KF (1998) Repeated administration of cocaine alters dopamine uptake and release in the striatum nucleus accumbens. Ann N Y Acad Sci 844:346-355.

Cole BJ, Robbins TW (1989) Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi on performance of a 5-choice serial reaction time task in rats: implications for theories of selective attention and arousal. Behav Brain Res 33:165-179.

Collins P, Wilkinson LS, Everitt BJ, Robbins TW, Roberts AC (2000) The effect of dopamine depletion from the caudate nucleus of the common marmoset (Callithrix jacchus) on tests of prefrontal cognitive function. Behav Neurosci 114:3-17.

Corbit LH, Muir JL, Balleine BW (2001) The role of the nucleus accumbens in instrumental conditioning: Evidence of a functional dissociation between accumbens core and shell. J Neurosci 21:3251-3260.

Cornish JL, Kalivas PW (2000) Glutamate transmission in the nucleus accumbens mediates relapse in cocaine addiction. J Neurosci 20:RC89.

Courtney SM, Ungerleider LG, Keil K, Haxby JV (1996) Object and spatial visual working memory activate separate neural systems in human cortex. Cereb Cortex 6:39-49.

Crider A, Solomon PR, McMahon MA (1982) Disruption of selective attention in the rat following chronic d-amphetamine administration: relationship to schizophrenic attention disorder. BiolPsychiatry 17:351-361.

Criswell HE, Ridings A (1983) Intravenous self-administration of morphine by naive mice. Pharmacol Biochem Behav 18:467-470.

Crofts HS, Dalley JW, Collins P, Van Denderen JC, Everitt BJ, Robbins TW, Roberts AC (2001) Differential effects of 6-OHDA lesions of the frontal cortex and caudate nucleus on the ability to acquire an attentional set. Cereb Cortex 11:1015-1026.

Dackis CA, Gold MS (1985) New concepts in cocaine addiction: the dopamine depletion hypothesis. Neurosci Biobehav Rev 9:469-477.

Dackis CA, Kampman KM, Lynch KG, Pettinati HM, O'Brien CP (2005) A double-blind, placebo-controlled trial of modafinil for cocaine dependence. Neuropsychopharmacology 30:205-211.

Dalley JW, Cardinal RN, Robbins TW (2004) Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. Neurosci Biobehav Rev 28:771-784.

Dalley JW, Laane K, Pena Y, Theobald DE, Everitt BJ, Robbins TW (2005a) Attentional and motivational deficits in rats withdrawn from intravenous self-administration of cocaine or heroin. Psychopharmacology (Berl) 182:579-587.

Dalley JW, Theobald DE, Berry D, Milstein JA, Laane K, Everitt BJ, Robbins TW (2005b) Cognitive sequelae of intravenous amphetamine self-administration in rats: evidence for selective effects on attentional performance. Neuropsychopharmacology 30:525-537.

de Lima MN, Luft T, Roesler R, Schroder N (2006) Temporary inactivation reveals an essential role of the dorsal hippocampus in consolidation of object recognition memory. Neurosci Lett 405:142-146.

De Vries TJ, Schoffelmeer AN, Binnekade R, Raaso H, Vanderschuren LJ (2002) Relapse to cocaine- and heroin-seeking behavior mediated by dopamine D2 receptors is time-dependent and associated with behavioral sensitization. Neuropsychopharmacology 26:18-26.

Del Arco A, Mora F (2002) NMDA and AMPA/kainate glutamatergic agonists increase the extracellular concentrations of GABA in the prefrontal cortex of the freely moving rat: modulation by endogenous dopamine. Brain Res Bull 57:623-630.

Del Arco A, Mora F (2005) Glutamate-dopamine in vivo interaction in the prefrontal cortex modulates the release of dopamine and acetylcholine in the nucleus accumbens of the awake rat. J Neural Transm 112:97-109.

Del Olmo N, Higuera-Matas A, Miguens M, Garcia-Lecumberri C, Borcel E, Solis JM, Ambrosio E (2006) Hippocampal synaptic plasticity and water maze learning in cocaine self-administered rats. Ann N Y Acad Sci 1074:427-437.

Demb JB, Desmond JE, Wagner AD, Vaidya CJ, Glover GH, Gabrieli JD (1995) Semantic encoding and retrieval in the left inferior prefrontal cortex: a functional MRI study of task difficulty and process specificity. J Neurosci 15:5870-5878.

Deneau G, Yanagita T, Seevers MH (1969) Self-administration of psychoactive substances by the monkey. Psychopharmacologia 16:30-48.

Deroche-Gamonet V, Belin D, Piazza PV (2004) Evidence for addiction-like behavior in the rat.[see comment]. Science 305:1014-1017.

Di Chiara G (2002) Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. Behav Brain Res 137:75-114.

Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 85:5274-5278.

Di Pietro NC, Black YD, Kantak KM (2006) Context-dependent prefrontal cortex regulation of cocaine self-administration and reinstatement behaviors in rats. Eur J Neurosci 24:3285-3298.

Dias R, Robbins TW, Roberts AC (1996) Primate analogue of the Wisconsin Card Sorting Test: effects of excitotoxic lesions of the prefrontal cortex in the marmoset. Behav Neurosci 110:872-886.

Divac I (1968) Effects of prefrontal and caudate lesions on delayed response in cats. Acta Biol Exp (Warsz) 28:149-167.

Divac I (1972) Delayed alternation in cats with lesions of the prefrontal cortex and the caudate nucleus. Physiol Behav 8:519-522.

Divac I, Markowitsch HJ, Pritzel M (1978) Behavioral and anatomical consequences of small intrastriatal injections of kainic acid in the rat. Brain Res 151:523-532.

Dobrossy MD, Svendsen CN, Dunnett SB (1995) The effects of bilateral striatal lesions on the acquisition of an operant test of short term memory. Neuroreport 6:2049-2053.

Dobrossy MD, Svendsen CN, Dunnett SB (1996) Bilateral striatal lesions impair retention of an operant test of short-term memory. Brain Res Bull 41:159-165.

Dominguez-Escriba L, Hernandez-Rabaza V, Soriano-Navarro M, Barcia JA, Romero FJ, Garcia-Verdugo JM, Canales JJ (2006) Chronic cocaine exposure impairs progenitor proliferation but spares survival and maturation of neural precursors in adult rat dentate gyrus. Eur J Neurosci 24:586-594.

Dong Y, Nasif FJ, Tsui JJ, Ju WY, Cooper DC, Hu XT, Malenka RC, White FJ (2005) Cocaine-induced plasticity of intrinsic membrane properties in prefrontal cortex pyramidal neurons: adaptations in potassium currents. J Neurosci 25:936-940.

Drapeau E, Mayo W, Aurousseau C, Le Moal M, Piazza PV, Abrous DN (2003) Spatial memory performances of aged rats in the water maze predict levels of hippocampal neurogenesis. Proc Natl Acad Sci U S A 100:14385-14390.

Dunnett SB, Nathwani F, Brasted PJ (1999) Medial prefrontal and neostriatal lesions disrupt performance in an operant delayed alternation task in rats. Behav Brain Res 106:13-28.

Easton CJ, Babuscio T, Carroll KM (2007) Treatment retention and outcome among cocaine-dependent patients with and without active criminal justice involvement. J Am Acad Psychiatry Law 35:83-91.

Edwards S, Whisler KN, Fuller DC, Orsulak PJ, Self DW (2007) Addiction-related alterations in D1 and D2 dopamine receptor behavioral responses following chronic cocaine self-administration. Neuropsychopharmacology 32:354-366.

Elkashef A, Fudala PJ, Gorgon L, Li SH, Kahn R, Chiang N, Vocci F, Collins J, Jones K, Boardman K, Sather M (2006) Double-blind, placebo-controlled trial of selegiline transdermal system (STS) for the treatment of cocaine dependence. Drug Alcohol Depend 85:191-197.

Epstein DH, Hawkins WE, Covi L, Umbricht A, Preston KL (2003) Cognitive-behavioral therapy plus contingency management for cocaine use: findings during treatment and across 12-month follow-up. Psychol Addict Behav 17:73-82.

Erb S, Funk D, Le AD (2003) Prior, repeated exposure to cocaine potentiates locomotor responsivity to central injections of corticotropin-releasing factor (CRF) in rats. Psychopharmacology (Berl) 170:383-389.

Erb S, Lopak V, Smith C (2004) Cocaine pre-exposure produces a sensitized and context-specific c-fos mRNA response to footshock stress in the central nucleus of the AMYGDALA. Neuroscience 129:719-725.

Erb S, Kayyali H, Romero K (2006) A study of the lasting effects of cocaine pre-exposure on anxiety-like behaviors under baseline conditions and in response to central injections of corticotropin-releasing factor. Pharmacol Biochem Behav 85:206-213.

Ernst M, Bolla K, Mouratidis M, Contoreggi C, Matochik JA, Kurian V, Cadet JL, Kimes AS, London ED (2002) Decision-making in a risk-taking task: a PET study. Neuropsychopharmacology 26:682-691.

Ernst T, Chang L, Oropilla G, Gustavson A, Speck O (2000) Cerebral perfusion abnormalities in abstinent cocaine abusers: a perfusion MRI and SPECT study. Psychiatry Res 99:63-74.

Evans AH, Pavese N, Lawrence AD, Tai YF, Appel S, Doder M, Brooks DJ, Lees AJ, Piccini P (2006) Compulsive drug use linked to sensitized ventral striatal dopamine transmission. Ann Neurol 59:852-858.

Everitt BJ, Dickinson A, Robbins TW (2001) The neuropsychological basis of addictive behaviour. Brain Res Brain Res Rev 36:129-138.

Featherstone RE, McDonald RJ (2004a) Dorsal striatum and stimulus-response learning: lesions of the dorsolateral, but not dorsomedial, striatum impair acquisition of a simple discrimination task. Behav Brain Res 150:15-23.

Featherstone RE, McDonald RJ (2004b) Dorsal striatum and stimulus-response learning: lesions of the dorsolateral, but not dorsomedial, striatum impair acquisition of a stimulus-response-based instrumental discrimination task, while sparing conditioned place preference learning. Neuroscience 124:23-31.

Featherstone RE, McDonald RJ (2005) Lesions of the dorsolateral striatum impair the acquisition of a simplified stimulus-response dependent conditional discrimination task. Neuroscience 136:387-395.

Febo M, Segarra AC, Tenney JR, Brevard ME, Duong TQ, Ferris CF (2004) Imaging cocaine-induced changes in the mesocorticolimbic dopaminergic system of conscious rats. J Neurosci Methods 139:167-176.

Febo M, Segarra AC, Nair G, Schmidt K, Duong TQ, Ferris CF (2005) The neural consequences of repeated cocaine exposure revealed by functional MRI in awake rats. Neuropsychopharmacology 30:936-943.

Fein G, Di Sclafani V, Meyerhoff DJ (2002) Prefrontal cortical volume reduction associated with frontal cortex function deficit in 6-week abstinent crack-cocaine dependent men. Drug Alcohol Depend 68:87-93.

Ferrario CR, Gorny G, Crombag HS, Li Y, Kolb B, Robinson TE (2005) Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. Biol Psychiatry 58:751-759.

Fiez JA, Raife EA, Balota DA, Schwarz JP, Raichle ME, Petersen SE (1996) A positron emission tomography study of the short-term maintenance of verbal information. J Neurosci 16:808-822.

Fishbein DH, Eldreth DL, Hyde C, Matochik JA, London ED, Contoreggi C, Kurian V, Kimes AS, Breeden A, Grant S (2005) Risky decision making and the anterior cingulate cortex in abstinent drug abusers and nonusers. Brain Res Cogn Brain Res 23:119-136.

Fletcher PJ, Tenn CC, Rizos Z, Lovic V, Kapur S (2005) Sensitization to amphetamine, but not PCP, impairs attentional set shifting: reversal by a D1 receptor agonist injected into the medial prefrontal cortex. Psychopharmacology (Berl) 183:190-200.

Fletcher PJ, Tenn CC, Sinyard J, Rizos Z, Kapur S (2007) A sensitizing regimen of amphetamine impairs visual attention in the 5-choice serial reaction time test: reversal by a D1 receptor agonist injected into the medial prefrontal cortex. Neuropsychopharmacology 32:1122-1132.

Floresco SB, Phillips AG (2001) Delay-dependent modulation of memory retrieval by infusion of a dopamine D1 agonist into the rat medial prefrontal cortex. Behav Neurosci 115:934-939.

Floresco SB, Magyar O, Ghods-Sharifi S, Vexelman C, Tse MT (2006) Multiple dopamine receptor subtypes in the medial prefrontal cortex of the rat regulate set-shifting. Neuropsychopharmacology 31:297-309.

Fontana D, Post RM, Weiss SR, Pert A (1993) The role of D1 and D2 dopamine receptors in the acquisition and expression of cocaine-induced conditioned increases in locomotor behavior. Behav Pharmacol 4:375-387.

Franklin TR, Acton PD, Maldjian JA, Gray JD, Croft JR, Dackis CA, O'Brien CP, Childress AR (2002) Decreased gray matter concentration in the insular, orbitofrontal, cingulate, and temporal cortices of cocaine patients. Biol Psychiatry 51:134-142.

Frey PW, Colliver JA (1973) Senstivity and responsivitiy meausres for discrimination learning. Learning & Motivation 4:327-342.

Friedman HR, Goldman-Rakic PS (1988) Activation of the hippocampus and dentate gyrus by working-memory: a 2-deoxyglucose study of behaving rhesus monkeys. J Neurosci 8:4693-4706.

Friedman HR, Goldman-Rakic PS (1994) Coactivation of prefrontal cortex and inferior parietal cortex in working memory tasks revealed by 2DG functional mapping in the rhesus monkey. J Neurosci 14:2775-2788.

Funahashi S, Bruce CJ, Goldman-Rakic PS (1989) Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. J Neurophysiol 61:331-349.

Fuster JM, Alexander GE (1971) Neuron activity related to short-term memory. Science 173:652-654.

Galizio M, Rosenthal D, Stein FA (1983) Sensation seeking, reinforcement, and student drug use. Addict Behav 8:243-252.

Giancola PR, Martin CS, Tarter RE, Pelham WE, Moss HB (1996a) Executive cognitive functioning and aggressive behavior in preadolescent boys at high risk for substance abuse/dependence. J Stud Alcohol 57:352-359.

Giancola PR, Moss HB, Martin CS, Kirisci L, Tarter RE (1996b) Executive cognitive functioning predicts reactive aggression in boys at high risk for substance abuse: a prospective study. Alcohol Clin Exp Res 20:740-744.

Gillen RW, Kranzler HR, Bauer LO, Burleson JA, Samarel D, Morrison DJ (1998) Neuropsychologic findings in cocaine-dependent outpatients. Prog Neuropsychopharmacol Biol Psychiatry 22:1061-1076.

Glickstein SB, Desteno DA, Hof PR, Schmauss C (2005) Mice lacking dopamine D2 and D3 receptors exhibit differential activation of prefrontal cortical neurons during tasks requiring attention. Cereb Cortex 15:1016-1024.

Goeders NE, Kuhar MJ (1987) Chronic cocaine administration induces opposite changes in dopamine receptors in the striatum and nucleus accumbens. Alcohol Drug Res 7:207-216.

Goldman-Rakic PS (1995) Architecture of the prefrontal cortex and the central executive. Ann N Y Acad Sci 769:71-83.

Goldman-Rakic PS (1996) Regional and cellular fractionation of working memory. Proc Natl Acad Sci U S A 93:13473-13480.

Goldstein RZ, Volkow ND (2002) Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. Am J Psychiatry 159:1642-1652.

Goldstein RZ, Leskovjan AC, Hoff AL, Hitzemann R, Bashan F, Khalsa SS, Wang GJ, Fowler JS, Volkow ND (2004) Severity of neuropsychological impairment in cocaine and alcohol addiction: association with metabolism in the prefrontal cortex. Neuropsychologia 42:1447-1458.

Gould E, Tanapat P (1999) Stress and hippocampal neurogenesis. Biol Psychiatry 46:1472-1479.

Gould E, Tanapat P, Hastings NB, Shors TJ (1999a) Neurogenesis in adulthood: a possible role in learning. Trends Cogn Sci 3:186-192.

Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ (1999b) Learning enhances adult neurogenesis in the hippocampal formation. Nat Neurosci 2:260-265.

Graff-Guerrero A, Willeit M, Ginovart N, Mamo D, Mizrahi R, Rusjan P, Vitcu I, Seeman P, Wilson AA, Kapur S (2007) Brain region binding of the D(2/3) agonist [(11)C]-(+)-PHNO and the D(2/3) antagonist [(11)C]raclopride in healthy humans. Hum Brain Mapp.

Granon S, Passetti F, Thomas KL, Dalley JW, Everitt BJ, Robbins TW (2000) Enhanced and impaired attentional performance after infusion of D1 dopaminergic receptor agents into rat prefrontal cortex. J Neurosci 20:1208-1215.

Grant S, Contoreggi C, London ED (2000) Drug abusers show impaired performance in a laboratory test of decision making. Neuropsychologia 38:1180-1187.

Green RH, Beatty WW, Schwartzbaum JS (1967) Comparative effects of septo-hippocampal and caudate lesions on avoidance behavior in rats. J Comp Physiol Psychol 64:444-452.

Grove WM, Eckert ED, Heston L, Bouchard TJ, Jr., Segal N, Lykken DT (1990) Heritability of substance abuse and antisocial behavior: a study of monozygotic twins reared apart. Biol Psychiatry 27:1293-1304.

Gynther LM, Carey G, Gottesman, II, Vogler GP (1995) A twin study of non-alcohol substance abuse. Psychiatry Res 56:213-220.

Hall J, Parkinson JA, Connor TM, Dickinson A, Everitt BJ (2001) Involvement of the central nucleus of the amygdala and nucleus accumbens core in mediating Pavlovian influences on instrumental behaviour. Eur J Neurosci 13:1984-1992.

Hammer RP, Jr., Pires WS, Markou A, Koob GF (1993) Withdrawal following cocaine self-administration decreases regional cerebral metabolic rate in critical brain reward regions. Synapse 14:73-80.

Hannon R, Bader A (1974) A comparison of frontal pole, anterior median and caudate nucleus lesions in the rat. Physiol Behav 13:513-521.

Hara Y, Pickel VM (2005) Overlapping intracellular and differential synaptic distributions of dopamine D1 and glutamate N-methyl-D-aspartate receptors in rat nucleus accumbens. J Comp Neurol 492:442-455.

Hedou G, Homberg J, Feldon J, Heidbreder CA (2001) Expression of sensitization to amphetamine and dynamics of dopamine neurotransmission in different laminae of the rat medial prefrontal cortex. Neuropharmacology 40:366-382.

Heidbreder CA, Thompson AC, Shippenberg TS (1996) Role of extracellular dopamine in the initiation and long-term expression of behavioral sensitization to cocaine. J Pharmacol Exp Ther 278:490-502.

Hemby SE, Tang W, Muly EC, Kuhar MJ, Howell L, Mash DC (2005) Cocaine-induced alterations in nucleus accumbens ionotropic glutamate receptor subunits in human and non-human primates. J Neurochem 95:1785-1793.

Hernandez L, Hoebel BG (1988) Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. Life Sci 42:1705-1712.

Hester R, Garavan H (2004) Executive dysfunction in cocaine addiction: evidence for discordant frontal, cingulate, and cerebellar activity. J Neurosci 24:11017-11022.

Heyne A, Wolffgramm J (1998) The development of addiction to d-amphetamine in an animal model: same principles as for alcohol and opiate. Psychopharmacology (Berl) 140:510-518.

Hitri A, Casanova MF, Kleinman JE, Wyatt RJ (1994) Fewer dopamine transporter receptors in the prefrontal cortex of cocaine users. Am J Psychiatry 151:1074-1076.

Hnasko TS, Sotak BN, Palmiter RD (2005) Morphine reward in dopamine-deficient mice. Nature 438:854-857.

Hoff AL, Riordan H, Morris L, Cestaro V, Wieneke M, Alpert R, Wang GJ, Volkow N (1996) Effects of crack cocaine on neurocognitive function. Psychiatry Res 60:167-176.

Huang CC, Lin HJ, Hsu KS (2007a) Repeated cocaine administration promotes long-term potentiation induction in rat medial prefrontal cortex. Cereb Cortex 17:1877-1888.

Huang CC, Yang PC, Lin HJ, Hsu KS (2007b) Repeated cocaine administration impairs group II metabotropic glutamate receptor-mediated long-term depression in rat medial prefrontal cortex. J Neurosci 27:2958-2968.

Hurd YL, Herkenham M (1993) Molecular alterations in the neostriatum of human cocaine addicts. Synapse 13:357-369.

Hurd YL, Weiss F, Koob G, Ungerstedt U (1990) The influence of cocaine self-administration on in vivo dopamine and acetylcholine neurotransmission in rat caudate-putamen. Neurosci Lett 109:227-233.

Hutcheson DM, Parkinson JA, Robbins TW, Everitt BJ (2001) The effects of nucleus accumbens core and shell lesions on intravenous heroin self-administration and the acquisition of drug-seeking behaviour under a second-order schedule of heroin reinforcement. Psychopharmacology (Berl) 153:464-472.

Imam SZ, Duhart HM, Skinner JT, Ali SF (2005) Cocaine induces a differential dose-dependent alteration in the expression profile of immediate early genes, transcription factors, and caspases in PC12 cells: a possible mechanism of neurotoxic damage in cocaine addiction. Ann N Y Acad Sci 1053:482-490.

Ito R, Dalley JW, Robbins TW, Everitt BJ (2002) Dopamine release in the dorsal striatum during cocaine-seeking behavior under the control of a drug-associated cue. J Neurosci 22:6247-6253.

Jackson ME, Frost AS, Moghaddam B (2001) Stimulation of prefrontal cortex at physiologically relevant frequencies inhibits dopamine release in the nucleus accumbens. J Neurochem 78:920-923.

Jentsch JD, Olausson P, De La Garza R, 2nd, Taylor JR (2002) Impairments of reversal learning and response perseveration after repeated, intermittent cocaine administrations to monkeys. Neuropsychopharmacology 26:183-190.

Jones SR, Lee TH, Wightman RM, Ellinwood EH (1996) Effects of intermittent and continuous cocaine administration on dopamine release and uptake regulation in the striatum: in vitro voltammetric assessment. Psychopharmacology (Berl) 126:331-338.

Jung BJ, Dawson R, Jr., Sealey SA, Peris J (1999) Endogenous GABA release is reduced in the striatum of cocaine-sensitized rats. Synapse 34:103-110.

Kabbaj M, Devine DP, Savage VR, Akil H (2000) Neurobiological correlates of individual differences in novelty-seeking behavior in the rat: differential expression of stress-related molecules. J Neurosci 20:6983-6988.

Kalivas PW, Duffy P (1990) Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. Synapse 5:48-58.

Kampman KM, Pettinati H, Lynch KG, Sparkman T, O'Brien CP (2003) A pilot trial of olanzapine for the treatment of cocaine dependence. Drug Alcohol Depend 70:265-273.

Kampman KM, Pettinati H, Lynch KG, Dackis C, Sparkman T, Weigley C, O'Brien CP (2004) A pilot trial of topiramate for the treatment of cocaine dependence. Drug Alcohol Depend 75:233-240.

Kampman KM, Dackis C, Lynch KG, Pettinati H, Tirado C, Gariti P, Sparkman T, Atzram M, O'Brien CP (2006) A double-blind, placebo-controlled trial of amantadine, propranolol, and their combination for the treatment of cocaine dependence in patients with severe cocaine withdrawal symptoms. Drug Alcohol Depend 85:129-137.

Kantak KM, Green-Jordan K, Valencia E, Kremin T, Eichenbaum HB (2001) Cognitive task performance after lidocaine-induced inactivation of different sites within the basolateral amygdala and dorsal striatum. Behav Neurosci 115:589-601.

Kantak KM, Udo T, Ugalde F, Luzzo C, Di Pietro N, Eichenbaum HB (2005) Influence of cocaine self-administration on learning related to prefrontal cortex or hippocampus functioning in rats. Psychopharmacology (Berl) 181:227-236.

Karoum F, Suddath RL, Wyatt RJ (1990) Chronic cocaine and rat brain catecholamines: long-term reduction in hypothalamic and frontal cortex dopamine metabolism. Eur J Pharmacol 186:1-8.

Kellendonk C, Simpson EH, Polan HJ, Malleret G, Vronskaya S, Winiger V, Moore H, Kandel ER (2006) Transient and selective overexpression of dopamine D2 receptors in the striatum causes persistent abnormalities in prefrontal cortex functioning. Neuron 49:603-615.

Kelley AE (1999) Functional specificity of ventral striatal compartments in appetitive behaviors. Ann N Y Acad Sci 877:71-90.

Kelley AE, Berridge KC (2002) The neuroscience of natural rewards: relevance to addictive drugs. J Neurosci 22:3306-3311.

Kelley BJ, Yeager KR, Pepper TH, Beversdorf DQ (2005) Cognitive impairment in acute cocaine withdrawal. Cogn Behav Neurol 18:108-112.

Kenny PJ, Chen SA, Kitamura O, Markou A, Koob GF (2006) Conditioned withdrawal drives heroin consumption and decreases reward sensitivity. J Neurosci 26:5894-5900.

Khan ZU, Gutierrez A, Martin R, Penafiel A, Rivera A, De La Calle A (1998) Differential regional and cellular distribution of dopamine D2-like receptors: an immunocytochemical study of subtype-specific antibodies in rat and human brain. J Comp Neurol 402:353-371.

Khroyan TV, Barrett-Larimore RL, Rowlett JK, Spealman RD (2000) Dopamine D1- and D2-like receptor mechanisms in relapse to cocaine-seeking behavior: effects of selective antagonists and agonists. J Pharmacol Exp Ther 294:680-687.

Kilts CD, Schweitzer JB, Quinn CK, Gross RE, Faber TL, Muhammad F, Ely TD, Hoffman JM, Drexler KP (2001) Neural activity related to drug craving in cocaine addiction. Arch Gen Psychiatry 58:334-341.

Kippin TE, Fuchs RA, See RE (2006) Contributions of prolonged contingent and noncontingent cocaine exposure to enhanced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl) 187:60-67.

Kirisci L, Vanyukov M, Tarter R (2005) Detection of youth at high risk for substance use disorders: a longitudinal study. Psychol Addict Behav 19:243-252.

Kitamura O, Wee S, Specio SE, Koob GF, Pulvirenti L (2006) Escalation of methamphetamine self-administration in rats: a dose-effect function. Psychopharmacology (Berl) 186:48-53.

Kiyatkin EA (1994) Enhanced locomotor reactivity to apomorphine following repeated cocaine treatment. Pharmacol Biochem Behav 49:247-251.

Kiyatkin EA, Stein EA (1995) Fluctuations in nucleus accumbens dopamine during cocaine self-administration behavior: an in vivo electrochemical study. Neuroscience 64:599-617.

Kleven MS, Perry BD, Woolverton WL, Seiden LS (1990) Effects of repeated injections of cocaine on D1 and D2 dopamine receptors in rat brain. Brain Res 532:265-270.

Knackstedt LA, Kalivas PW (2007) Extended access to cocaine self-administration enhances drug-primed reinstatement but not behavioral sensitization. J Pharmacol Exp Ther 322:1103-1109.

Knapp CM, Printseva B, Cottam N, Kornetsky C (2002) Effects of cue exposure on brain glucose utilization 8 days after repeated cocaine administration. Brain Res 950:119-126.

Kodsi MH, Swerdlow NR (1994) Quinolinic acid lesions of the ventral striatum reduce sensorimotor gating of acoustic startle in rats. Brain Res 643:59-65.

Kolb B (1990) Prefrontal Cortex. Cambridge, MA: MIT Press.

Kondrad RL, Burk JA (2004) Transient disruption of attentional performance following escalating amphetamine administration in rats. Psychopharmacology (Berl) 175:436-442.

Konishi S, Kawazu M, Uchida I, Kikyo H, Asakura I, Miyashita Y (1999) Contribution of working memory to transient activation in human inferior prefrontal cortex during performance of the Wisconsin Card Sorting Test. Cereb Cortex 9:745-753.

Koob GF (1992) Neural mechanisms of drug reinforcement. Ann N Y Acad Sci 654:171-191.

Koob GF, Le HT, Creese I (1987) The D1 dopamine receptor antagonist SCH 23390 increases cocaine self-administration in the rat. Neurosci Lett 79:315-320.

Kosten TR, Scanley BE, Tucker KA, Oliveto A, Prince C, Sinha R, Potenza MN, Skudlarski P, Wexler BE (2006) Cue-induced brain activity changes and relapse in cocaine-dependent patients. Neuropsychopharmacology 31:644-650.

Kubler A, Murphy K, Garavan H (2005) Cocaine dependence and attention switching within and between verbal and visuospatial working memory. Eur J Neurosci 21:1984-1992.

Kubota K, Niki H (1971) Prefrontal cortical unit activity and delayed alternation performance in monkeys. J Neurophysiol 34:337-347.

Lang WJ, Latiff AA, McQueen A, Singer G (1977) Self administration of nicotine with and without a food delivery schedule. Pharmacol Biochem Behav 7:65-70.

Lanser MG, Ellenbroek BA, Zitman FG, Heeren DJ, Cools AR (2001) The role of medial prefrontal cortical dopamine in spontaneous flexibility in the rat. Behav Pharmacol 12:163-171.

Lecca D, Cacciapaglia F, Valentini V, Acquas E, Di Chiara G (2007) Differential neurochemical and behavioral adaptation to cocaine after response contingent and noncontingent exposure in the rat. Psychopharmacology (Berl) 191:653-667.

Letchworth SR, Nader MA, Smith HR, Friedman DP, Porrino LJ (2001) Progression of changes in dopamine transporter binding site density as a result of cocaine self-administration in rhesus monkeys. J Neurosci 21:2799-2807.

Leuner B, Gould E, Shors TJ (2006) Is there a link between adult neurogenesis and learning? Hippocampus 16:216-224.

Levine MS, Hull CD, Buchwald NA, Villablanca JR (1978) Effects of caudate nuclei or frontal cortical ablations in kittens: motor activity and visual discrimination performance in neonatal and juvenile kittens. Exp Neurol 62:555-569.

Leyton M, Casey KF, Delaney JS, Kolivakis T, Benkelfat C (2005) Cocaine craving, euphoria, and self-administration: a preliminary study of the effect of catecholamine precursor depletion. Behav Neurosci 119:1619-1627.

Leyton M, Boileau I, Benkelfat C, Diksic M, Baker G, Dagher A (2002) Amphetamine-induced increases in extracellular dopamine, drug wanting,

and novelty seeking: a PET/[11C]raclopride study in healthy men. Neuropsychopharmacology 27:1027-1035.

Lim DK, Yu ZJ, Hoskins B, Rockhold RW, Ho IK (1990) Effects of acute and subacute cocaine administration on the CNS dopaminergic system in Wistar-Kyoto and spontaneously hypertensive rats: II. Dopamine receptors. Neurochem Res 15:621-627.

Little KY, Patel UN, Clark TB, Butts JD (1996) Alteration of brain dopamine and serotonin levels in cocaine users: a preliminary report. Am J Psychiatry 153:1216-1218.

Little KY, Krolewski DM, Zhang L, Cassin BJ (2003) Loss of striatal vesicular monoamine transporter protein (VMAT2) in human cocaine users. Am J Psychiatry 160:47-55.

Little KY, Zhang L, Desmond T, Frey KA, Dalack GW, Cassin BJ (1999) Striatal dopaminergic abnormalities in human cocaine users. Am J Psychiatry 156:238-245.

Little KY, McLaughlin DP, Zhang L, McFinton PR, Dalack GW, Cook EH, Jr., Cassin BJ, Watson SJ (1998) Brain dopamine transporter messenger RNA and binding sites in cocaine users: a postmortem study. Arch Gen Psychiatry 55:793-799.

Liu X, Matochik JA, Cadet JL, London ED (1998) Smaller volume of prefrontal lobe in polysubstance abusers: a magnetic resonance imaging study. Neuropsychopharmacology 18:243-252.

London ED, Cascella NG, Wong DF, Phillips RL, Dannals RF, Links JM, Herning R, Grayson R, Jaffe JH, Wagner HN, Jr. (1990) Cocaine-induced reduction of glucose utilization in human brain. A study using positron emission tomography and [fluorine 18]-fluorodeoxyglucose. Arch Gen Psychiatry 47:567-574.

Loose R, Kaufmann C, Tucha O, Auer DP, Lange KW (2006) Neural networks of response shifting: influence of task speed and stimulus material. Brain Res 1090:146-155.

Lu D, Mahmood A, Qu C, Goussev A, Schallert T, Chopp M (2005) Erythropoietin enhances neurogenesis and restores spatial memory in rats after traumatic brain injury. J Neurotrauma 22:1011-1017.

Lu XY, Ghasemzadeh MB, Kalivas PW (1998) Expression of D1 receptor, D2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens. Neuroscience 82:767-780.

Lyons D, Friedman DP, Nader MA, Porrino LJ (1996) Cocaine alters cerebral metabolism within the ventral striatum and limbic cortex of monkeys. J Neurosci 16:1230-1238.

Macedo DS, Correia EE, Vasconcelos SM, Aguiar LM, Viana GS, Sousa FC (2004) Cocaine treatment causes early and long-lasting changes in muscarinic and dopaminergic receptors. Cellular & Molecular Neurobiology 24:129-136.

Macey DJ, Rice WN, Freedland CS, Whitlow CT, Porrino LJ (2004) Patterns of functional activity associated with cocaine self-administration in the rat change over time. Psychopharmacology (Berl) 172:384-392.

Maggos CE, Tsukada H, Kakiuchi T, Nishiyama S, Myers JE, Kreuter J, Schlussman SD, Unterwald EM, Ho A, Kreek MJ (1998) Sustained withdrawal allows normalization of in vivo [11C]N-methylspiperone dopamine D2 receptor binding after chronic binge cocaine: a positron emission tomography study in rats. Neuropsychopharmacology 19:146-153.

Makris N, Gasic GP, Seidman LJ, Goldstein JM, Gastfriend DR, Elman I, Albaugh MD, Hodge SM, Ziegler DA, Sheahan FS, Caviness VS, Jr., Tsuang MT, Kennedy DN, Hyman SE, Rosen BR, Breiter HC (2004) Decreased absolute amygdala volume in cocaine addicts. Neuron 44:729-740.

Malcolm R, LaRowe S, Cochran K, Moak D, Herron J, Brady K, Hedden S, Woolson R, Halushka P (2005) A controlled trial of amlodipine for cocaine dependence: a negative report. J Subst Abuse Treat 28:197-204.

Malison RT, Best SE, van Dyck CH, McCance EF, Wallace EA, Laruelle M, Baldwin RM, Seibyl JP, Price LH, Kosten TR, Innis RB (1998) Elevated

striatal dopamine transporters during acute cocaine abstinence as measured by [123I] beta-CIT SPECT. Am J Psychiatry 155:832-834.

Manns JR, Hopkins RO, Reed JM, Kitchener EG, Squire LR (2003) Recognition memory and the human hippocampus. Neuron 37:171-180.

Manschreck TC, Schneyer ML, Weisstein CC, Laughery J, Rosenthal J, Celada T, Berner J (1990) Freebase cocaine and memory. Compr Psychiatry 31:369-375.

Mansour A, Meador-Woodruff JH, Bunzow JR, Civelli O, Akil H, Watson SJ (1990) Localization of dopamine D2 receptor mRNA and D1 and D2 receptor binding in the rat brain and pituitary: an in situ hybridization-receptor autoradiographic analysis. J Neurosci 10:2587-2600.

Mansour A, Meador-Woodruff JH, Zhou QY, Civelli O, Akil H, Watson SJ (1991) A comparison of D1 receptor binding and mRNA in rat brain using receptor autoradiographic and in situ hybridization techniques. Neuroscience 45:359-371.

Mantsch JR, Yuferov V, Mathieu-Kia AM, Ho A, Kreek MJ (2004) Effects of extended access to high versus low cocaine doses on self-administration, cocaine-induced reinstatement and brain mRNA levels in rats. Psychopharmacology (Berl) 175:26-36.

Martinez D, Broft A, Foltin RW, Slifstein M, Hwang DR, Huang Y, Perez A, Frankle WG, Cooper T, Kleber HD, Fischman MW, Laruelle M (2004) Cocaine dependence and d2 receptor availability in the functional subdivisions of the striatum: relationship with cocaine-seeking behavior. Neuropsychopharmacology 29:1190-1202.

Mash DC, Ouyang Q, Qin Y, Pablo J (2005) Norepinephrine transporter immunoblotting and radioligand binding in cocaine abusers. J Neurosci Methods 143:79-85.

Mash DC, Pablo J, Ouyang Q, Hearn WL, Izenwasser S (2002) Dopamine transport function is elevated in cocaine users. J Neurochem 81:292-300.

Mathiesen C, Caesar K, Lauritzen M (2000) Temporal coupling between neuronal activity and blood flow in rat cerebellar cortex as indicated by field potential analysis. J Physiol 523 Pt 1:235-246.

Mathiesen C, Caesar K, Akgoren N, Lauritzen M (1998) Modification of activity-dependent increases of cerebral blood flow by excitatory synaptic activity and spikes in rat cerebellar cortex. J Physiol 512 (Pt 2):555-566.

Matochik JA, London ED, Eldreth DA, Cadet JL, Bolla KI (2003) Frontal cortical tissue composition in abstinent cocaine abusers: a magnetic resonance imaging study. Neuroimage 19:1095-1102.

McFarland K, Kalivas PW (2001) The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 21:8655-8663.

McFarland K, Lapish CC, Kalivas PW (2003) Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 23:3531-3537.

McKay JR, Lynch KG, Shepard DS, Pettinati HM (2005) The effectiveness of telephone-based continuing care for alcohol and cocaine dependence: 24-month outcomes. Arch Gen Psychiatry 62:199-207.

Meador-Woodruff JH, Little KY, Damask SP, Mansour A, Watson SJ (1993) Effects of cocaine on dopamine receptor gene expression: a study in the postmortem human brain. Biol Psychiatry 34:348-355.

Meek PS, Clark HW, Solana VL (1989) Neurocognitive impairment: the unrecognized component of dual diagnosis in substance abuse treatment. J Psychoactive Drugs 21:153-160.

Mehta MA, Sahakian BJ, McKenna PJ, Robbins TW (1999) Systemic sulpiride in young adult volunteers simulates the profile of cognitive deficits in Parkinson's disease. Psychopharmacology (Berl) 146:162-174.

Mehta MA, Manes FF, Magnolfi G, Sahakian BJ, Robbins TW (2004) Impaired set-shifting and dissociable effects on tests of spatial working memory following the dopamine D2 receptor antagonist sulpiride in human volunteers. Psychopharmacology (Berl) 176:331-342.

Meiergerd SM, Schenk JO, Sorg BA (1997) Repeated cocaine and stress increase dopamine clearance in the rat medial prefrontal cortex. Brain Res 773:203-207.

Merikangas KR, Stolar M, Stevens DE, Goulet J, Preisig MA, Fenton B, Zhang H, O'Malley SS, Rounsaville BJ (1998) Familial transmission of substance use disorders. Arch Gen Psychiatry 55:973-979.

Miller L (1985) Neuropsychological assessment of substance abusers: review and recommendations. J Subst Abuse Treat 2:5-17.

Mittenberg W, Motta S (1993) Effects of chronic cocaine abuse on memory and learning. Arch Clin Neuropsychol 8:477-483.

Moeller FG, Dougherty DM, Barratt ES, Schmitz JM, Swann AC, Grabowski J (2001) The impact of impulsivity on cocaine use and retention in treatment. J Subst Abuse Treat 21:193-198.

Mogensen J, Iversen IH, Divac I (1987) Neostriatal lesions impaired rats' delayed alternation performance in a T-maze but not in a two-key operant chamber. Acta Neurobiol Exp (Wars) 47:45-54.

Montague PR, Hyman SE, Cohen JD (2004) Computational roles for dopamine in behavioural control. Nature 431:760-767.

Moore RJ, Vinsant SL, Nader MA, Porrino LJ, Friedman DP (1998a) Effect of cocaine self-administration on dopamine D2 receptors in rhesus monkeys. Synapse 30:88-96.

Moore RJ, Vinsant SL, Nader MA, Porrino LJ, Friedman DP (1998b) Effect of cocaine self-administration on striatal dopamine D1 receptors in rhesus monkeys. Synapse 28:1-9.

Morrow TJ, Paulson PE, Danneman PJ, Casey KL (1998) Regional changes in forebrain activation during the early and late phase of formalin nociception: analysis using cerebral blood flow in the rat. Pain 75:355-365.

Muller U, von Cramon DY, Pollmann S (1998) D1- versus D2-receptor modulation of visuospatial working memory in humans. J Neurosci 18:2720-2728.

Nader MA, Czoty PW (2005) PET imaging of dopamine D2 receptors in monkey models of cocaine abuse: genetic predisposition versus environmental modulation. Am J Psychiatry 162:1473-1482.

Nader MA, Daunais JB, Moore T, Nader SH, Moore RJ, Smith HR, Friedman DP, Porrino LJ (2002) Effects of cocaine self-administration on striatal dopamine systems in rhesus monkeys: initial and chronic exposure. Neuropsychopharmacology 27:35-46.

Nader MA, Morgan D, Gage HD, Nader SH, Calhoun TL, Buchheimer N, Ehrenkaufer R, Mach RH (2006) PET imaging of dopamine D2 receptors during chronic cocaine self-administration in monkeys. Nat Neurosci 9:1050-1056.

Nasif FJ, Sidiropoulou K, Hu XT, White FJ (2005) Repeated cocaine administration increases membrane excitability of pyramidal neurons in the rat medial prefrontal cortex. Journal of Pharmacology & Experimental Therapeutics 312:1305-1313.

Neill DB, Boggan WO, Grossman SP (1974) Impairment of avoidance performance by intrastriatal administration of 6-hydroxydopamine. Pharmacol Biochem Behav 2:97-103.

Ng JP, Hubert GW, Justice JB, Jr. (1991) Increased stimulated release and uptake of dopamine in nucleus accumbens after repeated cocaine administration as measured by in vivo voltammetry. J Neurochem 56:1485-1492.

Nogueira L, Kalivas PW, Lavin A (2006) Long-term neuroadaptations produced by withdrawal from repeated cocaine treatment: role of dopaminergic receptors in modulating cortical excitability. J Neurosci 26:12308-12313.

O'Malley S, Adamse M, Heaton RK, Gawin FH (1992) Neuropsychological impairment in chronic cocaine abusers. AmJDrug Alcohol Abuse 18:131-144.

Oberg RG, Divac I (1975) Dissociative effects of selective lesions in the caudate nucleus of cats and rats. Acta Neurobiol Exp (Wars) 35:647-659.

Ohata M, Takei H, Fredericks WR, Rapoport SI (1982) Effects of immobilization stress on cerebral blood flow and cerebrovascular permeability in spontaneously hypertensive rats. J Cereb Blood Flow Metab 2:373-379.

Ornstein TJ, Iddon JL, Baldacchino AM, Sahakian BJ, London M, Everitt BJ, Robbins TW (2000) Profiles of cognitive dysfunction in chronic amphetamine and heroin abusers. 23:113-126.

Owen AM, Roberts AC, Polkey CE, Sahakian BJ, Robbins TW (1991) Extra-dimensional versus intra-dimensional set shifting performance following frontal lobe excisions, temporal lobe excisions or amygdalo-hippocampectomy in man. Neuropsychologia 29:993-1006.

Owen AM, Roberts AC, Hodges JR, Summers BA, Polkey CE, Robbins TW (1993) Contrasting mechanisms of impaired attentional set-shifting in patients with frontal lobe damage or Parkinson's disease. Brain 116 (Pt 5):1159-1175.

Parkinson JA, Willoughby PJ, Robbins TW, Everitt BJ (2000) Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs Pavlovian approach behavior: further evidence for limbic cortical-ventral striatopallidal systems. Behav Neurosci 114:42-63.

Parkinson JA, Olmstead MC, Burns LH, Robbins TW, Everitt BJ (1999) Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-amphetamine. J Neurosci 19:2401-2411.

Paterson NE, Markou A (2003) Increased motivation for self-administered cocaine after escalated cocaine intake. Neuroreport 14:2229-2232.

Pattij T, Janssen MC, Vanderschuren LJ, Schoffelmeer AN, van Gaalen MM (2007) Involvement of dopamine D1 and D2 receptors in the nucleus accumbens core and shell in inhibitory response control. Psychopharmacology (Berl) 191:587-598.

Paulson PE, Robinson TE (1996) Regional differences in the effects of amphetamine withdrawal on dopamine dynamics in the striatum. Analysis of circadian patterns using automated on-line microdialysis. Neuropsychopharmacology 14:325-337.

Paxinos G, Watson C (1998) The Rat Brain in Stereotaxic Coordinates, 4th Edition. San Diego: Academic.

Pearlson GD, Jeffery PJ, Harris GJ, Ross CA, Fischman MW, Camargo EE (1993) Correlation of acute cocaine-induced changes in local cerebral blood flow with subjective effects. Am J Psychiatry 150:495-497.

Pecina S, Cagniard B, Berridge KC, Aldridge JW, Zhuang X (2003) Hyperdopaminergic mutant mice have higher "wanting" but not "liking" for sweet rewards. J Neurosci 23:9395-9402.

Pecins-Thompson M, Peris J (1993) Behavioral and neurochemical changes caused by repeated ethanol and cocaine administration. Psychopharmacology (Berl) 110:443-450.

Pelloux Y, Everitt BJ, Dickinson A (2007) Compulsive drug seeking by rats under punishment: effects of drug taking history. Psychopharmacology (Berl) 194:127-137.

Perez MF, White FJ, Hu XT (2006) Dopamine D(2) receptor modulation of K(+) channel activity regulates excitability of nucleus accumbens neurons at different membrane potentials. J Neurophysiol 96:2217-2228.

Peris J (1996) Repeated cocaine injections decrease the function of striatal gamma-aminobutyric acid(A) receptors. J Pharmacol Exp Ther 276:1002-1008.

Peris J, Boyson SJ, Cass WA, Curella P, Dwoskin LP, Larson G, Lin LH, Yasuda RP, Zahniser NR (1990) Persistence of neurochemical changes in dopamine systems after repeated cocaine administration. J Pharmacol Exp Ther 253:38-44.

Peterson JD, Wolf ME, White FJ (2000) Altered responsiveness of medial prefrontal cortex neurons to glutamate and dopamine after withdrawal from repeated amphetamine treatment. Synapse 36:342-344.

Pettit HO, Ettenberg A, Bloom FE, Koob GF (1984) Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. Psychopharmacology (Berl) 84:167-173.

Pettit HO, Pan HT, Parsons LH, Justice JB, Jr. (1990) Extracellular concentrations of cocaine and dopamine are enhanced during chronic cocaine administration. J Neurochem 55:798-804.

Pezze MA, Dalley JW, Robbins TW (2007) Differential roles of dopamine D1 and D2 receptors in the nucleus accumbens in attentional performance on the five-choice serial reaction time task. Neuropsychopharmacology 32:273-283.

Pickens R, Thompson T (1968) Cocaine-reinforced behavior in rats: effects of reinforcement magnitude and fixed-ratio size. J Pharmacol Exp Ther 161:122-129.

Pickens R, Harris WC (1968) Self-administration of d-amphetamine by rats. Psychopharmacologia 12:158-163.

Ploeger GE, Spruijt BM, Cools AR (1994) Spatial localization in the Morris water maze in rats: acquisition is affected by intra-accumbens injections of the dopaminergic antagonist haloperidol. Behav Neurosci 108:927-934.

Pontieri FE, Mainero C, La Riccia M, Passarelli F, Orzi F (1995) Functional correlates of repeated administration of cocaine and apomorphine in the rat. Eur J Pharmacol 284:205-209.

Prado-Alcala RA, Grinberg ZJ, Arditti ZL, Garcia MM, Prieto HG, Brust-Carmona H (1975) Learning deficits produced by chronic and reversible lesions of the corpus striatum in rats. Physiol Behav 15:283-287.

Przewlocka B, Lason W (1995) Adaptive changes in the proenkephalin and D2 dopamine receptor mRNA expression after chronic cocaine in the nucleus accumbens and striatum of the rat. Eur Neuropsychopharmacol 5:465-469.

Qin Y, Ouyang Q, Pablo J, Mash DC (2005) Cocaine abuse elevates alpha-synuclein and dopamine transporter levels in the human striatum. Neuroreport 16:1489-1493.

Raichle ME, Fiez JA, Videen TO, MacLeod AM, Pardo JV, Fox PT, Petersen SE (1994) Practice-related changes in human brain functional anatomy during nonmotor learning. Cereb Cortex 4:8-26.

Rawson RA, Huber A, McCann M, Shoptaw S, Farabee D, Reiber C, Ling W (2002) A comparison of contingency management and cognitive-behavioral approaches during methadone maintenance treatment for cocaine dependence. Arch Gen Psychiatry 59:817-824.

Rawson RA, McCann MJ, Flammino F, Shoptaw S, Miotto K, Reiber C, Ling W (2006) A comparison of contingency management and cognitive-behavioral approaches for stimulant-dependent individuals. Addiction 101:267-274.

Reading PJ, Dunnett SB, Robbins TW (1991) Dissociable roles of the ventral, medial and lateral striatum on the acquisition and performance of a complex visual stimulus-response habit. Behav Brain Res 45:147-161.

Redish AD (2004) Addiction as a computational process gone awry. Science 306:1944-1947.

Riedel G, Harrington NR, Hall G, Macphail EM (1997) Nucleus accumbens lesions impair context, but not cue, conditioning in rats. Neuroreport 8:2477-2481.

Rinaldi A, Mandillo S, Oliverio A, Mele A (2007) D1 and D2 receptor antagonist injections in the prefrontal cortex selectively impair spatial learning in mice. Neuropsychopharmacology 32:309-319.

Risner ME (1975) Intravenous self-administration of d- and l-amphetamine by dog. Eur J Pharmacol 32:344-348.

Risner ME, Jones BE (1975) Self-administration of CNS stimulants by dog. Psychopharmacologia 43:207-213.

Robbins TW (1996) Dissociating executive functions of the prefrontal cortex. Philos Trans R Soc Lond B Biol Sci 351:1463-1470; discussion 1470-1461.

Roberts DC, Corcoran ME, Fibiger HC (1977) On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. Pharmacol Biochem Behav 6:615-620.

Robinson S, Sandstrom SM, Denenberg VH, Palmiter RD (2005) Distinguishing whether dopamine regulates liking, wanting, and/or learning about rewards. Behav Neurosci 119:5-15.

Robinson TE, Becker JB (1982) Behavioral sensitization is accompanied by an enhancement in amphetamine-stimulated dopamine release from striatal tissue in vitro. Eur J Pharmacol 85:253-254.

Robinson TE, Camp DM (1987) Long-lasting effects of escalating doses of d-amphetamine on brain monoamines, amphetamine-induced stereotyped behavior and spontaneous nocturnal locomotion. Pharmacol Biochem Behav 26:821-827.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 18:247-291.

Robinson TE, Berridge KC (2000) The psychology and neurobiology of addiction: an incentive-sensitization view. Addiction 95 Suppl 2:S91-117.

Robinson TE, Berridge KC (2003) Addiction. Annu Rev Psychol 54:25-53.

Robinson TE, Jurson PA, Bennett JA, Bentgen KM (1988) 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 462:211-222.

Robledo P, Maldonado-Lopez R, Koob GF (1992) Role of dopamine receptors in the nucleus accumbens in the rewarding properties of cocaine. Ann N Y Acad Sci 654:509-512.

Rogers RD, Robbins TW (2001) Investigating the neurocognitive deficits associated with chronic drug misuse. CurrOpinNeurobiol 11:250-257.

Rogers RD, Everitt BJ, Baldacchino A, Blackshaw AJ, Swainson R, Wynne K, Baker NB, Hunter J, Carthy T, Booker E, London M, Deakin JF, Sahakian

BJ, Robbins TW (1999) Dissociable deficits in the decision-making cognition of chronic amphetamine abusers, opiate abusers, patients with focal damage to prefrontal cortex, and tryptophan-depleted normal volunteers: evidence for monoaminergic mechanisms. Neuropsychopharmacology 20:322-339.

Rosselli M, Ardila A (1996) Cognitive effects of cocaine and polydrug abuse. JClinExpNeuropsychol 18:122-135.

Rossetti ZL, Hmaidan Y, Gessa GL (1992) Marked inhibition of mesolimbic dopamine release: a common feature of ethanol, morphine, cocaine and amphetamine abstinence in rats. Eur J Pharmacol 221:227-234.

Sanberg PR, Pisa M, Fibiger HC (1979) Avoidance, operant and locomotor behavior in rats with neostriatal injections of kainic acid. Pharmacol Biochem Behav 10:137-144.

Santucci AC, Capodilupo S, Bernstein J, Gomez-Ramirez M, Milefsky R, Mitchell H (2004) Cocaine in adolescent rats produces residual memory impairments that are reversible with time. Neurotoxicol Teratol 26:651-661.

Sawaguchi T, Goldman-Rakic PS (1991) D1 dopamine receptors in prefrontal cortex: involvement in working memory. Science 251:947-950.

Sawaguchi T, Goldman-Rakic PS (1994) The role of D1-dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. J Neurophysiol 71:515-528.

Sawaguchi T, Matsumura M, Kubota K (1988) Dopamine enhances the neuronal activity of spatial short-term memory task in the primate prefrontal cortex. Neurosci Res 5:465-473.

Sayre SL, Schmitz JM, Stotts AL, Averill PM, Rhoades HM, Grabowski JJ (2002) Determining predictors of attrition in an outpatient substance abuse program. Am J Drug Alcohol Abuse 28:55-72.

Schaefer GJ, Michael RP (1986) Changes in response rates and reinforcement thresholds for intracranial self-stimulation during morphine withdrawal. Pharmacol Biochem Behav 25:1263-1269.

Schlussman SD, Ho A, Zhou Y, Curtis AE, Kreek MJ (1998) Effects of "binge" pattern cocaine on stereotypy and locomotor activity in C57BL/6J and 129/J mice. Pharmacol Biochem Behav 60:593-599.

Schmidt HD, Pierce RC (2006) Cooperative activation of D1-like and D2-like dopamine receptors in the nucleus accumbens shell is required for the reinstatement of cocaine-seeking behavior in the rat. Neuroscience 142:451-461.

Schmidt HD, Anderson SM, Pierce RC (2006) Stimulation of D1-like or D2 dopamine receptors in the shell, but not the core, of the nucleus accumbens reinstates cocaine-seeking behaviour in the rat. Eur J Neurosci 23:219-228.

Schoenbaum G, Saddoris MP, Ramus SJ, Shaham Y, Setlow B (2004) Cocaine-experienced rats exhibit learning deficits in a task sensitive to orbitofrontal cortex lesions. Eur J Neurosci 19:1997-2002.

Schroder N, O'Dell SJ, Marshall JF (2003) Neurotoxic methamphetamine regimen severely impairs recognition memory in rats. Synapse 49:89-96.

Schultz W (1998) Predictive reward signal of dopamine neurons. J Neurophysiol 80:1-27.

Schultz W (2001) Reward signaling by dopamine neurons. Neuroscientist 7:293-302.

Schultz W, Dickinson A (2000) Neuronal coding of prediction errors. Annu Rev Neurosci 23:473-500.

Schwartzbaum JS, Donovick PJ (1968) Discrimination reversal and spatial alternation associated with septal and caudate dysfunction in rats. J Comp Physiol Psychol 65:83-92.

Seamans JK, Floresco SB, Phillips AG (1998) D1 receptor modulation of hippocampal-prefrontal cortical circuits integrating spatial memory with executive functions in the rat. J Neurosci 18:1613-1621.

See RE, Elliott JC, Feltenstein MW (2007) The role of dorsal vs ventral striatal pathways in cocaine-seeking behavior after prolonged abstinence in rats. Psychopharmacology (Berl).

Seeman P, Tallerico T, Ko F (2003) Dopamine displaces [3H]domperidone from high-affinity sites of the dopamine D2 receptor, but not [3H]raclopride or [3H]spiperone in isotonic medium: Implications for human positron emission tomography. Synapse 49:209-215.

Seeman P, Tallerico T, Ko F (2004) Alcohol-withdrawn animals have a prolonged increase in dopamine D2high receptors, reversed by general anesthesia: relation to relapse? Synapse 52:77-83.

Seeman P, McCormick PN, Kapur S (2007) Increased dopamine D2(High) receptors in amphetamine-sensitized rats, measured by the agonist [(3)H](+)PHNO. Synapse 61:263-267.

Seeman P, Ulpian C, Wreggett KA, Wells JW (1984) Dopamine receptor parameters detected by [3H]spiperone depend on tissue concentration: analysis and examples. J Neurochem 43:221-235.

Seeman P, Tallerico T, Ko F, Tenn C, Kapur S (2002) Amphetamine-sensitized animals show a marked increase in dopamine D2 high receptors occupied by endogenous dopamine, even in the absence of acute challenges. Synapse 46:235-239.

Seeman P, Weinshenker D, Quirion R, Srivastava LK, Bhardwaj SK, Grandy DK, Premont RT, Sotnikova TD, Boksa P, El-Ghundi M, O'Dowd B F, George SR, Perreault ML, Mannisto PT, Robinson S, Palmiter RD, Tallerico T (2005) Dopamine supersensitivity correlates with D2High states, implying many paths to psychosis. Proc Natl Acad Sci U S A 102:3513-3518.

Selby MJ, Azrin RL (1998) Neuropsychological functioning in drug abusers. Drug Alcohol Depend 50:39-45.

Self DW, Barnhart WJ, Lehman DA, Nestler EJ (1996) Opposite modulation of cocaine-seeking behavior by D1- and D2-like dopamine receptor agonists. Science 271:1586-1589.

Shaham Y, Shalev U, Lu L, De Wit H, Stewart J (2003) The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology (Berl) 168:3-20.

Sharkey J, McBean DE, Kelly PA (1991) Acute cocaine administration: effects on local cerebral blood flow and metabolic demand in the rat. Brain Res 548:310-314.

Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E (2001) Neurogenesis in the adult is involved in the formation of trace memories. Nature 410:372-376.

Sim ME, Lyoo IK, Streeter CC, Covell J, Sarid-Segal O, Ciraulo DA, Kim MJ, Kaufman MJ, Yurgelun-Todd DA, Renshaw PF (2007) Cerebellar Gray Matter Volume Correlates with Duration of Cocaine Use in Cocaine-Dependent Subjects. Neuropsychopharmacology.

Simon H, Scatton B, Moal ML (1980) Dopaminergic A10 neurones are involved in cognitive functions. Nature 286:150-151.

Simpson DD, Joe GW, Fletcher BW, Hubbard RL, Anglin MD (1999) A national evaluation of treatment outcomes for cocaine dependence. Arch Gen Psychiatry 56:507-514.

Sinha R, Lacadie C, Skudlarski P, Fulbright RK, Rounsaville BJ, Kosten TR, Wexler BE (2005) Neural activity associated with stress-induced cocaine craving: a functional magnetic resonance imaging study. Psychopharmacology (Berl) 183:171-180.

Sofuoglu M, Poling J, Gonzalez G, Gonsai K, Kosten T (2006) Cocaine withdrawal symptoms predict medication response in cocaine users. Am J Drug Alcohol Abuse 32:617-627.

Sokoloff L (1978) Local cerebral energy metabolism: its relationships to local functional activity and blood flow. Ciba Found Symp:171-197.

Sokolowski JD, Salamone JD (1994) Effects of dopamine depletions in the medial prefrontal cortex on DRL performance and motor activity in the rat. Brain Res 642:20-28.

Sokolowski JD, McCullough LD, Salamone JD (1994) Effects of dopamine depletions in the medial prefrontal cortex on active avoidance and escape in the rat. Brain Res 651:293-299.

Sorg BA, Kalivas PW (1993) Effects of cocaine and footshock stress on extracellular dopamine levels in the medial prefrontal cortex. Neuroscience 53:695-703.

Sorg BA, Davidson DL, Kalivas PW, Prasad BM (1997) Repeated daily cocaine alters subsequent cocaine-induced increase of extracellular dopamine in the medial prefrontal cortex. J Pharmacol Exp Ther 281:54-61.

Stam CJ, de Bruin JP, van Haelst AM, van der Gugten J, Kalsbeek A (1989) Influence of the mesocortical dopaminergic system on activity, food hoarding, social-agonistic behavior, and spatial delayed alternation in male rats. Behav Neurosci 103:24-35.

Stefani MR, Moghaddam B (2006) Rule learning and reward contingency are associated with dissociable patterns of dopamine activation in the rat prefrontal cortex, nucleus accumbens, and dorsal striatum. J Neurosci 26:8810-8818.

Stoof JC, Kebabian JW (1984) Two dopamine receptors: biochemistry, physiology and pharmacology. Life Sci 35:2281-2296.

Strickland TL, Mena I, Villanueva-Meyer J, Miller BL, Cummings J, Mehringer CM, Satz P, Myers H (1993) Cerebral perfusion and neuropsychological consequences of chronic cocaine use. J Neuropsychiatry Clin Neurosci 5:419-427.

Sun W, Rebec GV (2005) The role of prefrontal cortex D1-like and D2-like receptors in cocaine-seeking behavior in rats. Psychopharmacology (Berl) 177:315-323.

Sun W, Rebec GV (2006) Repeated cocaine self-administration alters processing of cocaine-related information in rat prefrontal cortex. J Neurosci 26:8004-8008.

Taghzouti K, Louilot A, Herman JP, Le Moal M, Simon H (1985) Alternation behavior, spatial discrimination, and reversal disturbances following 6-hydroxydopamine lesions in the nucleus accumbens of the rat. Behav Neural Biol 44:354-363.

Takahashi RN, Singer G (1979) Self-administration of delta 9-tetrahydrocannabinol by rats. Pharmacol Biochem Behav 11:737-740.

Takahata R, Moghaddam B (1998) Glutamatergic regulation of basal and stimulus-activated dopamine release in the prefrontal cortex. J Neurochem 71:1443-1449.

Tang WX, Fasulo WH, Mash DC, Hemby SE (2003) Molecular profiling of midbrain dopamine regions in cocaine overdose victims. J Neurochem 85:911-924.

Tarter RE (1988) Are there inherited behavioral traits that predispose to substance abuse? J Consult Clin Psychol 56:189-196.

Tarter RE, Moss H, Blackson T, Vanyukov M, Brigham J, Loeber R (1998) Disaggregating the liability for drug abuse. NIDA Res Monogr 169:227-243.

Thomas MJ, Beurrier C, Bonci A, Malenka RC (2001) Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. Nat Neurosci 4:1217-1223.

Thompson T, Schuster CR (1964) Morphine Self-Administration, Food-Reinforced, and Avoidance Behaviors in Rhesus Monkeys. Psychopharmacologia 5:87-94.

Tomasi D, Goldstein RZ, Telang F, Maloney T, Alia-Klein N, Caparelli EC, Volkow ND (2007) Widespread disruption in brain activation patterns to a working memory task during cocaine abstinence. Brain Res.

Tost H, Meyer-Lindenberg A, Klein S, Schmitt A, Hohn F, Tenckhoff A, Ruf M, Ende G, Rietschel M, Henn FA, Braus DF (2006) D2 antidopaminergic modulation of frontal lobe function in healthy human subjects. Biol Psychiatry 60:1196-1205.

Trantham-Davidson H, Neely LC, Lavin A, Seamans JK (2004) Mechanisms underlying differential D1 versus D2 dopamine receptor regulation of inhibition in prefrontal cortex. J Neurosci 24:10652-10659.

Trueman RC, Brooks SP, Dunnett SB (2005) Implicit learning in a serial choice visual discrimination task in the operant 9-hole box by intact and striatal lesioned mice. Behav Brain Res 159:313-322.

Trulson ME, Ulissey MJ (1987) Chronic cocaine administration decreases dopamine synthesis rate and increases [3H] spiroperidol binding in rat brain. Brain Res Bull 19:35-38.

Tsai SJ, Huang YH, Chang LS, Yang YC, Sim CB (1995) Alterations in local cerebral glucose utilization in rats after chronic amphetamine administration without subsequent challenge. Psychiatry Res 57:65-73.

Tseng KY, O'Donnell P (2004) Dopamine-glutamate interactions controlling prefrontal cortical pyramidal cell excitability involve multiple signaling mechanisms. J Neurosci 24:5131-5139.

Tseng KY, O'Donnell P (2007) D2 dopamine receptors recruit a GABA component for their attenuation of excitatory synaptic transmission in the adult rat prefrontal cortex. Synapse 61:843-850.

Tsuang MT, Lyons MJ, Eisen SA, Goldberg J, True W, Lin N, Meyer JM, Toomey R, Faraone SV, Eaves L (1996) Genetic influences on DSM-III-R drug abuse and dependence: a study of 3,372 twin pairs. Am J Med Genet 67:473-477.

Tsukada H, Kreuter J, Maggos CE, Unterwald EM, Kakiuchi T, Nishiyama S, Futatsubashi M, Kreek MJ (1996) Effects of binge pattern cocaine administration on dopamine D1 and D2 receptors in the rat brain: an in vivo study using positron emission tomography. J Neurosci 16:7670-7677.

Ueda S, Sakakibara S, Yoshimoto K (2005) Effect of long-lasting serotonin depletion on environmental enrichment-induced neurogenesis in adult rat hippocampus and spatial learning. Neuroscience 135:395-402.

Ujike H, Akiyama K, Otsuki S (1990) D-2 but not D-1 dopamine agonists produce augmented behavioral response in rats after subchronic treatment with methamphetamine or cocaine. Psychopharmacology (Berl) 102:459-464.

Unterwald EM, Fillmore J, Kreek MJ (1996) Chronic repeated cocaine administration increases dopamine D1 receptor-mediated signal transduction. Eur J Pharmacol 318:31-35.

Unterwald EM, Ho A, Rubenfeld JM, Kreek MJ (1994) Time course of the development of behavioral sensitization and dopamine receptor up-regulation during binge cocaine administration. J Pharmacol Exp Ther 270:1387-1396.

Vaillant GE (1966) A 12-year follow-up of New York narcotic addicts. 3. Some social and psychiatric characteristics. Arch Gen Psychiatry 15:599-609.

van den Bree MB, Johnson EO, Neale MC, Pickens RW (1998) Genetic and environmental influences on drug use and abuse/dependence in male and female twins. Drug Alcohol Depend 52:231-241.

van Gorp WG, Wilkins JN, Hinkin CH, Moore LH, Hull J, Horner MD, Plotkin D (1999) Declarative and procedural memory functioning in abstinent cocaine abusers. Arch Gen Psychiatry 56:85-89.

van Praag H, Christie BR, Sejnowski TJ, Gage FH (1999) Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci U S A 96:13427-13431.

van Praag H, Shubert T, Zhao C, Gage FH (2005) Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci 25:8680-8685.

Vanderschuren LJ, Everitt BJ (2004) Drug seeking becomes compulsive after prolonged cocaine self-administration. Science 305:1017-1019.

Vanderschuren LJ, Di Ciano P, Everitt BJ (2005) Involvement of the dorsal striatum in cue-controlled cocaine seeking. J Neurosci 25:8665-8670.

Verdejo-Garcia A, Perez-Garcia M (2007) Profile of executive deficits in cocaine and heroin polysubstance users: common and differential effects on separate executive components. Psychopharmacology (Berl) 190:517-530.

Verdejo-Garcia A, Bechara A, Recknor EC, Perez-Garcia M (2006) Executive dysfunction in substance dependent individuals during drug use and abstinence: an examination of the behavioral, cognitive and emotional correlates of addiction. J Int Neuropsychol Soc 12:405-415.

Verin M, Partiot A, Pillon B, Malapani C, Agid Y, Dubois B (1993) Delayed response tasks and prefrontal lesions in man--evidence for self generated patterns of behaviour with poor environmental modulation. Neuropsychologia 31:1379-1396.

Volkow ND, Fowler JS, Wang GJ, Swanson JM (2004) Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. Mol Psychiatry 9:557-569.

Volkow ND, Mullani N, Gould KL, Adler S, Krajewski K (1988) Cerebral blood flow in chronic cocaine users: a study with positron emission tomography. British Journal of Psychiatry 152:641-648.

Volkow ND, Hitzemann R, Wang GJ, Fowler JS, Wolf AP, Dewey SL, Handlesman L (1992) Long-term frontal brain metabolic changes in cocaine abusers. Synapse 11:184-190.

Volkow ND, Fowler JS, Wolf AP, Hitzemann R, Dewey S, Bendriem B, Alpert R, Hoff A (1991) Changes in brain glucose metabolism in cocaine dependence and withdrawal. Am J Psychiatry 148:621-626.

Volkow ND, Fowler JS, Wang GJ, Hitzemann R, Logan J, Schlyer DJ, Dewey SL, Wolf AP (1993) Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. Synapse 14:169-177.

Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Childress AR, Jayne M, Ma Y, Wong C (2006) Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. J Neurosci 26:6583-6588.

Volkow ND, Fowler JS, Wolf AP, Schlyer D, Shiue CY, Alpert R, Dewey SL, Logan J, Bendriem B, Christman D, et al. (1990) Effects of chronic cocaine abuse on postsynaptic dopamine receptors. Am J Psychiatry 147:719-724.

Von Huben SN, Davis SA, Lay CC, Katner SN, Crean RD, Taffe MA (2006) Differential contributions of dopaminergic D1- and D2-like receptors to cognitive function in rhesus monkeys. Psychopharmacology (Berl) 188:586-596.

Waelti P, Dickinson A, Schultz W (2001) Dopamine responses comply with basic assumptions of formal learning theory. Nature 412:43-48.

Wagner MK (2001) Behavioral characteristics related to substance abuse and risk-taking, sensation-seeking, anxiety sensitivity, and self-reinforcement. Addict Behav 26:115-120.

Wais PE, Wixted JT, Hopkins RO, Squire LR (2006) The hippocampus supports both the recollection and the familiarity components of recognition memory. Neuron 49:459-466.

Wang GJ, Volkow ND, Fowler JS, Cervany P, Hitzemann RJ, Pappas NR, Wong CT, Felder C (1999) Regional brain metabolic activation during craving elicited by recall of previous drug experiences. Life Sci 64:775-784.

Wang Y, Goldman-Rakic PS (2004) D2 receptor regulation of synaptic burst firing in prefrontal cortical pyramidal neurons. Proc Natl Acad Sci U S A 101:5093-5098.

Watanabe M, Kodama T, Hikosaka K (1997) Increase of extracellular dopamine in primate prefrontal cortex during a working memory task. J Neurophysiol 78:2795-2798.

Weeks JR (1962) Experimental morphine addiction: method for automatic intravenous injections in unrestrained rats. Science 138:143-144.

West AR, Grace AA (2002) Opposite influences of endogenous dopamine D1 and D2 receptor activation on activity states and electrophysiological properties of striatal neurons: studies combining in vivo intracellular recordings and reverse microdialysis. J Neurosci 22:294-304.

White FJ, Hu XT, Zhang XF, Wolf ME (1995) Repeated administration of cocaine or amphetamine alters neuronal responses to glutamate in the mesoaccumbens dopamine system. J Pharmacol Exp Ther 273:445-454.

Wik G, Fredrikson M, Fischer H (1997) Evidence of altered cerebral blood-flow relationships in acute phobia. Int J Neurosci 91:253-263.

Wilcox RA, Robinson TE, Becker JB (1986) Enduring enhancement in amphetamine-stimulated striatal dopamine release in vitro produced by prior exposure to amphetamine or stress in vivo. Eur J Pharmacol 124:375-376.

Williams GV, Goldman-Rakic PS (1995) Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. Nature 376:572-575.

Williams JM, Steketee JD (2005a) Time-dependent effects of repeated cocaine administration on dopamine transmission in the medial prefrontal cortex. Neuropharmacology 48:51-61.

Williams JM, Steketee JD (2005b) Effects of repeated cocaine on the release and clearance of dopamine within the rat medial prefrontal cortex. Synapse 55:98-109.

Wilson FA, Scalaidhe SP, Goldman-Rakic PS (1993) Dissociation of object and spatial processing domains in primate prefrontal cortex. Science 260:1955-1958.

Winterer G, Weinberger DR (2004) Genes, dopamine and cortical signal-to-noise ratio in schizophrenia. Trends Neurosci 27:683-690.

Wise RA (1984) Neural mechanisms of the reinforcing action of cocaine. NIDA Res Monogr 50:15-33.

Wise RA, Bozarth MA (1985) Brain mechanisms of drug reward and euphoria. Psychiatr Med 3:445-460.

Wise RA, Rompre PP (1989) Brain dopamine and reward. Annu Rev Psychol 40:191-225.

Wise RA, Leone P, Rivest R, Leeb K (1995) Elevations of nucleus accumbens dopamine and DOPAC levels during intravenous heroin self-administration. Synapse 21:140-148.

Wolffgramm J, Heyne A (1995) From controlled drug intake to loss of control: the irreversible development of drug addiction in the rat. Behav Brain Res 70:77-94.

Wong DF, Kuwabara H, Schretlen DJ, Bonson KR, Zhou Y, Nandi A, Brasic JR, Kimes AS, Maris MA, Kumar A, Contoreggi C, Links J, Ernst M, Rousset O, Zukin S, Grace AA, Rohde C, Jasinski DR, Gjedde A, London ED (2006) Increased Occupancy of Dopamine Receptors in Human Striatum during Cue-Elicited Cocaine Craving. Neuropsychopharmacology.

Woolverton WL, Virus RM (1989) The effects of a D1 and a D2 dopamine antagonist on behavior maintained by cocaine or food. Pharmacol Biochem Behav 32:691-697.

Wyvell CL, Berridge KC (2000) Intra-accumbens amphetamine increases the conditioned incentive salience of sucrose reward: enhancement of reward "wanting" without enhanced "liking" or response reinforcement. J Neurosci 20:8122-8130.

Yamaguchi M, Suzuki T, Seki T, Namba T, Juan R, Arai H, Hori T, Asada T (2004) Repetitive cocaine administration decreases neurogenesis in adult rat hippocampus. Ann N Y Acad Sci 1025:351-362.

Yamamuro Y, Hori K, Iwano H, Nomura M (1994) The relationship between learning performance and dopamine in the prefrontal cortex of the rat. Neurosci Lett 177:83-86.

Zhang Y, Schlussman SD, Ho A, Kreek MJ (2003) Effect of chronic "binge cocaine" on basal levels and cocaine-induced increases of dopamine in

the caudate putamen and nucleus accumbens of C57BL/6J and 129/J mice. Synapse 50:191-199.

Zola SM, Squire LR (2001) Relationship between magnitude of damage to the hippocampus and impaired recognition memory in monkeys. Hippocampus 11:92-98.

Zola SM, Squire LR, Teng E, Stefanacci L, Buffalo EA, Clark RE (2000) Impaired recognition memory in monkeys after damage limited to the hippocampal region. J Neurosci 20:451-463.

Zola-Morgan S, Squire LR, Rempel NL, Clower RP, Amaral DG (1992) Enduring memory impairment in monkeys after ischemic damage to the hippocampus. J Neurosci 12:2582-2596.