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Approval Sheet

The Transitional States of Drug Addiction in Rhesus Monkeys

By

Porche´ Kirkland Henry
Doctor of Philosophy

Graduate Division of Biological and Biomedical Sciences
Molecular and Systems Pharmacology

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The Transitional States of Drug Addiction in Rhesus Monkeys

By

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B.S. Spelman College, 2003

Advisor: Leonard L. Howell, Ph.D.

An abstract of
A dissertation submitted to the Faculty of the Graduate School of Emory University
in partial fulfillment of the requirements of the degree of
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Abstract

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By Porche' Kirkland Henry

Long-term cocaine use leads to a compulsive pattern of drug intake and an increased propensity to relapse during periods of abstinence. Such compulsive, escalated use has been associated with a number of physiological changes within the dopamine system. This project was designed to identify endpoints associated with the transition from recreational use to periods of extensive drug use and withdrawal by characterizing changes in behavior, neurochemistry, and functional brain activation as a function of drug history. Rhesus monkeys trained to self-administer cocaine were provided limited cocaine access restricting total daily intake. After several weeks under the limited access condition, an extended access condition was added during which subjects had the opportunity to escalate daily drug intake. Bimonthly reinstatement experiments conducted during the periods of limited and extended access determined changes in vulnerability to reinstate extinguished drug seeking behavior as cocaine exposure increased over time. Data from reinstatement tests were then complemented with direct and indirect measures of dopamine system function with *in vivo* microdialysis, acoustic startle, and FDG-PET imaging. Sensitivity to psychostimulants on enhancing extracellular dopamine in the striatum, altering the behavioral-stimulant response to acoustic startle, and modulating the pattern of cortical brain activation were also determined after each access period and following a period of drug withdrawal. Extended access to cocaine self-administration lead to increased cocaine intake but did not result in escalation. Surprisingly, there was no effect of drug history on cocaine-induced reinstatement or sensitivity to psychostimulants on startle amplitude. There were, however, diminished drug-induced increases in striatal dopamine as measured with dialysis and enhanced brain activity throughout the frontal cortex and striatum over time. Collectively, these studies created a neurochemical profile of cocaine's effect on the brain of rhesus monkeys with a prolonged history of drug self-administration. Conclusions made from this project are potentially a step towards developing better animal models in which to evaluate prospective pharmacotherapies.

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This dissertation is dedicated to the memory of my grandmother and friend, W. Lois Brumby.

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CHAPTER I

Introduction

Cocaine abuse and addiction represent both an economically and socially draining public health problem. In 2007, an estimated 2.1 million Americans aged 12 and older reported being current cocaine users and an estimated 1.6 million were classified with dependence on the drug (National Survey on Drug Use and Health, 2007). The cost of this negligence is paid in the form of increased crime, violence, poverty, neonatal drug exposure, and spread of infectious disease. The development of more effective strategies for prevention and treatment of cocaine dependence is therefore of critical importance.

The initial use of cocaine begins as voluntary drug experimentation. As exposure becomes more frequent, use of the drug becomes far less recreational and develops more into an overwhelming obsession. Persistent fixation on acquiring and using increasingly more cocaine is perpetuated by the intense craving that is induced by the use of the drug. Cocaine exposure brings about long-lasting changes in the function of the dopaminergic systems that mediate reward circuitry (Weiss et al., 2001), or the network of neuronal responses to rewarding stimuli. The likely neuroadaptation to this continued cocaine abuse can result in compulsive behavior that is indicative of an addictive drug state. The outcome is an inability to self-limit drug intake which is presented as a progressively escalating pattern of cocaine use. Users begin to isolate

themselves socially and an ever-increasing binging routine is initiated (Gawin, 1991; Gawin and Ellinwood, 1989). During binges, episodes of frequent dosing are followed by periods of drug abstinence for days, weeks, or even months, only to repeat the cycle again. This transition from recreational cocaine abuse to debilitating cocaine addiction is therefore brought about by neuronal changes due to frequent drug exposure.

Addiction to controlled substances, such as cocaine, is a life-long illness that requires regular, frequent management and support. Probability of relapse is high and very common. The propensity to relapse despite prolonged periods of withdrawal is a universal hallmark of addiction. It would be of great benefit if treatments were developed that would not only assist in the cessation of cocaine use but would also aid in the maintenance of abstinence. Animal models of drug self-administration are unique and practical tools in which to study the maladaptive behavior associated with drug taking because of the commonality of drug effects across several species. Voluntary intake, binge phases, and relapse are just some of the patterns of drug use that can not only be mimicked in the animal model but can also be controlled and manipulated in order to gain a better understanding of the underlying mechanisms of addiction (Everitt and Robbins, 2000).

A model of escalating cocaine intake was developed in the rodent to study what effect increasing psychomotor stimulant intake would have on subsequent drug taking behavior, relapse susceptibility, and drug sensitivity (Ahmed and Koob, 1998, 1999). It was believed that any changes noted as a result of a binge-like pattern of stimulant self-administration would be reflective of a transition from drug use to drug dependence, an

essential feature of drug addiction. Animals permitted various access times to cocaine self-administration each day responded differently depending on their length of exposure. With limited access (1hr/day), cocaine intake remains stable across multiple test sessions, whereas with extended access (6hrs/day), cocaine intake gradually escalates over time. Subsequent dose-response curves reveal that animals that are given the extended access to drug also display elevated responding for a range of drug doses compared to animals that are given only limited access. In addition, when animals with a history of extended cocaine access are placed under the more restricted schedule, responses for cocaine remain elevated rather than returning to levels previously seen in the limited access group. This persistent escalation in drug intake following a period of extended access represents a long-lasting change in steady-state levels of cocaine self-administration due to the repeated, long-term exposure.

Similar rodent studies have also manipulated daily drug availability to create experimental drug histories characteristic of addiction-like patterns of behavior. In addition to the escalated drug consumption, rats allowed extended access to cocaine exhibit many behaviors similar to those described for cocaine-dependent humans. Long-term drug cocaine consumption in the rodent model has been reported to be associated with heightened susceptibility to drug-induced reinstatement (Ahmed and Cador, 2006; Deroche-Gamonet et al., 2004; Ferrario et al., 2005; Keiflin et al., 2008; Kippin et al., 2006; Knackstedt and Kalivas, 2007; Mantsch et al., 2004), increased motivation for drug self-administration (Ben-Shahar et al., 2008; Morgan and Roberts, 2004), and persistent drug seeking in the face of environmental adversity

(Vanderschuren and Everitt, 2004). These studies suggest that varying drug exposure is especially effective in demonstrating the transition from causal to compulsive cocaine use.

However, as expected, it is often difficult to translate studies in rodents to that of the human condition. It therefore has been the goal of this study, to extend findings from rodent self-administration models into the more closely human-related primate model. Rhesus monkeys have been used extensively in pharmacological studies and have been chosen for this study on the basis of their availability and extensive database. They share many distinct neuroanatomical and behavioral similarities with humans making them a very practical species in which to study the patterns of cocaine addiction and the resulting neurobehavioral changes. Rhesus monkeys trained to self-administer cocaine were provided limited daily access to cocaine to establish stable, long-term self-administration behavior. They were then switched to an extended access condition where cocaine was available for a longer period of time providing an opportunity to escalate drug intake. Similar to rodent models, this differential access to cocaine created a unique drug history from which investigations were made as to the acute versus chronic effects of cocaine exposure.

Unlike the rodent models in which rats are provided increasing periods of drug access over several days, this study incorporated a within-subject, longitudinal study designed to examine the effects of three distinct levels of cocaine access over the span of several months. The study design was intended to more closely model important aspects of the human process of addiction. The human condition has been described as

an increasing series of compulsive cocaine binges. Users will often binge several days a week, with each binge lasting for a number of hours (Gawin, 1991; Gawin and Ellinwood, 1989). Subjects in this study were taken from a state of drug naivety through months of limited cocaine access (recreational, casual drug use) followed by the inclusion of periodical extended access sessions several months, and subsequently, drug abstinence.

Related studies on the progression of cocaine addiction have focused on the escalating patterns of drug self-administration behavior (Mantsch et al., 2004) or the altered incentive value or motivation changes associated with chronic cocaine exposure (Ben-Shahar et al., 2004; Mateo et al., 2005). However, no nonhuman primate studies had been done utilizing the reinstatement paradigm as a dependent measure of behavioral changes indicative of the transitional stages of addiction. Repeated reinstatement probes throughout the course of this study captured any difference between the limited and extended access conditions, as well as, within each access conditions as a function of drug history. It was expected that if prolonged cocaine exposure leads to the development of behavioral changes characteristic of addiction, then the underlying neurochemistry associated with this behavior should similarly be sensitive to drug history. Therefore, *in vivo* neurochemistry, acoustic startle responses, and functional brain activation studies were conducted at each stage of this study to correlate behavioral effects to any neuroadaptive responses in dopamine system function.

Ideally, the conclusions derived from this study will extend findings from the animal model to a better understanding of the human condition. To date, there are no efficacious treatments for stimulant abuse and dependence; however, defining the altered states in different stages of addiction could potentially affect the success of potential medications. Identifying the characteristics that distinguish these different stages will be critical for the development of future pharmacotherapies.

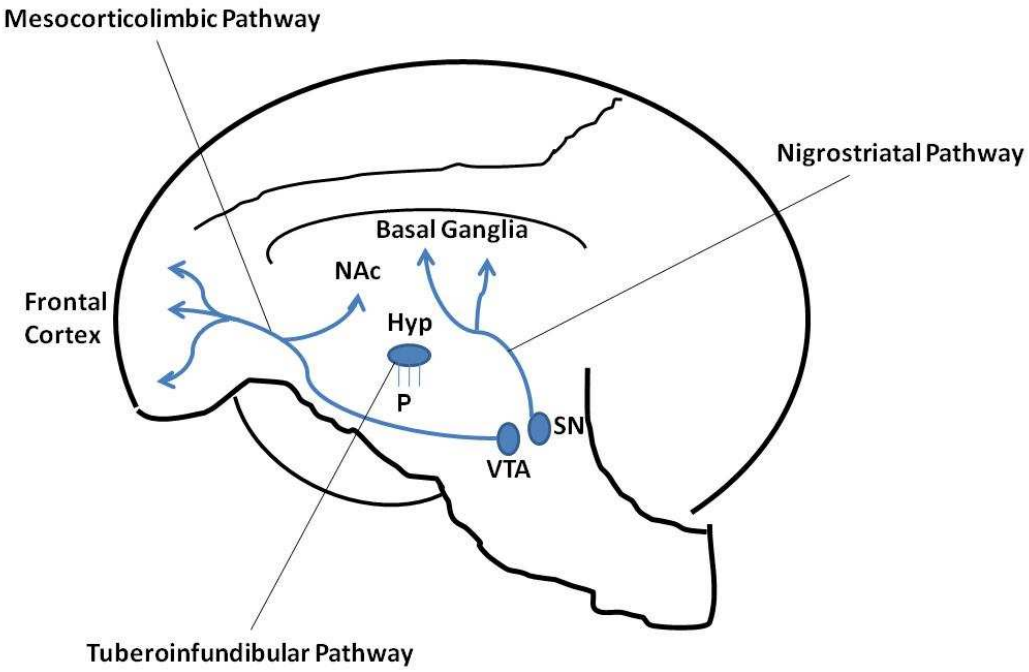
CHAPTER II

Background

A. Dopamine and Cocaine Reinforcement

Dopamine is a principal member of the catecholamine family of diffuse modulatory neurotransmitters in the central nervous system. The pathway of catecholaminergic neurons arise from a core of cell bodies in the midbrain and brain stem and project widely across the brain to disperse neurotransmitters to the striatum, the cortex, and parts of the limbic system. All catecholaminergic neurons contain the enzyme tyrosine hydroxylase, a catalyst in the first step in catecholamine synthesis. Tyrosine hydroxylase converts tyrosine to l-dopa followed by decarboxylation to form dopamine. Additionally, neurons that contain norepinephrine also contain the enzyme dopamine β-hydroxylase (DBH) which converts dopamine to norepinephrine. There are three main neuronal pathways for dopamine-containing neurons (Figure 2.1). The tuberoinfundibular pathway comprises of a group of short neurons in the hypothalamus that project to the pituitary gland and function mainly in endocrine control. The nigrostriatal pathway accounts for the majority of the dopamine neurons in the brain. Cell bodies of this pathway originate in the substantia nigra, pars compacta, and project axons that terminate in the caudate and putamen, a part of the basal ganglia involved with the coordination of movement. The nigrostriatal fibers run through the medial forebrain bundle parallel with the afferents of the mesocortical/mesolimbic neurons. Cell bodies of the mesocorticolimbic pathway originate in the ventral tegmental area

Figure 2.1 Dopamine pathways in the brain. The location of the main groups of cell bodies and fiber tracts are shown in blue. (Hyp – Hypothalamus, NAc – Nucleus Accumbens, P – Pituitary Gland, SN – Substania Nigra, VTA – Ventral Tegmental Area)


Mesocorticolimbic Pathway
Nigrostriatal Pathway
Basal Ganglia
NAc
Frontal
Cortex
Hyp
P
SN
VTA
Tuberoinfundibular Pathway

(VTA) of the midbrain and project to the frontal cortex and parts of the limbic system, including the nucleus accumbens. This system mediates species-specific behavior and emotion and it is the pathway associated with the perception of rewarding stimuli such as food, sex, and drugs (Rang et al., 2003).

The concentration of dopamine in the brain is strictly regulated. Release of dopamine along its neuronal pathways is quickly recaptured by presynaptic dopamine transporters (DAT) to maintain tight control of dopamine receptor activation throughout the system (Figure 2.2). The neurotransmitter is then repackaged into synaptic vesicles or metabolized and eliminated. The main pathway of dopamine metabolism is by monoamine oxidase (MAO) or catechol-O-methyl-transferase (COMT) (Figure 2.3). The resulting metabolic products, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), are then eliminated as waste. Postsynaptically, dopamine binds to the D_1 and D_2 dopamine receptor families, which are distinguished by their activation or inhibition of adenylate cyclase, respectively. The catalytic activity of the adenylate cyclase enzyme controls the concentration of cAMP (cyclic 3',5'-adenosine monophosphate) within the cells which, in turn, regulates many aspects of cellular function through the activation of various protein kinases. All dopamine receptors are G-protein-coupled transmembrane receptors targeting adenylate cyclase, but the family is further divided into subtypes based on their pharmacology, sequence homology, and coupling to adenylate cyclase (Jaber et al., 1996). The D_1-like family consists of the D_1 and D_5 receptors and the D_2-like family comprises D_2, D_3, and D_4 receptors.

Figure 2.2 Cocaine in the brain. Under normal conditions, dopamine is released by the presynaptic neuron into the synapse where it binds to postsynaptic dopamine receptors. Dopamine then cycles back into the presynaptic neuron through the dopamine transporter where it is repackaged into vesicles or metabolized and eliminated. If cocaine is present, it binds to the dopamine transporter and blocks reuptake of dopamine resulting in buildup of the neurotransmitter in the synapse and indirectly increasing dopamine neurotransmission.

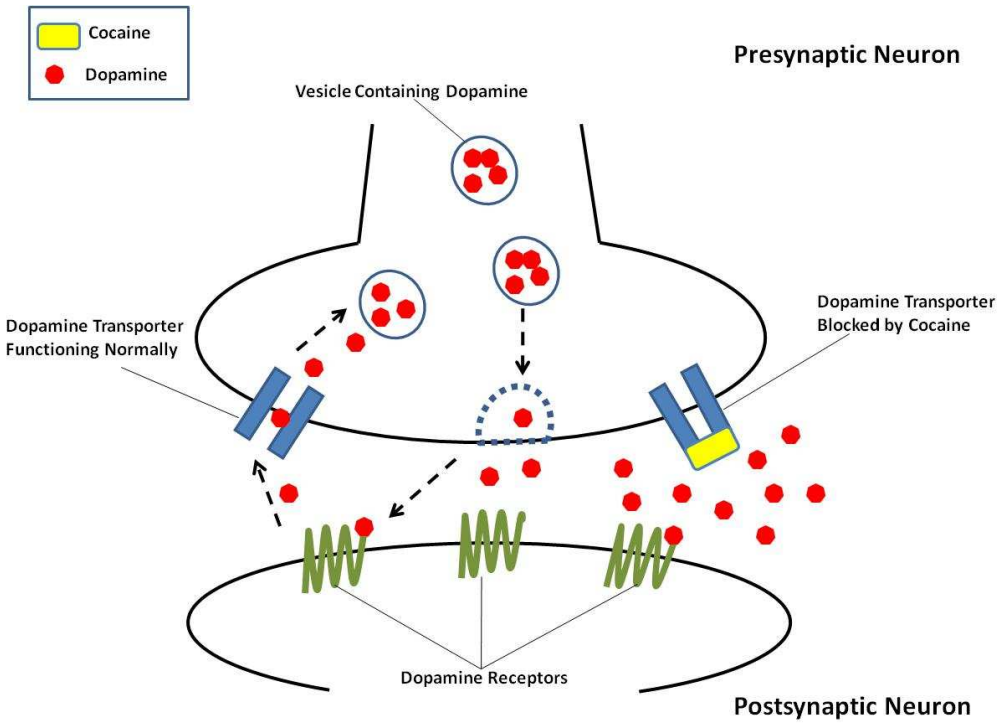
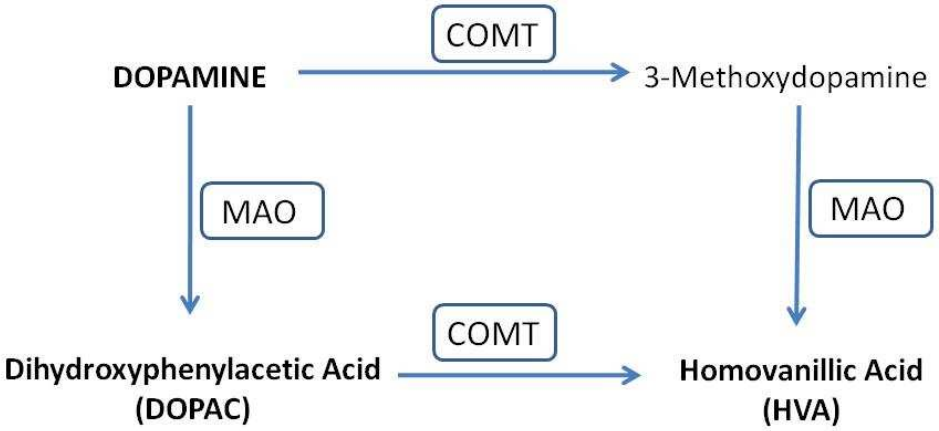

Cocaine
Dopamine
Presynaptic Neuron
Vesicle Containing Dopamine
Dopamine Transporter
Functioning Normally
Dopamine Transporter
Blocked by Cocaine
Dopamine Receptors
Postsynaptic Neuron

Figure 2.3 The main pathways for dopamine metabolism in the brain. (MAO – Monoamine Oxidase, COMT – Catechol-O-methyl-transferase)


COMT
DOPAMINE
3-Methoxydopamine
MAO
MAO
COMT
Dihydroxyphenylacetic Acid
(DOPAC)
Homovanillic Acid
(HVA)

The stimulant and euphoric effects of cocaine are produced through its interaction with presynaptic monoamine transporters resulting in increased extracellular levels of neurotransmitters and prolonged postsynaptic neurotransmission (Figure 2.2). Although cocaine has been shown to bind to all monoamine transporters, including serotonin, its ability to maintain self- administration and function as a reinforcer is attributed primarily to its blockade of dopamine reuptake through the dopamine transporter and indirectly increasing dopamine neurotransmission (Kuhar et al., 1991; Ritz et al., 1987). Specifically, *in vitro* binding studies have shown a direct correlation between the affinity of cocaine for the dopamine transporter and its ability to maintain drug self-administration in animals (Cline et al., 1992; Wilcox et al., 1999). Animals will also self-regulate infusions of cocaine in an effort to maintain a constant concentration of dopamine during administration of cocaine. This elevation of dopamine is also context-dependent, with significantly greater dopamine concentrations being observed in response-contingent delivery of cocaine rather than administration by an experimenter. The reinforcing effects can however be attenuated with selective antrograde neurotoxic lesions of the dopamine terminals of mesolimbic projections or with similar lesions of cell bodies in the ventral tegmental area (Roberts et al., 1977; Roberts and Koob, 1982). Additionally, *in vivo* studies that directly measure the extracellular concentrations of dopamine demonstrate dose-dependent increases in both the caudate and nucleus accumbens following injections of cocaine (Bradberry and Roth, 1989; Hurb et al., 1990). Finally, doses of cocaine commonly abused by humans lead to significant blockade of the dopamine transporter at a level that also induced

self-reports of feeling “high” in cocaine abusers (Volkow et al., 1997a). Pharmacological studies, coupled with direct and indirect dopamine measures through techniques such as microdialysis, selective neurotoxic lesioning, and brain imagining, have implicated the mesolimbic dopaminergic pathway in cocaine reinforcement.

Although the significance of increased dopamine neurotransmission has been well established for cocaine reinforcement, the affinity of cocaine for the norepinephrine and serotonin transporters indicates that these neruotransmitters might play a modulatory role. Afferents from the locus coeruleus (norepinephrine cell bodies in the pons) project throughout the cortex, hippocampus, and cerebellum. Norepinephrine input has also been found to stimulate dopamine release by both directly increasing the firing rate of the VTA (Grenhoff et al., 1993; Liprando et al., 2004) and through innervation of the prefrontal cortex which then sends excitatory glutamatergic projections back to the VTA and the nucleus accumbens (Rossetti et al., 1998; Swanson and Hartman, 1975; You et al., 1998). In fact, selective neurotoxic lesions of the locus coeruleus neurons attenuate dopamine cell activity (Tassin et al., 1979) and therefore decrease the concentration of dopamine released into the striatum (Lategan et al., 1990). Psychomotor stimulant-induced locomotor activity and performance on the conditioned place preference paradigm (two behavioral traits that are predictors of the abuse potential of drugs) are also altered by norepinephrine transmission. For example, behavioral responses to amphetamine, a dopamine transporter blocker and potent dopamine releaser, are diminished by infusions of the noradrenergic receptor antagonist, prazosin, directly into the prefrontal cortex (Blanc et al., 1994; Darracq et al.,

1998). However the contribution of norepinephrine on the primary reinforcement of cocaine is less clear. Depletion of norepinephrine, norepinephrine antagonists, and selective blockade of norepinephrine transporters all fail to alter reinforcement of cocaine-maintained behavior (Roberts et al., 1977; Wee et al., 2006; Woolverton, 1987). There is, on the other hand, evidence that norepinephrine may play a critical role in other aspects of cocaine reinforcement, such as stimulant-induced reinstatement of extinguished cocaine administration (Zhang and Kosten, 2005). Taken together, these studies demonstrate that the response to may cocaine involve a connection between the norepinephrine and dopamine pathways (Weinshenker and Schroeder, 2007).

Similar to norepinephrine, serotonin neurons of the raphe nuclei have also been shown to project onto the cell bodies and terminals of dopamine neurons throughout pathways of the mesocorticolimbic system (Herve et al., 1987; Phelix and Broderick, 1995). Serotonin innervation of dopamine neurons, however, has a differential effect on the rewarding and behavioral effects of cocaine. Enhancing serotonin neurotransmission either directly with serotonin receptor agonists or indirectly with selective serotonin reuptake inhibitors (SSRI) decreases the firing rate of dopamine neurons resulting in an attenuation of cocaine-induced increases in dopamine and a subsequent decrease in the reinforcing effect of cocaine (Czoty et al., 2002; Gobert et al., 2000). Antagonizing serotonin transmission, however, enhances the stimulant effects of cocaine (Howell and Byrd, 1995). In fact, lesioning of serotonin neurons does not disrupt cocaine reinforcement (Roberts et al., 1994) and unlike dopamine transport blockers, SSRI compounds are not in themselves reinforcing (Howell and Byrd, 1995).

The dopamine hypothesis for the reinforcement of cocaine maintained behavior describes a mechanism of action that is dependent on the activity of dopamine neurons in the mesocorticolimbc system, particularly afferents to the nucleus accumbens. The increase dopamine in terminal regions of this pathway leads to an enhanced post-synaptic response that mediates the reinforcing and stimulant properties of cocaine. Norepinephrine and serotonin modulate dopamine transmission by altering neuronal firing rate and neurotransmitter release throughout the dopamine system.

B. Self-Administration

Animal models of drug self-administration play a critical role in determining the reinforcing effectiveness and abuse liability of drugs. Animals trained to self-administer are conditioned to perform a behavioral task such as a lever press or nose poke to receive an infusion of drug. The viability of the self-administration paradigm as a research tool derives from the finding that most drugs that are reinforcing and therefore reliably engender drug taking in animals are drugs that are also abused by humans. The schedule parameters that govern self-administration dictate the sequence and total number of required responses necessary to receive a drug infusion. Under simple schedules, drug infusions typically follow a fixed number of responses (i.e. fixed-ratio (FR) schedule) or the first response to occur after a fixed period of time has elapsed (i.e. fixed-interval (FI) schedule). Combining simple schedules into second-order schedules, in which responding is maintained not only by the drug but also by drug-paired stimuli, allows for the study of more complex behavioral sequences that more accurately reflect human patterns of drug taking.

Under the second-order schedule used in the current study, a drug infusion can be earned after a FI of time has elapsed. During the FI, an FR requirement is also in effect. Each time the FR requirement is met, an environmental cue such as a light or tone is also presented. Completion of the FR at the end of the FI results in an infusion of drug and presentation of the secondary stimulus. The secondary stimulus serves as a conditioned reinforcer that signals the availability of drug and as a result helps to maintain the behavior during the extended schedule. Incorporating drug-paired stimuli into second-order schedules of drug self-administration makes the model more similar to the human condition. Stimuli associated with drug use, such as drug paraphernalia, can induce craving and trigger relapse events in cocaine abusers (O'Brien et al., 1990; Panlilio et al., 2005). Benefits of the second-order schedule include a robust pattern and rate of behavioral responding that is remarkably reproducible while only providing a limited number of drug reinforcers. Throughout this project both a simple FR schedule and a second-order schedule of reinforcement were used to create a history of cocaine self-administration that models the drug taking patterns encountered by drug-dependent subjects. This drug-taking history set the ground work for determining how the behavioral and neurochemical effects of cocaine change in rhesus monkeys at different phases of drug exposure.

-- Reinstatement as a Model of Relapse

Reinstatement refers to the resumption of drug seeking behavior after drug self-administration has been extinguished. The purpose of the extinction phase is to reduce responding for drug by removal of the drug infusions and any drug-paired stimuli (ie the

stimulus light). Once self-administration behavior has been extinguished, acute re-exposure to the drug and the drug-associated cues induce reinstatement of drug-seeking behavior. In a clinical setting, retrospective recall of relapse events and self-reports of craving suggests that exposure to drug or drug cues are conditions that will similarly induce relapse in abstinent, drug-dependent subjects. In animal studies, dopamine D_2 receptor agonists and indirect dopamine agonists (dopamine reuptake blockers like cocaine and amphetamine) reinstate extinguished self-administration behavior. This effect can in turn be blocked by dopamine receptor antagonists providing evidence that the dopamine system may also be involved in drug-primed reinstatement. In the present study, as cocaine intake progressed with longer access to cocaine self-administration, several tests for cocaine-induced reinstatement were conducted to determine the role of cocaine history in relapse to drug seeking. The ability of cocaine to induce reinstatement of extinguished drug-taking behavior was evaluated over repeated determinations. Progressive alterations in reinstatement behavior associated with cocaine history may involve neuroadaptive changes within the mesocortiolimbic dopamine system as a result of long-term cocaine exposure. It was therefore hypothesized that long-term access to cocaine would enhance the cocaine-primed reinstatement effects with repeated determinations throughout cocaine self-administration history. Several studies designed to assess the subsequent function of the dopamine system were then carried out to determine the neurobiological mechanisms mediating any changes in response to the pychostimlant as a function of drug history.

C. Direct Measures of Dopamine with in vivo Microdialysis

Microdialysis provides a means of sampling and monitoring the extracellular concentration of neurotransmitters in discrete brain regions *in vivo* following pharmacological manipulations. An accurate temporal profile of the onset and duration of drugs that elevate neurotransmitter levels can be acquired with short sampling intervals. The collected dialysate can then be analyzed immediately by high performance liquid chromatography. For the purpose of this project, changes in dopamine and its major metabolites, 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the dorsal striatum of awake rhesus monkeys were quantified following systemic administration of cocaine and amphetamine, two indirect dopamine agonists. The large area of dopaminergic innervation to the caudate nucleus made it an ideal target region for microdialysis studies. The caudate is very accessible via stereotaxic surgery and provides an excellent brain structure to monitor drug effects on the extracellular concentration of dopamine. Although the caudate is not implicated in the reinforcing effects of cocaine, changes in the function of dopamine neurons as a function of drug history would also be reflected in the dense dopamine projections to the caudate. The biggest concern with microdialysis studies is that repeated insertion of dialysis probes into the caudate could damage local tissue and the surrounding cellular regions potentially confounding experimental results. Steps are taken to ensure that this damage is minimal by limiting the frequency and duration of site access to that which is necessary for the completion of the study. Also tissue integrity is tested with each experiment by perfusing artificial cerebral spinal fluid with a high concentration of

potassium through the probes to induce voltage-dependent neurotransmitter release. When the tissue is healthy, the magnitude of evoked release of dopamine is consistent with each experiment. Additionally, immunohistochemistry studies have shown an absence of any significant tissue damage in the brains of monkeys previously implanted with probes for microdialysis (Czoty et al., 2000).

Microdialysis was used to determine the effect of cocaine self-administration history on basal dopamine levels and to characterize changes in the sensitivity to psychomotor stimulants as a function of drug intake as determined by altered responsiveness to cocaine and amphetamine. Positron emission tomography (PET) imaging studies with [^{11}C]raclopride, a competitive D2 dopamine receptor agonist, in humans have shown that cocaine addiction is associated with decreases in dopamine system function (Volkow et al., 1997b). Compared to control subjects, cocaine-dependent subjects have reduced dopamine release into the striatum following administration of methylphenidate, a drug that, like cocaine, increases dopamine transmission by blocking reuptake through the transporter. This dose of methylphenidate also induced intense restlessness and craving for cocaine in this group. The reduction in [^{11}C]raclopride binding after methylphenidate administration was lower in cocaine-dependent subjects suggesting less competition at the D_2 dopamine receptor and therefore less dopamine release. The decrease in release indicates that long-term cocaine use results in persistent changes to dopamine terminals and may account for the repetitive, compulsive drug taking behavior (binging) recognized with cocaine dependence. Therefore, it was hypothesized that compared to the drug naïve state, increases in

cocaine intake by allowing longer access to drug self-administration would result in consistent basal dopamine and metabolite levels but an attenuation in drug-induced increases in dopamine would be observed over time. This hypothesis would be consistent with the human literature documenting hypofuntionality of the dopamine system (Volkow et al., 2004, 2007) and directly correlate to changes in the pattern of cocaine self-administration behavior and to changes in the propensity to reinstate extinguished drug-taking behavior.

D. The Acoustic Startle Response

The acoustic startle response is a motor reflex in response to an intense, abrupt auditory stimulus (Koch, 1999). The reflex is an innate defense mechanism in all mammalian species and initiates preparation for a fight or flight response to potential danger. The startle reflex consists of a quick stiffening of the face, neck, and skeletal muscles and an acceleration of the heart rate. The magnitude of the acoustic startle reflex can be modulated by a variety of external and internal variables resulting in either an enhanced or attenuated response. For example, the acoustic startle response can be potentiated by fear-conditioning. Animals are conditioned to associate a neutral stimulus, such as a light or tone, with an aversive stimulus such as a mild electrical footshock (Davis et al, 1993). When presented with the neutral stimulus alone, the fear associated with the stimulus is determined by a modulation of their acoustic startle response. Repeated presentations of the aversive stimulus can also enhance the startle response above initial startle measures resulting in sensitization of the acoustic startle response after the footshocks (Davis, 1989). Animals can, in turn; habituate to multiple

presentations of the acoustic stimuli themselves both within and between startle sessions, diminishing the amplitude of the response over time (Koch, 1999). Presenting a non-startling stimulus seconds before the acoustic stimulus also reduces the magnitude of the resulting startle response (Koch, 1999). This prepulse inhibition of the startle response is used as a mechanism for measuring sensorimotor information processing. Moreover, the acoustic startle response can be modulated by various drugs that alter neurotransmitter systems.

A series of lesion and electrical stimulation studies identified a very simple sensory-motor reflex circuitry. The circuit involves auditory input to the cochlear root neurons (ear), to neurons in the reticular formation (brain stem), and finally to motorneurons in the spinal cord (Lang et al., 2000; Lee et al., 1996). The acoustic startle response is likewise sensitive to dopaminergic modulation by both direct and indirect dopamine agonists (Davis, 1985; Davis and Aghajanian, 1976; Davis et al., 1975; Gordon and Rosen, 1999; Meloni and Davis, 1999). The nuclei of the basal ganglia, including the striatum, play a major role in impulse control and motor functions, and neurons in the striatum are particularly sensitive to the excitatory effects of dopamine receptor agonists (Rang et al., 2003). In 2000, Meloni and Davis demonstrated that rats with 6-hydroxydopamine lesions of the sustantia nigra projections to the striatum showed markedly greater responses to acoustic startle when challenged with dopamine receptor agonists. Additionally, enhanced startle response by an acute agonist injection, such as cocaine and amphetamine, can in turn be attenuated by dopamine receptor antagonists, such as haloperidol (Davis, 1985; Davis and Aghajanian, 1976).

Dopamine agonists however decrease startle elicited electrically directly to neurons within the acoustic startle pathway (Davis et al., 1986b). Therefore, dopamine is believed to enhance the startle response by increasing the sensitivity of neurons within the startle pathway through projections from the striatum rather than direct activation of motor synapses (Davis, 1980).

Norepinephrine and serotonin also play an excitatory role in the startle response although the magnitude of the effect is not as large as dopamine (Davis, 1980). Drugs that increase norepinephrine by stimulating its release or blocking its reuptake through transporters enhance the acoustic startle response (Davis et al., 1977; Davis et al., 1979), whereas depletion of norepinephrine through lesions in the locus coeruleus or drugs that block its conversion from dopamine attenuates the response (Geyer et al., 1976; Kokkinidis and Anisman, 1978). Depression of startle can also be achieved by blockade of α and β adrenergic receptors with antagonists such as phenoxybenzamine and propranolol (Pohorecky et al., 1976). Serotonin, on the other hand, has dual effects on the acoustic startle response. Depletion, either pharmacologically by blocking the synthesis of serotonin or with electrolytic lesions of the raphe nuclei, is associated with increases in startle (Carlton and Advokat, 1973; Geyer et al., 1976). In fact, rats provided a tryptophan-free diet for several days had startle amplitudes 60% higher than control rats (Walters et al., 1979). Tryptophan is the precursor of serotonin but it is not made by the body and must be supplied through the diet. A systemic injection of tryptophan in the animals that had been depleted of serotonin through the restricted diet restored the startle response to normal levels within an hour of the injection.

Alternatively, increasing serotonin transmission by blocking its degradation or with serotonin agonists also increases the acoustic startle response (Davis et al., 1980). This effect is limited to stimulation of serotonin neurons in the lower brainstem that project primarily to the spinal cord.

The magnitude of the acoustic startle response has a non-zero baseline that can be easily be increased or decreased by experimental or pharmacological manipulations of neurochemical transmission. The plasticity of the acoustic startle response generalizes to other animals, including humans, and makes it a useful behavioral tool for quickly assessing neurological dysfunction. Chronic cocaine users in various phases of drug withdrawal have shown diminished responses to startle stimuli (Efferen et al., 2000). This may be the result of persistent changes to the dopamine system as a consequence of an extensive drug taking history. For example, imaging studies have shown significant reductions in dopamine release and dopamine receptor availability indicating a possible hypofunctional dopamine system in cocaine abusers (Volkow et al., 2007). The attenuation in acoustic startle response was also noted in rodents when comparisons were made before and after 7 days of cocaine exposure (Gordon and Rosen, 1999). The unique drug self-administration history of the nonhuman primates in the present study allowed for the assessment of increasing levels of cocaine exposure on the response to a startling stimulus as a means of assessing dopaminergic function. Baseline measures of startle amplitude prior to cocaine self-administration, increasing drug assess, and drug withdrawal were used to determine transitional changes in the basal startle response. Additional measures of startle amplitude in response to acute

cocaine and amphetamine challenges further determined progressive changes in dopamine function.

As seen with chronic cocaine users, it was expected that baseline acoustic startle responses would be progressively diminished with increased cocaine exposure due to a hypofunctional dopamine system. The startle amplitude would also be attenuated in response to cocaine or amphetamine drug challenges as dopamine release diminishes with increased cocaine exposure. These changes were predicted to persist into abstinence which would parallel startle measurements from cocaine abusers which were collected during withdrawal.

E. Brain Energy Metabolism

Positron emission tomography (PET) technology can provide images of biochemical changes, particularly blood flow and energy metabolism, within the body without altering normal physiological processes through the detection of radioactive ligands introduced into the bloodstream (Herholz et al., 2004). Positrons (antimatter counterpart to electrons) produced from the nuclear decay of the radioactive isotope are annihilated when they encounter electrons resulting in the emission of photons of light. The origins of the positrons are found by detectors that record the photons creating an image of functional activity. Glucose is the primary energy source for the brain. Its utilization is variable and cerebral blood flow changes frequently to adjust to regional metabolic demands as dictated by neuronal activity. The greatest rate of metabolism occurs in the cortex and the basal ganglia, areas of high neuronal activity and dopamine innervation. The use of radioactive fluorodeoxyglucose (FDG) along with

PET technology has determined that regional increases in brain energy metabolism are directly related to behaviorally evoked changes in blood flow (Magistretti et al., 2000). The method is based on the ability of FDG to be taken up into brain tissue by glucose transporters and phosphorylated with kinetics similar to that of glucose, but unlike glucose-6-phosphate, deoxyglucose-6-phosphate cannot be metabolized further. The radioactive compound accumulates intracellularly, therefore allowing for measurement of glucose utilization. Pharmacological activation can increase glucose utilization and therefore FDG uptake to reveal functionally altered brain regions.

Imaging studies in both humans and nonhuman primates have shown that chronic cocaine self-administration is associated with altered neuronal activity in the striatum and frontal cortex (Beveridge et al., 2006; Volkow et al., 2007). The striatum is divided into functional domains based on the projections it receives from the cortex and midbrain structures. The dorsal striatum includes the caudate and putamen and receives input from motor and sensory cortices while the ventral striatum comprises the nucleus accumbens and receives input from orbital frontal and limbic cortices. A unique pattern of dopaminergic projections innervates both dorsal and ventral striatum from the substantial nigra and ventral tegmental area, respectively. Dopamine input from the substantia nigra and ventral tegmental is known to be associated with the behavioral-stimulant and reinforcing effects of drugs of abuse. Using the autoradiographic 2-[14C]deoxyglucose method, metabolic mapping following the acute administration of cocaine in drug naïve monkeys produced changes in glucose utilization throughout the ventral striatum and restricted portions of the dorsal striatum (Lyons et al., 1996).

However, a history of cocaine self-administration, from the initial phases of exposure through months of chronic exposure, progressively intensified the metabolic effects of cocaine encompassing broader regions of the striatum to include more of the caudate and putamen (Porrino et al., 2004). Similar effects were found with drug-dependent subjects using the PET-FDG technique. Polydrug users given an infusion of cocaine were measured for changes in the rate of glucose metabolism as compared to saline treatment. The caudate and putamen were a part of the 26 regions of interest that showed a significant change in the metabolic rate of glucose in response to acute administration of cocaine (London et al., 1990). Initial drug taking behavior is therefore reinforced by activation of the ventral striatum, an area involved in processing pleasurable responses to drugs in the brain. As drug use continues, the impact extends to sensorimotor functions in the dorsal striatum.

Other dopamine projections are also involved in the continued use of drugs. The prefrontal cortex receives direct dopamine input from the ventral tegmental area. The prefrontal brain regions including the anterior cingulate cortex and the orbitofrontal cortex are involved with impulse control, motivation, and compulsive behaviors. Disruption of the prefrontal cortex may be associated with the compulsive drug intake and intense motivation to take drugs that are key symptoms of addiction (Volkow and Fowler, 2000). Acute administration of cocaine in drug naïve monkeys altered activity in the ventral and medial surfaces of the prefrontal cortex. The pattern of cortical metabolism then occupied a far more expanse region of the prefrontal cortex to include largely the caudal portion of the orbitofrontal cortex when cocaine administration

followed a chronic pattern of cocaine self-administration history. These same regions of the prefrontal cortex that are affected by chronic cocaine exposure also innervate the nucleus accumbens of the ventral striatum (Kunishio and Haber, 1994). The progressive changes in the cortex in response to cocaine administration directly parallel the changes in functional activity within the striatum. Moreover, within 1-6 weeks of drug abstinence, significant alterations in the brain activity of the frontal lobes can also be seen in cocaine-dependent human subjects compared to normal controls, and this remains evident even after 3-4 months of drug withdrawal (Volkow et al., 1992). Disruptions in the metabolic activity were associated with lower levels of dopamine release and dopamine receptor availability in the striatum indicating deficits in the dopamine pathways of cocaine abusers (Volkow et al., 1993).

Region specific alterations in glucose metabolism suggest an overall disruption of dopamine system function in both humans and nonhuman primates with an extensive drug taking history. In the present study, FDG-PET studies were conducted longitudinally to map the metabolic changes that occur as the cocaine self-administration history progressed from drug naïve to chronic exposure, and finally into withdrawal. It was hypothesized that acute cocaine-induced increases in glucose metabolism would be restricted to the orbitofrontal cortex during the drug naïve state and expand to include all portions of the striatum and frontal cortex with increasing cocaine exposure. These changes would further complement additional measures of dopamine system function from *in vivo* microdialysis and acoustic startle experiments.

F. Summary

Cocaine addiction is the persistent, compulsive use of the drug despite negative consequences. Functional treatments for addiction from would be to adjust the inappropriate behavior in order to discontinue the harmful use of the drug and to prevent factors that trigger relapse during abstinence. Identifying the neurochemistry that is correlated with the altered behavior is necessary for the development of such treatments. The aim of this study was to develop a monkey model of the human pattern of drug taking during the progression of addiction to determine how increasing levels of cocaine exposure would affect vulnerability to reinstate drug-seeking behavior after drug self-administration has been extinguished. Behavioral measures were linked to potential changes in neurochemistry using *in vivo* microdialysis and changes in regional brain activation in response to cocaine challenge using PET neuroimaging.

CHAPTER III

Cocaine-induced reinstatement during limited and extended drug access conditions in rhesus monkeys

A. Introduction

Cocaine addiction is a chronic, relapsing disorder resulting in a condition where the individual is unable to remain abstinent for extended periods of time. Preventing relapse to drug use is one of the most difficult obstacles in treating cocaine addiction (O'Brien, 2005). There is substantial evidence that environmental stimuli associated with cocaine use and acute re-exposure to cocaine are critical determinants of relapse (Jaffe et al., 1989; Rohsenow et al., 1990). Drug self-administration in animals is a validated technique to model drug use in humans and the environmental cues associated with this behavior (Everitt and Robbins, 2000). Moreover, the reinstatement paradigm provides a well-characterized preclinical model with relevance toward understanding drug relapse in humans (Stewart and de Wit, 1987). In the reinstatement procedure, extinction of drug-maintained responding is followed by exposure to a stimulus (response-independent drug injections, drug-conditioned contextual cues or stressful stimuli) which restores drug-appropriate responding (for review, see Shalev et al., 2002). This procedure has been used to model the human condition of relapse since conditions that are reported to provoke relapse in humans will also reinstate extinguished drug taking behavior in laboratory animals (Shaham et al., 2003).

Extended access to cocaine self-administration in rodents has been proposed to model the escalating patterns of drug intake seen in cocaine dependence (Ahmed and Koob, 1998; Ahmed and Koob, 1999; Deroche-Gamonet et al., 2004; Koob et al., 2004). Rodents provided extended access to cocaine have been evaluated for changes in reinstatement modeling drug-seeking behavior under a variety of conditions. Groups extinguished from long-access cocaine self-administration (6hrs or more daily) reinstated to lower doses of cocaine than groups extinguished from short-access (less than 3hrs daily) exposure (Ahmed and Cador, 2006; Knackstedt and Kalivas, 2007; Mantsch et al., 2004). Additionally, Ferrario et al. (2005) demonstrated enhanced drug-seeking in rodents even after 47 days of abstinence indicating that the self-administration experience is associated with persistent increases in drug-seeking behavior. Altogether, researchers find in rodents that the length of cocaine access can profoundly influence the sensitivity to drug-induced reinstatement with long-access groups consistently displaying higher response rates relative to their short-access counterparts (Ahmed and Cador, 2006; Ferrario et al., 2005; Kippin et al., 2006; Knackstedt and Kalivas, 2007; Mantsch et al., 2004).

The aim of the present study was to develop a model of extended cocaine access in nonhuman primates in order to evaluate the potential effect of cocaine self-administration history on cocaine-induced reinstatement in rhesus monkeys. We hypothesized that cocaine-induced reinstatement would be enhanced persistently in monkeys given extended cocaine access over the course of several months. Using a long-term, within-subject design, rhesus monkeys were initially trained to self-

administer cocaine under a second-order schedule. During limited access conditions, the number of cocaine infusions earned during 1 hour test sessions was limited to 5. Following the limited access condition, daily sessions included an additional 2 hours of cocaine access under a fixed-ratio schedule of reinforcement 3 days per week. Reinstatement probes were performed during the limited and extended access conditions to evaluate the potential consequences of drug intake on subsequent reinstatement effects.

B. Materials and Methods

Subjects

Six adult (6-8 years old) male rhesus monkeys (Macaca mulatta) weighing between 13 and 15 kg served as subjects. All monkeys were experimentally naïve at the start of the present experiments. Between daily experimental sessions, monkeys were individually housed, provided access to food daily (Purina Monkey Chow, fresh fruit and vegetables), and unlimited access to water. Animal use procedures were in strict accordance with the NIH "Guide for the Care and Use of Laboratory Animals" and were approved by the Institutional Animal Care and Use Committee of Emory University.

Catheter implantation

Monkeys were surgically implanted with chronic indwelling intravenous catheters. Implantation was done under a combination of Telazol and isoflurane (1.0-2.0%) anesthesia using aseptic techniques. One end of a silicone catheter was inserted into either the femoral or jugular vein and advanced into the vena cava. The distal end of the catheter was routed subcutaneously and attached to a vascular access port

(Access Technologies, Skokie, IL) in the interscapular region. Preoperative antibiotic (ceftriaxone) and postoperative analgesic (flunixin meglumine) were administered according to veterinary staff direction. Catheters were flushed daily with 1.0 ml of heparinized (100 U/ml) saline to maintain patency.

Apparatus

During experimental sessions, each monkey was seated in a commercially available primate chair (Primate Products, Miami, FL). A response panel equipped with a lever and stimulus lights was attached to the front of the chair. The skin over the vascular access port was cleaned with 95% ethanol and betadine, and then a special right-angle Huber needle (Access Technologies, Skokie, IL) was inserted into the port. The chair was then enclosed in a ventilated, sound-attenuating chamber (Med Associates, St. Albans, VT). Polyvinyl-chloride (PVC) tubing connected the Huber needle to a motor-driven syringe pump (Model PhD 2000, Harvard Apparatus, Holliston, MA) located outside the test chamber containing the drug solution. When activated, the pump delivered a unit dose of 0.1 mg/kg/infusion (-) cocaine HCl (National Institute on Drug Abuse, Bethesda, MD) in a volume of 2.0 ml during a 7.0 second infusion. Med-PC (Med Associates, St. Albans, VT) software systems controlled all experimental events and data collection.

Self-administration

Each monkey was trained on a second-order, fixed-interval (FI) 600 second schedule with fixed ratio (FR) 20 response components (FI 600; FR 20:S). Responding was initiated using an FR 1 schedule such that each response in the presence of a red

light produced an i.v. infusion of 0.1 mg/kg cocaine and a 15 second illumination of a white light. There was a 2 hour limited hold in which the subject could complete the ratio requirement. Daily sessions were limited to 5 injections. The ratio value was increased gradually as responding increased, from the initial FR 1 to FR 2, FR 5, FR 10 and ultimately FR 20. When the schedule value reached FR 20, drug injections no longer followed the completion of each FR but instead followed the FR components completed during an FI 30 second schedule. A 2 second white light was presented upon completion of each FR 20 component during the interval. Once the criteria of at least 4 infusions for 3 consecutive days was met at this duration, the FI was increased to 60 seconds, and subsequently increased in 60 second intervals until the FI duration reached 600 seconds. Under the terminal schedule, each test session began with a 600 second presentation of a red stimulus light. During this 600 second interval, completion of an FR 20 response requirement changed the stimulus light from red to white for 2 seconds. At the end of the 600 second interval, the subject had a 60 second limited hold to complete one additional FR 20. Completion of this terminal FR 20 before the limited hold elapsed changed the stimulus light from red to white for 15 seconds and initiated a 0.1mg/kg infusion of cocaine. The infusion was followed by a 60 second timeout during which stimulus lights were extinguished and responding had no scheduled consequences. Each daily session consisted of 5 consecutive 600 second components allowing for a total drug intake of 0.5mg/kg of cocaine. The initial training phase required approximately 100 sessions. Once responding stabilized under the terminal schedule, subjects were maintained on this limited access condition (5 injections/day

during 1-hour sessions) for an additional 3 months. Note the 0.1 mg/kg/infusion maintenance dose of cocaine is typically on the ascending limb or the peak of the dose-response curve in rhesus monkeys maintained under the second-order schedule utilized (Howell et al., 2007; Lindsey et al., 2004).

The limited access condition was then followed by 3 months of extended access conditions during which monkeys had an opportunity 3 days a week to increase the amount of cocaine self-administered. On extended access days, sessions began with the second-order schedule, as described. This was followed by 2 additional hours on a simple FR 20 schedule for a total session length of 3 hours. During the additional 2 hours of extended access, a green stimulus light illuminated the chamber and each FR 20 completed resulted in the presentation of the 15-second white light and a 0.1 mg/kg infusion of cocaine. Each infusion received was followed by a 60 second timeout. Monkeys had the opportunity to take an additional 30 infusions (3.0 mg/kg total) during the extended access condition in addition to the 0.5 mg/kg available during the 1 hour second-order schedule.

Reinstatement

Every 4 weeks, reinstatement experiments were conducted during the limited and extended access conditions using the second-order schedule. Cocaine self-administration behavior was extinguished by substituting saline for cocaine in the infusion syringe and omitting the presentation of the drug-paired stimulus lights. Extinction sessions were otherwise identical to the cocaine self-administration protocol. Extinction sessions continued until response rates dropped below 20% of the rate

formerly maintained by cocaine self-administration. To reinstate extinguished self-administration behavior, animals were given a response-independent injection of the maintenance dose of cocaine (0.1 mg/kg) at the start of the session and the drug-paired stimulus lights were restored, but saline continued to be available in the infusion syringe. Response-independent injections of cocaine were administered each day until responding under reinstatement conditions declined to that of extinction levels (<20%). At the conclusion of each reinstatement protocol, monkeys returned to their limited or extended access self-administration condition to re-establish baseline levels of responding until the next scheduled reinstatement determination. During the limited access condition, responding was maintained by the second-order schedule only. During the extended access conditions, responding was maintained by the second-order and FR schedules as described previously.

Data analysis

Mean session response rates were determined for individual subjects by dividing the total number of responses emitted in the presence of the discriminative stimuli (red or green lights) by the total session duration. The magnitude of reinstatement was the peak response rate observed on the first day of each series of reinstatement determinations. The persistence of reinstatement was the number of days necessary to reduce response rate to extinction levels (<20% of cocaine baseline). Response rate data were derived for individual subjects and compared to their own baseline performance (percent of baseline) so that each subject served as its own control. Response rates were then averaged for each drug access condition for group statistical

analyses. Within each access condition, a repeated measures ANOVA was performed across each reinstatement determination with peak reinstatement effect and days to reach extinction criteria as the factors. Differences between limited and extended access conditions were analyzed by direct comparisons using Student's *t*-test. All statistical tests were performed as indicated with commercially available statistical analysis software (SigmaStat; Systat Software, Inc., Point Richmond. CA).

C. Results

Self-administration

During the first 3 months of stable self-administration under the second-order schedule, monkeys earned an average of 4.6±0.1 infusions of cocaine daily and maintained consistent rates of responding (averaging 0.90±0.15 responses/second). One-way repeated measures ANOVA for the number of infusions earned and daily response rates did not show any significant difference in either variable during the first 3 months of the limited access condition. As expected, monkeys consistently earned nearly all available cocaine infusions and maintained stable response rates over the 3 months of limited access. The average daily number of infusions during the last 5 days of months 1-3 was 4.3±0.2, 4.8±0.1, and 4.6±0.2, respectively. Mean response rate (responses/second) during the last 5 days of months 1-3 was 0.95±0.30, 0.93±0.23, and 0.82±0.28, respectively.

During extended access conditions, behavior during the second-order schedule remained consistent with that observed during limited access conditions. The average daily number of cocaine infusions earned was 4.3±0.2, and the mean response rate was 0.83±0.18. One-way repeated measures ANOVA for the number of infusions earned and response rates during the second-order schedule did not show any significant difference in either variable over 3 consecutive months of the extended access condition. Similarly, the number of infusions earned during the FR 20 schedule did not show any significant difference over 3 consecutive months of the extended access condition. The average daily number of infusions during the last 5 days of month 4-6 was 23.3±3.0, 20.5±3.3,

and 19.7±3.2, respectively. However, one-way repeated measures ANOVA revealed a decrease in the response rate during the FR 20 schedule after the first month of extended access [$F(2,8)=10.214$, $p=0.006$]. Mean response rate during the last 5 days of months 4-6 was 0.19±0.08, 0.10±0.03, and 0.08±0.02, respectively. Although monkeys were taking the same number of infusions during the FR 20 component, the pattern in which they took the infusions changed over time. Instead of taking all the infusions within the first hour of the component, they were now taking several infusions earlier in the 2-hour component and spacing the remaining infusions over the course of the session. Figure 3.1 shows the progressive change in response pattern during the FR 20 schedule over the 3 months of extended access in a representative monkey.

Reinstatement

After each block of extinction, responding was reinstated with a response-independent injection of the maintenance dose of cocaine (0.1 mg/kg) immediately prior to a saline self-administration session. The drug was administered on consecutive days until response rates declined to extinction levels. Peak response rates occurred on the first day of reinstatement and gradually declined over consecutive sessions. Figure 3.2 shows response rates on the first day of reinstatement as a percentage of each monkey's self-administration baseline. One-way repeated measures ANOVA did not reveal any significant difference in mean response rates over consecutive months of limited or extended access conditions. Lastly, Figure 3.3 shows the persistence of cocaine-induced reinstatement. There was no significant difference in the number of sessions required to meet extinction criteria during the 3 months of limited or extended

access conditions. Regardless of self-administration history, the persistence of reinstatement was similar across multiple determinations, averaging 12.6±1.2 days over all blocks of reinstatement.

Figure 3.1 Cumulative records of the 2-hour FR 20 schedule during extended access conditions for subject RLq7. The records compare the distribution of responses and cocaine injections over 2 hours. Each diagonal line represents one infusion of 0.1 mg/kg cocaine and is followed by a 1 minute time-out.

Fixed-Ratio Self-Administration

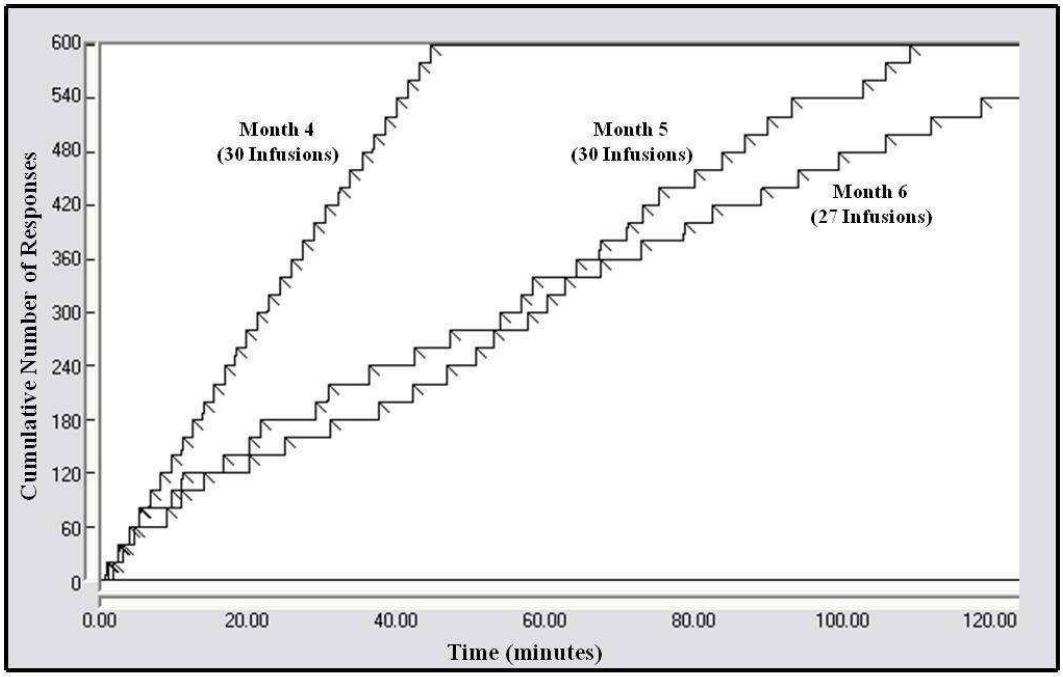


Figure 3.2 Magnitude of cocaine-induced reinstatement of operant behavior in monkeys following each month of limited (A) or extended (B) access conditions. Percent of baseline (last 5 days of cocaine self-administration each month) on the first day of reinstatement during each block of reinstatement is shown for individual monkeys. The numbers in parentheses are the group averages for each condition. Note that subject RLu7 did not reinstate during months 1 and 3.

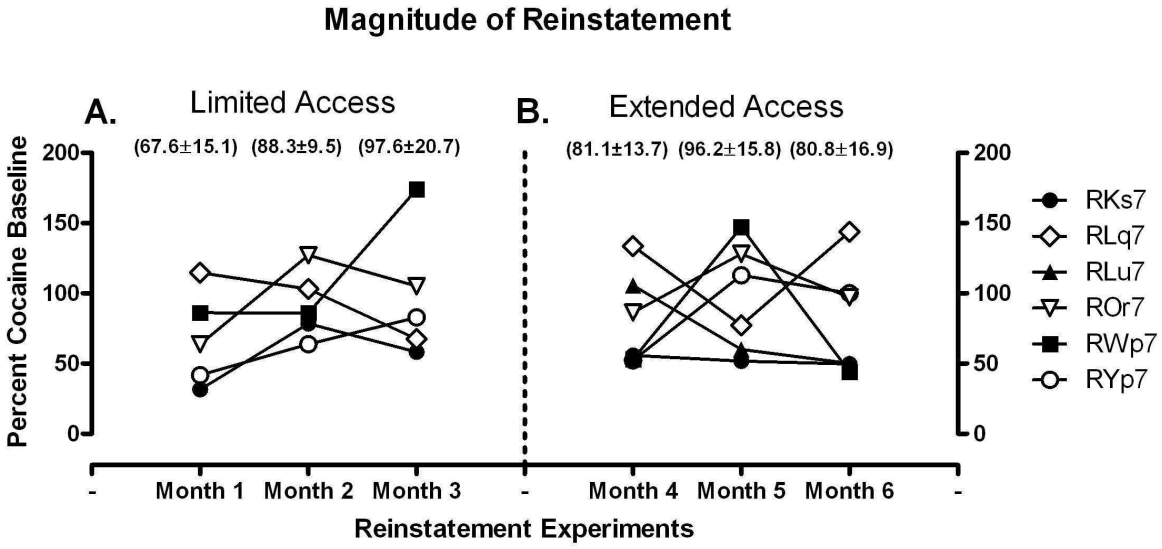
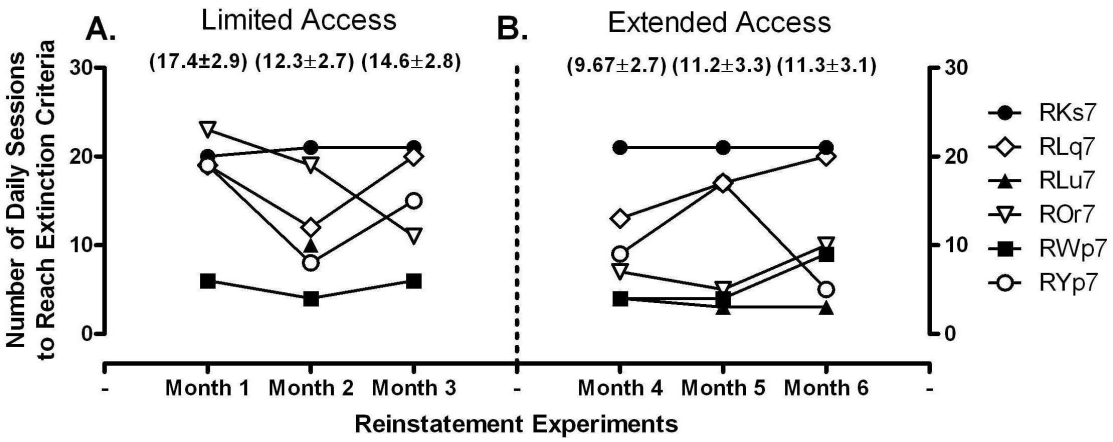

Magnitude of Reinstatement
A.
Limited Access
(67.6±15.1) (88.3±9.5) (97.6±20.7)
B.
Extended Access
(81.1±13.7) (96.2±15.8) (80.8±16.9)
Percent Cocaine Baseline
200
150
100
50
0
Month 1
Month 2
Month 3
Month 4
Month 5
Month 6
Reinstatement Experiments
RKs7
RLq7
RLu7
ROr7
RWp7
RYp7

Figure 3.3 Persistence of cocaine-induced reinstatement following each month of limited (A) or extended (B) access conditions. The number of daily sessions required to return to extinction criteria (<20% of cocaine maintained responding) during each block of reinstatement is shown for individual monkeys. The numbers in parentheses are the group averages for each experiment.

Persistence of Reinstatement



D. Discussion

The present study evaluated the potential influence of cocaine self-administration history on cocaine-induced reinstatement in nonhuman primates. Rhesus monkeys trained on a second-order schedule of cocaine self-administration had limited drug access for 3 months followed by a period of increased drug intake under an FR schedule during 3 months of extended drug access. As drug intake increased from limited to extended access conditions, self-administration behavior under the second-order schedule was stable over the course of the study. Cocaine-induced reinstatement was limited to a single dose but evaluated monthly during each access condition to characterize any progressive changes as a result of chronic drug exposure. The results indicate that the magnitude and persistence of reinstatement under the second order schedule were remarkably stable even when supplemental drug intake was provided over several months.

Numerous studies indicate that prolonged cocaine access can potentiate cocaine-induced reinstatement in rodents (Ahmed and Cador, 2006; Ferrario et al., 2005; Kippin et al., 2006; Knackstedt and Kalivas, 2007; Mantsch et al., 2004) suggesting that increases in drug intake induce a sensitized response to drug-induced reinstatement. However, there has been recent evidence in rodents highlighting the importance of behavioral history on cocaine-induced reinstatement. In one study (Kippin et al., 2006), rats that self-administered cocaine for 6h/day for 14 days showed more robust reinstatement than rats that received response-independent infusions of an equivalent dose of cocaine. As cocaine intake was matched across subjects, it was

the operant conditioning history that apparently enhanced responding during drug-induced reinstatement. Keiflin et al. (2008) also found that operant conditioning history influenced the levels of responding during cocaine-induced reinstatement. In the latter study, two groups of rats maintained similar cocaine intake while their responding was varied by either increasing the unit dose or the FR requirement. The subjects were then evaluated for dose-dependent changes in cocaine-induced reinstatement. For both groups, the conditions that produced the greatest rates of responding during self-administration were associated with enhanced responding during drug-induced reinstatement. Taken together, these studies suggest that operant conditioning history is a major determinant of drug-induced reinstatement.

In the current study, cocaine intake was limited under a second-order schedule of reinforcement. An important property of second-order schedules is that response-dependent, drug-paired stimuli can maintain high rates of operant behavior. Moreover, performance maintained by second-order schedules is remarkably stable over extended periods, and frequency of reinforcement is relatively insensitive to changes in response rate (Schindler et al., 2002; Spear et al., 1991). This consistency was seen during the second-order schedule of both the limited and extended access conditions. All animals received extensive self-administration training on the second-order schedule over 5-6 months in order to establish stable behavioral baselines prior to the initiation of extinction and reinstatement determinations under limited access conditions. Once responding was stabilized, each month of limited access resulted in a similar number of drug infusions and consistent rates of responding. Even after increased drug intake

during the FR schedule of the extended access sessions, the number of infusions and response rates during the preceding second-order schedule remained constant each month and comparable to that seen during the 3 month limited access condition. Hence, the consistency in drug-induced reinstatement may be attributed to the stability of the performance maintained by the second-order schedule employed and the extensive self-administration training prior to reinstatement determinations. Also, it is important to note that the paired brief-stimulus was not presented during the extinction sessions. Accordingly, the conditioned reinforcing effects of the drug-paired stimulus during reinstatement sessions may have contributed to the stability of behavior observed.

Interestingly, there was no escalation in drug intake during the extended access condition. The FR schedule allowed subjects to increase their cocaine intake without the limitations imposed by the second-order component, and compared to the limited access condition, drug intake increased markedly. There was also a progressive change in the pattern of responding during the FR schedule during each month of extended access, resulting in significant reductions in mean session response rates. However, this change in pattern of responding was not reflected in daily cocaine intake. Drug intake during the FR schedule was remarkably stable over the 3 months of extended access. This contrasts with a number of rodent studies reporting an escalation in cocaine intake during extended access conditions (Ahmed and Koob, 1999; Ferrario et al., 2005; Knackstedt and Kalivas, 2007). The stability of drug intake in the present study may reflect species differences or important procedural differences. Rodent studies

reporting an escalation in cocaine intake typically have used the same FR schedule of reinforcement during limited and extended access conditions. In the present study, the monkeys had an extensive history of cocaine self-administration under a second-order schedule before supplemental drug intake was provided intermittently under a separate FR schedule.

In summary, the results obtained indicate that rhesus monkeys with an extensive cocaine self-administration history under a second-order schedule of reinforcement show robust but consistent cocaine-induced reinstatement effects. Extended access to cocaine was not sufficient to enhance reinstatement of extinguished self-administration. Both the magnitude and persistence of reinstatement were remarkably stable. Similarly, others have reported that supplemental cocaine intake under an FR schedule did not alter the reinforcing effectiveness of cocaine in rhesus monkeys (Czoty et al., 2006). The short-term stability of cocaine-induced reinstatement over repeated determinations has been documented previously in nonhuman primates (Khroyan et al., 2000). The present study extends these findings by documenting the long-term stability of reinstatement effects even following an intervening period of supplemental drug intake under a different schedule of reinforcement. Collectively, the results further demonstrate that operant conditioning history and the schedule of reinforcement employed can have a major influence on subsequent drug-induced reinstatement.

Chapter IV

Effects of cocaine self-administration history on in vivo striatal dopamine neurochemistry and acoustic startle in rhesus monkeys

A. Introduction

The transition from infrequent and controlled cocaine use to dependence may involve lasting changes in neurobiological function as a consequence of persistent drug use. These changes manifest in the increased consumption of drug over time and the inability to modulate that intake despite the aversive consequences (Gawin and Ellinwood, 1989; Leshner, 1997; Siegel, 1984). Animal models of drug self-administration have been used to demonstrate many of the major hallmarks of drug abuse. In rodents, short periods of cocaine access (1 hr) produce a consistent pattern of self-administration behavior with a moderate level of drug intake, whereas prolonged cocaine availability (6 hr or more) can result in a progressive escalation in drug intake similar to human patterns of cocaine abuse (Ahmed and Koob, 1998; Ahmed and Koob, 1999). Using this model of chronic, elevated cocaine intake may be useful in characterizing changes in abuse-related behavior and neurobiological adaptations as a function of drug history.

Psychostimulants, such as cocaine and amphetamine, increase extracellular levels of monoamines. However, it is the mesocortiolimbic dopamine projections to the striatum and cortex that play the primary role in the reinforcing effects of cocaine (Wise and Bozarth, 1985). Studies comparing short versus long access to cocaine self-

administration in rodents have found noticeable differences in dopamine system function with prolonged cocaine exposure. Rodents provided long access are more sensitive to the behavioral-suppressant effects of a dopamine receptor antagonist suggesting a change in receptor number or function as a result of increased cocaine intake (Ahmed and Koob, 2004). Rodents provided long access also show deficits in cognitive function following extended drug experience (Briand et al., 2008) and significant decreases in dopamine D2 receptor mRNA and protein levels (Briand et al., 2008; Mantsch et al., 2004). Synaptic reorganization is also evident with increased density of dendritic spines on neurons in the prefrontal cortex and the nucleus accumbens (Ferrario et al., 2005).

Acoustic startle provides a simple and reliable behavioral measure to characterize the consequence of cocaine self-administration history on the status of the dopamine system and sensitivity to the acute effects of psychostimulants. Acoustic startle is a sensorimotor reflex in response to an intense, abrupt auditory stimulus. The reflex is an innate defense mechanism and is mediated by a well-characterized circuit that involves three synapses onto the cochlear root neurons, neurons in the reticularis pontis, and the spinal motoneurons (Davis et al., 1982; Lang et al., 2000; Lee et al., 1996). This response is likewise sensitive to dopaminergic modulation by both direct and indirect dopamine agonists (Davis, 1985; Davis et al., 1986b; Davis et al., 1975; Harty and Davis, 1985; Meloni and Davis, 1999). Moreover, chronic cocaine users in various phases of drug withdrawal have been shown to have diminished responses to startle stimuli

(Efferen et al., 2000). This may be the result of persistent changes to the dopamine system as a consequence of an extensive drug taking history.

The present study investigated the effect of extended i.v. cocaine self-administration history on subsequent dopamine system function in nonhuman primates. Rhesus monkeys were chosen as subjects because of their close physiological and behavioral relationship to humans, as well as for their longevity which allows for a more rigorous, within-subject design that covers months of cocaine access as documented in humans. Naïve monkeys were trained on a fixed ratio (FR) 20 schedule of cocaine reinforcement. Following acquisition of self-administration, cocaine access was limited to 1 hr/day for 10 weeks (limited access), and then increased to 4 hr/day of extended access for 10 weeks (extended access). Extended access was then followed by 4 weeks of withdrawal from cocaine self-administration. Basal levels of striatal extracellular dopamine and its primary metabolites, as well as the effectiveness of cocaine and amphetamine to elevate dopamine, were determined prior to cocaine self-administration, during limited and extended access conditions, and following the withdrawal period. *In vivo* microdialysis was complemented with behavioral measures of acoustic startle. Baseline measures of startle amplitude prior to cocaine self-administration, during limited and extended access conditions, and withdrawal were used to determine any progressive changes in basal startle response. In addition, measures of startle amplitude in response to cocaine and amphetamine challenges also assessed progressive changes in sensitivity to psychostimulants. The overall objective was to determine, *in vivo,*

whether the tone and responsiveness of the dopamine system changes as a result of cocaine self-administration history.

B. Materials and Methods

Subjects

Four male and two female adult (6-8 years old) rhesus monkeys (Macaca mulatta) weighing between 9 and 15 kg were used. All monkeys were experimentally naïve at the start of the present experiments. Between daily experimental sessions, monkeys were individually housed and provided *ad libitum* access to food (Purina Monkey Chow, fresh fruit and vegetables), and water daily. Animal use procedures were in strict accordance with the National Institutes of Health's "Guide for the Care and Use of Laboratory Animals" and were approved by the Institutional Animal Care and Use Committee of Emory University.

Self-Administration

Monkeys were surgically implanted with chronic indwelling intravenous catheters. Implantation was done under a combination of Telazol and isoflurane (1.0-2.0%) anesthesia using aseptic techniques. One end of a silicone catheter was inserted into either the femoral or jugular vein and advanced into the vena cava. The distal end of the catheter was routed subcutaneously and attached to a vascular access port (Access Technologies, Skokie, IL) in the intrascapular region. Preoperative antibiotic (Rochephin) and postoperative analgesic (Banamine) were administered according to veterinary staff direction. Catheters were flushed daily with 1.0 ml of heparinized (100 U/ml) saline to maintain patency.

During self-administration sessions, each monkey was seated in a commercially available primate chair (Primate Products, Miami, FL). A response panel equipped with a lever and stimulus lights was attached to the front of the chair. The skin over the vascular access port was cleaned with 95% ethanol and betadine, and then a special right-angle Huber needle (Access Technologies, Skokie, IL) was inserted into the port. The chair was then enclosed in a ventilated, sound-attenuating chamber (Med Associates, St. Albans, VT). Polyvinyl-chloride (PVC) tubing connected the Huber needle to a motor-driven syringe pump (Model PhD 2000; Harvard Apparatus, Holliston, MA) located outside the test chamber containing the drug solution. When activated, the pump delivered a unit dose of 0.1 mg/kg/infusion (-) cocaine HCl (National Institute on Drug Abuse, Bethesda, MD) in a volume of 2.0 ml during a 7.0 second infusion. Med-PC (Med Associates, St. Albans, VT) software systems controlled all experimental events and data collection.

Each monkey was trained on an FR schedule of drug self-administration. FR self-administration training began with an FR 1 and increased sequentially when at least 3 infusions were earned in three consecutive testing days at each FR until FR 20 was reached. Only 5 infusions were available for each session during FR self-administration training. It took on average about 30 sessions to complete FR self-administration training. Each session began with the presentation of a red stimulus light. The completion of a FR response requirement changed the stimulus light from red to white for 15 seconds and initiated a 0.1mg/kg infusion of cocaine. The infusion was followed by a 60-second timeout during which stimulus lights were extinguished and responding

had no scheduled consequences. At the end of the timeout, the red light was presented again to initiate the next FR component. Once the terminal schedule was reached (FR 20), the sessions were limited to 1 hour each day, 6 days/week, but no limit was placed on the number of available infusions. Subjects were maintained on this limited access schedule for 60 sessions. The limited access condition was then followed by 60 sessions on an extended access schedule during which the session length was increased to 4 hours each day with a limit of 60 available infusions to prevent adverse effects. Finally, the limited access condition was reinstated to determine if the history of extended access resulted in a persistent escalation of cocaine self-administration. Four weeks of withdrawal from cocaine self-administration then followed the limited access probe condition.

In vivo Microdialysis

For *in vivo* microdialysis experiments, subjects were implanted bilaterally with guide cannulae (CMA 12; CMA Microdialysis, Stockholm, Sweden) targeted to the caudate nucleus, a target region of dense dopamine terminals. Before surgery, monkeys were sedated with Telazol then supplemented with inhaled isoflurane (1.0-2.0%) to maintain depth of anesthesia during the procedure. A stereotaxic apparatus was used for proper positioning of the animal's head and to ensure insertion of the guide cannulae to the appropriate depth based on coordinates derived from a standard macaque atlas. A small burr hole for the guide cannulae was made above the caudate nucleus using a trephine drill. Once implanted, Teflon screws and cranioplastic cement held the guides securely in place, and a protective chamber with removable cap (IPI-J1

and ICO-J0; Crist Instrument Company, Inc., Hagerstown, MD) prevented access to the site by the monkeys. Analgesics and antibiotics were prescribed as necessary to alleviate any discomfort associated with surgery, and a two week recovery period preceded any planned microdialysis experiments. Three subjects lost their dialysis chambers prior to the completion of all baseline experiments in the drug naïve state and were therefore not included in the data presented.

Four sets of microdialysis experiments were conducted throughout this study. Changes in dopamine levels in the caudate in response to an acute drug challenge were determined before the initiation of self-administration in the drug naïve state, during limited access, extended access, and again after the withdrawal period. During each set of experiments, the monkeys were seated in a primate chair and placed inside a ventilated, sound-attenuating chamber as previously described. The cap of the chamber was removed and 24mm microdialysis probes (CMA 12, custom made) were inserted into both guide cannulae. A microinjection pump (Harvard Apparatus, Holliston, MA) located outside the chamber continuously delivered artificial cerebrospinal fluid (aCSF) (Na_2HPO_4, 1.0mM; NaCl, 150mM; KCl, 3mM; CaCl, 1.3mM; MgCl, 1.0mM; and ascorbic acid, 0.15mM) through the probes via FEP Teflon tubing (CMA, 1.2µl internal volume/100mm length) at a perfusion flow rate of 2.0µl/min. Following a 1 hour equilibrium period, 6 samples of baseline dialysate (basal samples) were collected before an acute i.v. injection of either cocaine (0.3 or 1.0 mg/kg) or amphetamine (0.1 or 0.3mg/kg) was administered through the vascular access port. Samples of dialysate were then collected every 10 minutes for 2 hours following the injection. Finally, a high

potassium (K^+) aCSF solution containing 54mM KCl and 103mM NaCl was substituted for the standard aCSF at the end of the session to induce volatage-dependent dopamine release. This release of dopamine served as an indication of site viability across several experimental determinations. Additionally, the probes were tested *in vitro* before and after each *in vivo* experiment to determine the probe efficiency and performance.

Small-bore high-pressure liquid chromatography (HPLC) and electrochemical detection quantified the levels of dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), according to well-established analytical procedures (Czoty et al., 2000). The HPLC system consisted of a small-bore (3mm internal diameter x 100mm) column (5µm C_{18} stationary phase; Thermo Hypersil; Keystone Scientific Operations, Bellefonte, PA) with commercially available mobile phase (ESA Biosciences, Inc., Chelmsford, MA) delivered by an ESA model 582 solvent delivery pump at a flow of 0.6 µl/min. After loading the dialysis samples into the refrigerated sample tray (ESA model 542), they were automatically mixed with 3µl of ascorbate oxidase and 5µl of this mixture was injected into the HPLC system. Electrochemical analyses were performed with an ESA dual-channel analytical cell (model 5040) and guard cell (model 5020, potential = 350mV) and an ESA Coulchem II detector. The potential of channel 1 was set to -150mV for oxidation and channel 2 was set to 275mV for reduction.

A full range of dopamine standards (0.5-25nM) was analyzed before and after each set of experimental samples to evaluate the possible degradation of dopamine. Levels of dopamine below 0.5nM were considered below the limits of detection.

Chromatograms were generated using EZChrom Elite software (version 3.1, Scientific Software, Pleasanton, CA). This software was also used to analyze the chromatograms, by comparing the estimated concentration of dopamine and its metabolites to the standards. The change in dopamine and metabolite concentrations was then presented as a percentage of their basal levels as a function of drug dose over time. The percent change in dopamine and metabolite levels for the left and right caudate were averaged for each monkey at each dose of drug.

Acoustic Startle

A detailed account of the apparatus used to quantify acoustic startle responses in rhesus monkeys has been provided previously (Winslow et al., 2002). Briefly, startle testing was conducted in a sound-attenuating chamber equipped with a small ventilation fan and two wall-mounted, high-frequency audio speakers. Background noise (64 dB) was produced by a white noise generator (Lafayette, Model 15800, Lafayette, ID) and delivered through the speakers. The speakers also delivered the startle stimulus, a 50-ms burst of white noise of varying intensities. Whole-body startle responses were measured with an Endevco 2217E accelerometer connected to a Model 104 Endevco amplifier (Endevco, San Juan Capistrano, CA) inside the testing chamber. The accelerometer was center-mounted underneath the upper panel of a two panel platform. The panels were bolted together and separated by heavy compression springs at each corner. On startle testing days, each monkey was placed inside a custom-built lexan primate restraint box (25x25x56 cm) designed to support the monkey in an upright position while still allowing free movement within the apparatus. Subjects were

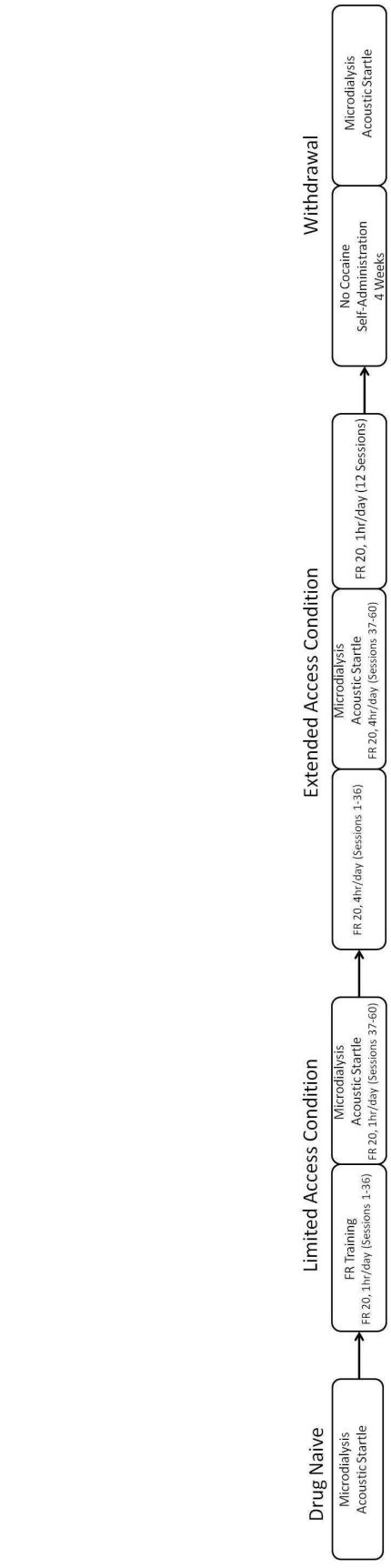
positioned on the platform inside the testing chamber. Movement of the restraint box, resulting from whole-body startle responses, displaced the accelerometer and produced a voltage signal that was integrated by the Endevco amplifier to produce an output voltage signal proportional to the chamber's displacement velocity. Startle amplitude was defined by the maximum accelerometer voltage generated within the first 200 ms of the acoustic stimulus presentation.

Four sets of acoustic startle experiments were conducted throughout this study. The stimulus-response relationship was determined before the initiation of self-administration in the drug naïve state, during limited access and extended access, and again after the withdrawal period. Within each set of experiments, a startle test session was conducted 3 days/week. A 24-min test session began with a 5 min acclimation period then consisted of six consecutive blocks of 3 stimulus intensities (95, 105, and 115 dB) for a total of 18 presentations. The order of the stimulus presentations was systematically varied for each block with an inter-stimulus interval of 60 seconds. Background movement was also sampled by measuring accelerometer voltage generated in the absence of any startle stimulus 30 seconds before each startle presentation. This measure was defined as basal activity to provide a measure of general activity. Two baseline test sessions were conducted prior to any drug sessions. Immediately prior to the start of a drug test session, intramuscular injections were given of either saline or drug (0.3, 1.0, 1.7mg/kg cocaine and 0.1, 0.3, 1.0mg/kg amphetamine). All saline and drug doses were randomized for each monkey and tested twice for a total of 16 test sessions for each set of startle experiments.

Study Timeline

Subjects underwent the first set of microdialysis and acoustic startle experiments in the drug naïve state (Figure 4.1). Microdialysis experiments were conducted once every 2 weeks and acoustic startle test sessions were conducted 3 times per week (Monday, Thursday, and Friday). Immediately following these initial determinations, subjects were trained under the FR schedule of cocaine self-administration. Once the terminal FR 20 schedule was reached, sessions were limited to 1 hour each day, 6 days/week. Subjects were maintained under this limited access condition for 60 test sessions. The second set of microdilaysis and acoustic startle experiments began after 36 test sessions were completed. On days when subjects were not involved in either microdialysis or acoustic startle experiments, cocaine self-administration sessions were conducted as normal. Subsequently, the extended access condition (FR 20, 4 hours each day) and the third set of microdialysis and acoustic startle experiments followed the same schedule described above for the limited access condition. The limited access condition was then reinstated for 12 sessions to evaluate changes in the level of cocaine intake due to a history of extended access to cocaine. Finally, subjects underwent 4 weeks of withdrawal during which all tests were suspended before a fourth set of microdialysis and acoustic startle experiments were conducted.

Figure 4.1 Study timeline for each drug access condition including each set of microdialysis and acoustic startle experiments. See Methods section for detailed explanation.


Drug Naive
Microdialysis
Acoustic Startle
Limited Access Condition
FR Training
FR 20, 1hr/day (Sessions 1-36)
Microdialysis
Acoustic Startle
FR 20, 1hr/day (Sessions 37-60)
Extended Access Condition
FR 20, 4hr/day (Sessions 1-36)
Microdialysis
Acoustic Startle
FR 20, 4hr/day (Sessions 37-60)
FR 20, 1hr/day (12 Sessions)
Withdrawal
No Cocaine
Self-Administration
4 Weeks
Microdialysis
Acoustic Startle

Data Analysis

We used a paired t-test to compare the average number of infusions earned during the first and tenth week of the extended access condition to determine if cocaine intake escalated during the 10 weeks of cocaine self-administration. In addition, daily intake during hour 1 for the first 12 sessions of extended access was also compared by one-way, repeated measures ANOVA.

For the microdialysis experiments, dopamine levels are expressed as nanomolar concentration in dialysate, unadjusted for probe recovery. Mean baseline dopamine concentrations for an individual were defined as the mean of the dopamine concentration of the 6 samples preceding drug treatment. The dopamine concentration for each 10 minute sample was expressed as a percentage of the mean baseline value. Individual subject means were well reflected in the group data. The peak dopamine responses for each set of microdialysis experiments were analyzed by one-way, repeated measures ANOVA to determine the effect cocaine self-administration history on the ability of acute administration of cocaine and amphetamine to increase dopamine concentrations above baseline. Mean baseline dopamine and metabolite data were also analyzed by one-way, repeated measures ANOVA to identify any significant effect of drug history on basal levels.

The startle amplitude for each stimulus intensity is the average of 2 test sessions conducted for each drug dose per animal. Each test session presented the stimulus intensities 6 times in random order. Within each set of startle experiments, a two-way, repeated measures ANOVA was conducted for cocaine and amphetamine with startle

amplitude response as the dependent measure. Differences between drug naïve, limited access, extended access, and withdrawal conditions at the mid-range dose of cocaine and amphetamine, were analyzed by an additional two-way, repeated measures ANOVA. The Tukey's post hoc test for multiple comparisons was used for all follow-up analyses.

C. Results

Self-Administration Behavior

Table 4.1. lists total levels of drug intake (mg/kg) in individual animals for each phase of cocaine access. Self-administration training sessions allowed for a maximum of 5 cocaine infusions per session (0.1 mg/kg/infusion). Training generally took about 30 sessions and total cocaine intake for all monkeys averaged 9.3±1.3 mg/kg. Following acquisition of self-administration, cocaine became available under limited access conditions. Self-administration sessions were performed 6 days per week and over the 60 sessions of limited access, cocaine intake averaged 82.4±13.3 mg/kg. When cocaine access was increased to 4 hr/day during the 60 sessions of extended access, total intake for each monkey increased considerably above their limited access levels averaging 245.0±27.8 mg/kg for the entire group.

Figure 4.2 illustrates, on the other hand, that as a group drug intake did not show any escalation over the course of the 10 week extended access. A paired t-test comparing the average intake during the first week 1 to the tenth week 10 revealed no significant difference in the level of intake throughout the extended access condition. Similarly, during the first hour of extended access sessions, cocaine intake remained unchanged from that seen during the 1 hour of limited access each week, further indicating the stability of self-administration behavior. As shown in the inset of Figure 4.2, there was also no increase in daily cocaine intake during hour 1 over the first 12 sessions of extended access. Finally, returning to limited access conditions for 12

sessions was accompanied by a level of cocaine intake indistinguishable from that seen during the first 10 weeks (data not shown).

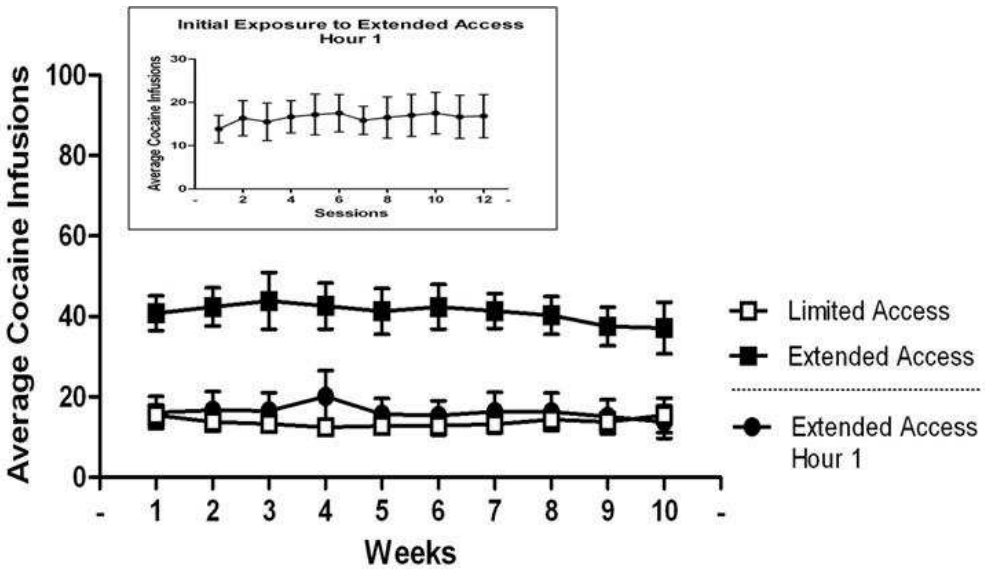
Table 4.1

Total Cocaine Intake (mg/kg) during FR 20 Self-Administration Training and Limited and Extended Access Conditions for Individual Monkeys

Monkey	Training (~30 days)	Limited Access (10 weeks)	Extended Access (10 weeks)
RBl6 (F)	13.80	113.70	345.80
RDn8 (F)	4.47	131.30	315.10
RJl8 (M)	8.90	74.40	225.60
RKn8 (M)	8.30	50.80	186.90
ROf8 (M)	10.90	65.70	202.20
RYt8 (M)	9.40	58.50	194.10
Mean	**9.3±1.3**	**82.4±13.3**	**245.0±27.84**

The letter in parentheses (F or M) indicates gender. Mean±SEM.

Figure 4.2 Average number of cocaine infusions (mean±SEM) earned each week of limited (open squares) and extended access (filled squares) conditions. Also shown is the average number of infusions earned during hour 1 of extended access (filled circles). Inset: Average number of cocaine infusions (mean±SEM) earned during hour 1 over the first 12 sessions of extended access.


Initial Exposure to Extended Access
Hour 1
Average Cocaine Infusions
Sessions
Average Cocaine Infusions
Weeks
Limited Access
Extended Access
Extended Access
Hour 1

In vivo Microdialysis

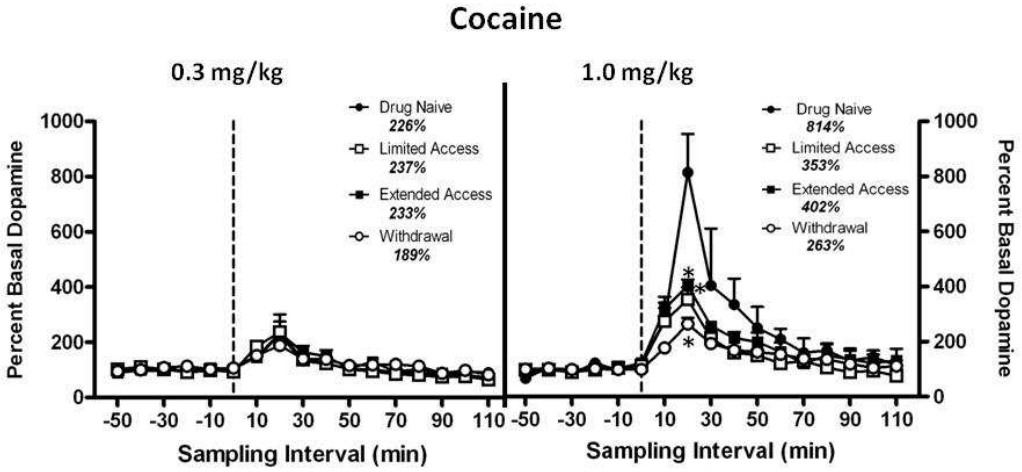
Cocaine (0.3 mg/kg and 1.0 mg/kg) or amphetamine (0.1 mg/kg and 0.3 mg/kg) elicited dose-dependent increases in extracellular dopamine (Figure 4.3A&B). The peak dopamine response (percent of basal levels) occurred within 20 minutes of the drug administration and gradually returned towards baseline levels over the remaining 2 hour session. As cocaine self-administration history progressed there was a significant decline in cocaine-induced increases in dopamine during limited and extended access and following withdrawal ($F(3,6)=10.935$; $p=0.008$) at the highest dose. There was also a nonsignificant downward trend in amphetamine-induced increases in dopamine during extended access and following withdrawal at the highest dose. There was no significant difference in the peak dopamine effect in response to the lower doses of cocaine (0.3 mg/kg) or amphetamine (0.1 mg/kg) at any time point. Note that the highest doses of cocaine (1.0 mg/kg) and amphetamine (0.3 mg/kg) also produced a comparable increase in dopamine at their peak in the drug naive state, 814% and 765% respectively (Figure 4.4). This potency comparison was used to determine dose selection in acoustic startle experiments.

Table 4.2 shows that measures of basal dopamine (average of the 6 baseline samples taken before administration of drug) remained consistent across multiple experiments within individual subjects and that similar levels of dopamine were observed in all 3 monkeys. There was no significant change in the nanomolar concentration of dopamine as a function of cocaine self-administration history, although levels were less than 70% of those observed in the drug naïve state during limited and

extended access conditions. Also included in Table 4.2 are the basal concentrations of the major dopamine metabolites, DOPAC and HVA. There was a significant decrease in the DOPAC metabolite as a function of cocaine self-administration history ($F(3,6)=8.056$, $p=0.016$). A similar decrease was observed with the HVA metabolite during limited and extended access ($F(2,4)=10.318$, $p=0.026$), but the metabolite level was no longer different from that observed in the drug naïve state following the withdrawal period. Note, however, that the ratio of dopamine:DOPAC and dopamine:HVA was consistent across all conditions. There was also no change in *in vitro* probe recovery of dopamine or its metabolites across multiple determinations (data not shown).

Figure 4.3 Effects of cocaine (A) and amphetamine (B) on extracellular levels of dopamine before drug self-administration (drug naïve), during limited and extended access, and following withdrawal (n=3). Dopamine levels are expressed as the mean (±SEM) percentage of baseline levels during 10-min sampling intervals beginning 1 hr after probe implantation. Dashed vertical line at time point 0-min represents administration of drug after collection of 6 baseline samples. The peak percent increase in dopamine in response to both doses of cocaine and amphetamine is indicated for each drug access condition. *****Indicates significant difference from drug naïve state at time point 20-min.

A.


Cocaine
0.3 mg/kg
1.0 mg/kg
Drug Naive
226%
Limited Access
237%
Extended Access
233%
Withdrawal
189%
Drug Naive
814%
Limited Access
353%
Extended Access
402%
Withdrawal
263%
Percent Basal Dopamine
Sampling Interval (min)
-50 -30 -10 10 30 50 70 90 110
0 200 400 600 800 1000

B.

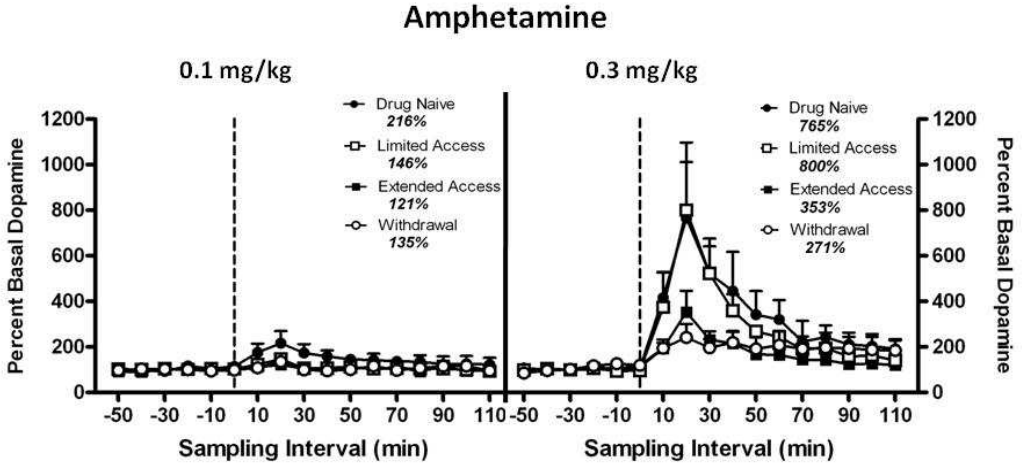

Amphetamine
0.1 mg/kg
0.3 mg/kg
Drug Naive
216%
Limited Access
146%
Extended Access
121%
Withdrawal
135%
Drug Naive
765%
Limited Access
800%
Extended Access
353%
Withdrawal
271%
Percent Basal Dopamine
Sampling Interval (min)
-50 -30 -10 10 30 50 70 90 110
0 200 400 600 800 1000 1200

Figure 4.4 The peak effect of 1.0 mg/kg cocaine and 0.3 mg/kg amphetamine on extracellular dopamine before drug self-administration in the drug naïve state. Dopamine levels are expressed as the mean (±SEM) percentage of baseline levels during 10-min sampling intervals beginning 1 hr after probe implantation. Dashed vertical line at time point 0-min represents administration of drug after collection of 6 baseline samples.

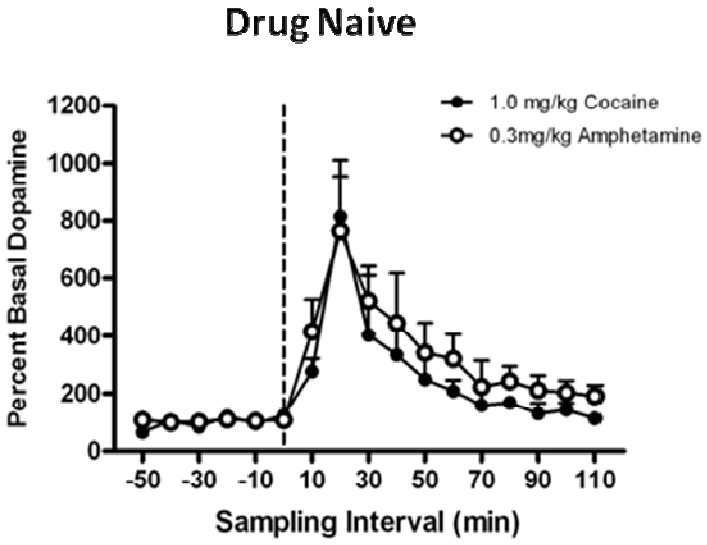

Drug Naive
1.0 mg/kg Cocaine
0.3mg/kg Amphetamine
Percent Basal Dopamine
1200
1000
800
600
400
200
0
-50
-30
-10
10
30
50
70
90
110
Sampling Interval (min)

Table 4.2

Baseline Dopamine and Metabolites

Drug Naïve					
	DA	**DOPAC**	**HVA**	**DA:DOPAC**	**DA:HVA**
RYt8	8.93±2.3	331.0±99.2	4210±1024	0.027	0.0021
RKn8	11.25±4.5	451.7±31.5	5381±635.9	0.025	0.0021
RDn8	14.00±4.0	232.4±51.5	4209±1367	0.060	0.0033
Mean	**10.88±0.4**	**355.0±11.0**	**4625±114.8**	**0.031**	**0.0024**
Limited Access					
	DA	**DOPAC**	**HVA**	**DA:DOPAC**	**DA:HVA**
RYt8	7.44±2.6	221.4±42.2	3610±651.8	0.034	0.0021
RKn8	9.16±3.3	250.0±28.2	4612±1399	0.037	0.0020
RDn8	4.93±3.1	175.1±33.0	2712±583.7	0.028	0.0018
Mean	**7.28±0.3**	**213.6±3.9***	**3678±104.7***	**0.034**	**0.0020**
Extended Access					
	DA	**DOPAC**	**HVA**	**DA:DOPAC**	**DA:HVA**
RYt8	8.39±4.4	194.8±128.7	3156±282.1	0.043	0.0027
RKn8	11.47±4.0	274.9±50.3	4814±754.1	0.042	0.0024
RDn8	2.53±1.0	105.1±61.6	3083±761.9	0.024	0.0008
Mean	**7.45±0.4**	**192.1±9.4***	**3711±121.4***	**0.038**	**0.0020**
Withdrawal					
	DA	**DOPAC**	**HVA**	**DA:DOPAC**	**DA:HVA**
RYt8	4.96±1.8	129.8±44.0	2765±676.2	0.038	0.0018
RKn8	17.81±5.6	317.4±107.2	6357±2305	0.056	0.0028
RDn8	5.438±3.3	150.6±90.2	3281±1023	0.036	0.0017
Mean	**10.48±0.7**	**215.5±12.0***	**4437±220.3**	**0.049**	**0.0024**

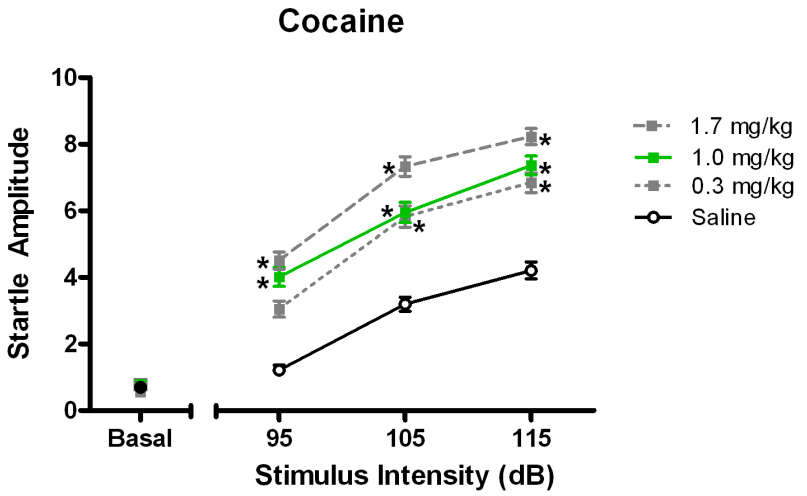
Data represent nM concentrations of dopamine and its metabolites, DOPAC and HVA, uncorrected for probe recovery, with SD for individual animals and SEM for the group mean.
*****Significantly different from drug naïve state.

Acoustic Startle

As expected, acoustic startle was robust and the startle amplitude increased with stimulus intensity. There was a significant main effect of acute cocaine challenge on acoustic startle (Drug Naïve [$F(3, 15)=35.713$; $p<0.001$]; Limited Access [$F(3, 15)=13.677$; $p<0.001$]; Extended Access [$F(3,15)=31.718$; $p<0.001$]; Withdrawal [$F(3,15)=14.991$; $p<0.001$]), that was not seen with amphetamine. No dose of amphetamine produced a startle response greater than the saline control even with repeated, long-term cocaine self-administration history. The first set of startle experiments during the drug naïve state is depicted in Figure 4.5A&B and is typical of the stimulus-response relationship seen during limited access, extended access, and following withdrawal. For each set of startle determinations, cocaine consistently induced a greater enhancement of startle than any dose of amphetamine. Although cocaine treatment enhanced the startle response above that of saline, the effect was not dose dependent nor did the effectiveness of cocaine change as cocaine self-administration history increased from limited to extended access conditions. Startle amplitude at the mid-range dose tested for both cocaine and amphetamine is graphed in Figure 4.6A&B for each set of startle experiments. Cocaine self-administration history had no effect on baseline startle or startle amplitude in response to acute stimulant challenges with either cocaine or amphetamine.

Figure 4.5 Effects of cocaine (A) and amphetamine (B) on average startle amplitude for drug naïve animals (n=6). Drug naïve subjects were given either saline or drug immediately prior to the start of an experimental session. Graphs depict mean startle amplitude for increasing drug doses as a function of acoustic stimulus intensities (dB). Data are collapsed across repeated blocks of stimulus presentation. *****Indicates a significant difference from saline at each stimulus intensity.

A.


Cocaine
Startle Amplitude
10
8
6
4
2
0
Basal
95
105
115
Stimulus Intensity (dB)
1.7 mg/kg
1.0 mg/kg
0.3 mg/kg
Saline

B.

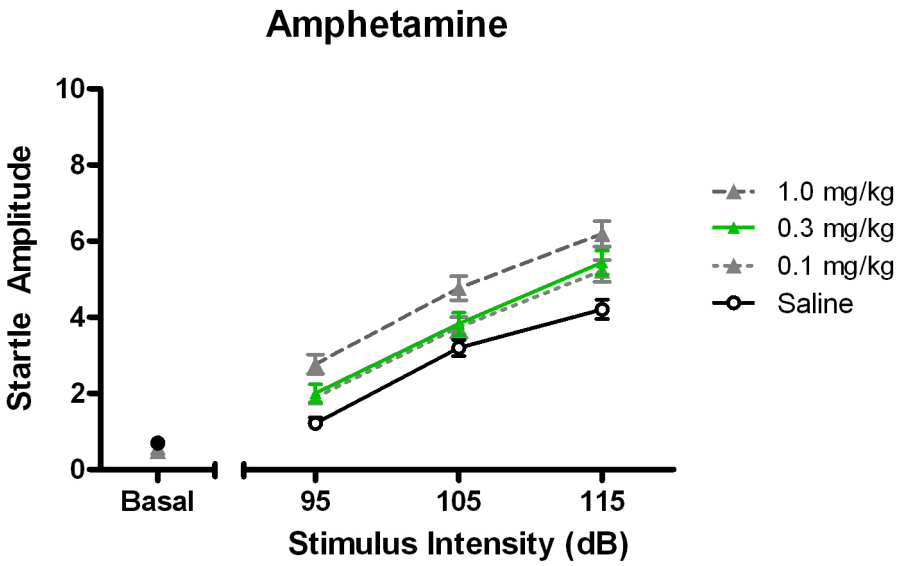
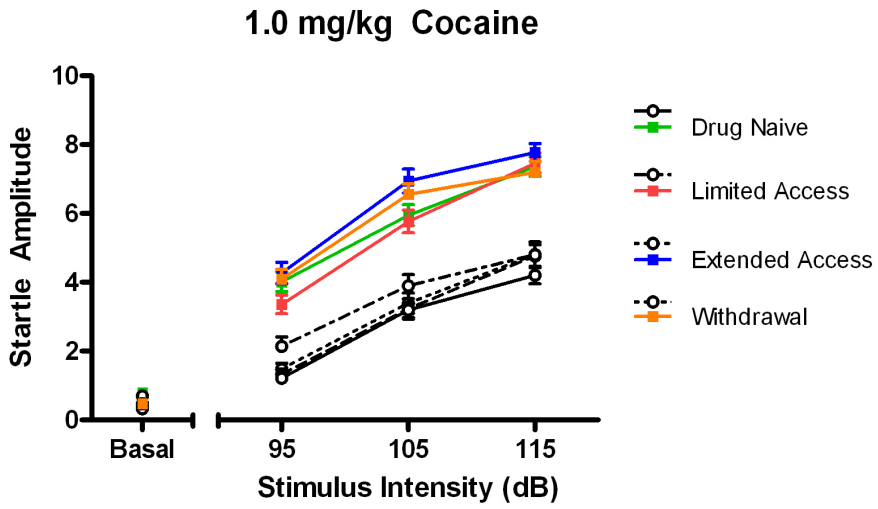
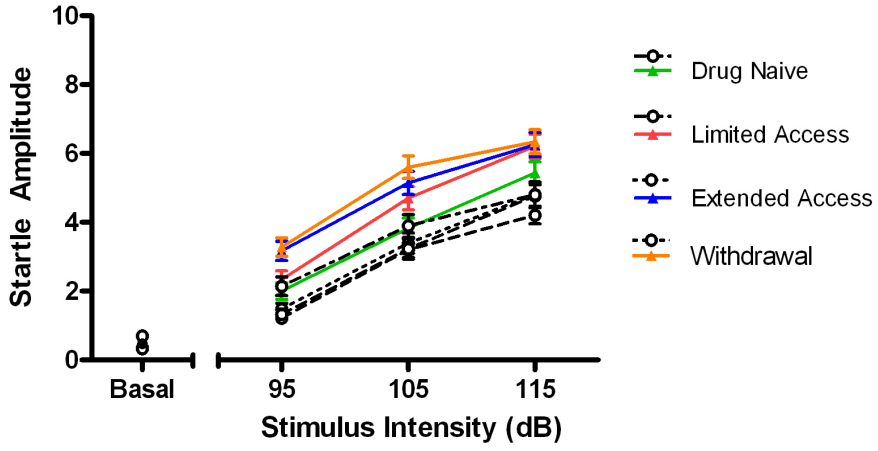

Amphetamine
Startle Amplitude
10
8
6
4
2
0
Basal
95
105
115
Stimulus Intensity (dB)
1.0 mg/kg
0.3 mg/kg
0.1 mg/kg
Saline

Figure 4.6 Average startle amplitude as a function of cocaine self-administration history (n=6). For each set of acoustic startle experiments (drug naïve, limited access, extended access, and withdrawal), subjects were given either saline or drug immediately prior to the start of an experimental session. Graphs depict mean startle amplitude as a function of acoustic stimulus intensities (dB) at the mid-range dose tested for cocaine (1.0 mg/kg) and amphetamine (0.3 mg/kg) for each set of startle experiments. Data are collapsed across repeated blocks of stimulus presentation. Open circles represent saline in both graphs.

A.


1.0 mg/kg Cocaine
Startle Amplitude
10
8
6
4
2
0
Basal
95
105
115
Stimulus Intensity (dB)
Drug Naive
Limited Access
Extended Access
Withdrawal

B.


0.3 mg/kg Amphetamine
Startle Amplitude
10
8
6
4
2
0
Basal
95
105
115
Stimulus Intensity (dB)
Drug Naive
Limited Access
Extended Access
Withdrawal

D. Discussion

The objective of the present study was to establish a well-documented history of cocaine self-administration in nonhuman primates and to evaluate potential changes in dopamine system function. Cocaine- and amphetamine-induced increases in striatal dopamine diminished following a history of cocaine self-administration, indicating tolerance to the neurochemical effects of these psychomotor stimulants. This diminished dopamine response was not associated with a significant decrease in basal dopamine, although there was a significant decrease in DOPAC and HVA levels following the limited access condition. These changes in dopamine neurochemistry were not dependent upon the emergence of an escalating pattern of drug intake. During the extended access condition, there was no escalation in the number of cocaine infusions earned over the 60 day period. This was surprising considering rodent studies employing a similar dosing regimen have repeatedly shown an escalating pattern of cocaine intake in subjects that have experienced up to 6 hrs of daily cocaine access (Ahmed and Koob, 1999; Ferrario et al., 2005; Knackstedt and Kalivas, 2007). In acoustic startle experiments, acute administration of cocaine enhanced the startle response, but there was no effect of self-administration history on baseline startle amplitude or sensitivity to cocaine-induced enhancement of startle. Overall, the present experiments indicate that nonhuman primates with a history of cocaine self-administration show evidence of tolerance to cocaine- and amphetamine-induced increases in striatial dopamine. This effect on dopamine neurochemistry, however, was not associated with

an escalation in drug intake and was not reflected in behavioral measures of acoustic startle.

Neuroimaging studies in cocaine dependent human subjects have associated long-term cocaine use with a decrease in dopamine neurotransmission. Functional changes in the dopamine system in dependent subjects can be characterized by marked decreases in drug-induced dopamine release, increases in dopamine transporter density, and decreases in dopamine receptor binding, consistent with a hypofunctional dopamine system (Malison et al., 1998; Martinez et al., 2007; Volkow et al., 1990; Volkow et al., 1997b). These human studies are consistent with findings reported in rodents showing a diminished dopamine response to cocaine following prolonged access to cocaine. An acute cocaine challenge in rodents with a history of continuous cocaine self-administration for 24 hrs failed to significantly increase dopamine levels above that seen in drug naïve animals (Mateo et al., 2005). Similar to the current study, there was also no difference in the cocaine-induced dopamine response between rodents provided extended access (6hrs) to cocaine self-administration and those with only limited access (1hr) (Ahmed et al., 2003). The strength of the current study was the use of a within-subject, longitudinal design that allowed for the evaluation of striatal dopamine from a drug naive state through two different conditions of cocaine self-administration and drug withdrawal in nonhuman primates.

Acoustic startle is an innate reflex elicited by a sudden and intense auditory stimulus. The reflex is mediated by a simple 3-synapse circuit (Davis et al., 1982; Lang et al., 2000; Lee et al., 1996) and is sensitive to dopaminergic modulation by both direct

and indirect dopamine agonists (Davis et al., 1986b; Harty and Davis, 1985; Meloni and Davis, 1999). Furthermore, chronic cocaine users have been shown to have diminished responses to startle stimuli presumably as a consequence of an extensive drug taking history (Efferen et al., 2000). We hypothesized that enduring neuroadaptive changes in the dopamine system due to chronic cocaine exposure would alter the responsiveness to acoustic startle. Moreover, we hypothesized that the enhanced startle amplitude following acute stimulant challenge would also be diminished, further indicating a tolerance to psychostimulants. The present study is the first to characterize the effect of cocaine self-administration history on subsequent acoustic startle responding had never before been accomplished in nonhuman primates. However, there was no evidence that cocaine self-administration history affected basal or stimulant-induced enhancement of startle amplitude. The consistency of startle-inducing acoustic and tactile stimuli (air puffs) following several days or weeks of continuous cocaine exposure is well documented by the literature derived from rodent studies. Compared to saline treated groups, maximum tactile startle responses were largely unchanged in rodents with a history of cocaine self-administration or chronic cocaine infusions (Barros and Miczek, 1996; Mansbach et al., 1994; Mutschler and Miczek, 1998). The magnitude of the acoustic startle response was similarly unaffected by long-term cocaine exposure whether self-administered or by chronic infusion (Mansbach et al., 1994). Similarly, there was no change in baseline responding to acoustic stimuli at any time point in this study.

Acute administration of cocaine enhanced the acoustic startle response, although the effect was not dose-dependent. A ceiling effect may explain the lack of a significant dose-related effect with cocaine. Interesting, sensitivity to cocaine-induced enhancement of startle was not influenced by cocaine self-administration history even though there was evidence of a decreased dopamine response determined with *in vivo* microdialysis. While the dopamine response to acute cocaine challenge was blunted with continued cocaine self-administration history, the increase observed may have been sufficient to modulate startle. Additionally, cocaine has equipotent affinity for the dopamine and serotonin transporters (Rothman and Baumann, 2003) and selective serotonin receptor agonists alone have been shown to increase the magnitude of the acoustic startle response in rodents (Davis et al., 1986a; Nanry and Tilson, 1989; Svensson and Ahlenius, 1983). A potential role for serotonin is supported by the ineffectiveness of amphetamine to enhance acoustic startle in the present study. Unlike cocaine, amphetamine has very low affinity for the serotonin transporter (Rothman and Baumann, 2003). Amphetamine is a potent dopamine transporter blocker and a releaser of dopamine (Sulzer et al., 1995), but acute administration of amphetamine did not have a significant effect on startle amplitude.

The rodent literature also suggests that the behavioral-stimulant effects of cocaine may be insensitive to changes induced by chronic cocaine self-administration. Rodents trained to self-administer cocaine under a long-access schedule (6hrs/session) escalate their drug intake over time, however they show the same level of cocaine-induced locomotor activity as their short access counterparts (1 hr/session) (Ahmed and

Cador, 2006; Ben-Shahar et al., 2004; Ben-Shahar et al., 2005; Ferrario et al., 2005; Knackstedt and Kalivas, 2007). Moreover, consistent behavioral-stimulant effects were observed even though long access to cocaine was associated with increased motivational effects of cocaine, increased spine density in the nucleus accumbens core, enhanced synaptic activity, and synaptic reorganization (Ben-Shahar et al., 2004; Ferrario et al., 2005; Knackstedt and Kalivas, 2007). Clearly, changes in neurochemistry are not necessarily reflected across all behavioral measures.

There was no escalation in drug intake during the extended access condition. Compared to the limited access condition, drug intake increased markedly when subjects were given three extra hours of drug access. However, drug intake was remarkably stable over the 60 days of extended access. Moreover, drug intake during the first hour of extended access was virtually identical to drug intake during the one-hour limited access condition. This contrasts with a number of rodent studies reporting an escalation in cocaine intake during extended access conditions (Ahmed and Koob, 1998; Ahmed and Koob, 1999; Ben-Shahar et al., 2004; Ferrario et al., 2005; Mantsch et al., 2004). Rodents provided long access to cocaine increase their drug intake significantly within five self-administration sessions and continue to increase thereafter. Furthermore, increases in cocaine intake are seen in rodents within the first hour of long access conditions whereas intake remains unchanged with repeated testing under limited access conditions. In the current study, a similar reinforcement schedule was used in the nonhuman primate model including prolonged self-administration sessions for 60 days providing ample opportunity for an escalated pattern of cocaine intake to

emerge. In fact, a consistent level of cocaine intake was also noted previously in rhesus monkeys provided supplemental drug intake under a FR schedule several days a week. Even with months of supplemental drug intake, there was no indication of an escalated pattern of cocaine intake over time (Henry and Howell, 2009, in press). Taken together, these nonhuman primate studies demonstrate that supplemental drug intake and extended access to cocaine self-administration do not result in escalation of cocaine intake. Nonetheless, the present findings did show a significant effect of cocaine self-administration history on subsequent neurochemical measures which lends support for the utility of the nonhuman primate as a model in which to study the impact of extended cocaine access on neurochemistry.

In summary, rhesus monkeys with a progressive history of cocaine self-administration developed evidence of tolerance to stimulant-induced increases in striatal dopamine as determined with *in vivo* microdialysis. This neuroadaptive response was not associated with an escalated pattern of cocaine intake. In contrast, behavioral measures of stimulant-enhanced acoustic startle responding were unaffected as cocaine exposure increased despite the blunted dopamine response to cocaine. The results of this study demonstrate that an escalated pattern of drug use is not necessary for drug history to alter the responsiveness of the dopamine system. Duration of cocaine access may be a more important variable in determining the neurobiological effects of cocaine self-administration history in nonhuman primates.

CHAPTER V

Acute brain metabolic effects of cocaine in rhesus monkeys with a history of cocaine self-administration

A. Introduction

The reinforcing effect of cocaine is due to a short-lived rise in dopamine levels in the ventral striatum, but repeated cocaine exposure leads to lasting changes in this neurobiological response which is believed to result in the compulsive drug taking behavior involved with drug addiction. The adverse psychological consequences of disruption to dopamine circuitry involve dopamine projections to the prefrontal cortex from the striatum and midbrain. The relevance of the prefrontal cortex to drug reinforcement is in its involvement in the motivation and compulsive drive to obtain and use drugs and in the persistent stimulus-reinforcement relationship that supports continued drug use in spite of the loss of any pleasurable effects (Volkow and Fowler, 2000). Positron emission tomography in cocaine abusers has revealed disruptions in dopamine brain function including decreases in striatal dopamine D2 receptor availability and dopamine release reflecting persistently altered dopamine function compared to control subjects (Volkow et al., 1993; Volkow et al., 1990; Volkow et al., 1997b). A history of cocaine use is also associated with reductions in glucose metabolism in both the striatum and orbitofrontal cortex of detoxified cocaine abusers further indicating changes in dopamine brain function (London et al., 1990; Volkow et al., 1992).

In contrast to the pattern of decreased dopamine brain activation displayed in the withdrawal state, acute psychostimulant administration causes variable changes in brain activity and metabolism in the frontal cortex of cocaine abusers relative to control subjects. In cocaine abusers, the administration of methylphenidate, a dopamine transport blocker, induced increases in dopamine release, activated orbitofrontal cortex, and increased craving for cocaine despite the decrease in dopamine system function (Volkow et al., 1999; Volkow et al., 2005). This increase in brain metabolism as measured by F-18 labeled fluorodeoxyglucose (FDG) PET imaging was determined in subjects that tended to have high dopamine D2 receptor availability whereas subjects with lower D2 receptor availability showed decreases in drug-induced metabolism (Volkow et al., 1999). In a study utilizing fMRI, cocaine-induced euphoria and craving was associated with increases in brain activation throughout the mesolimbic and mesocortical dopamine pathways in cocaine-dependent subjects including the prefrontal cortex and the anterior cingulate (Breiter et al., 1997; Kufahl et al., 2005). Other investigations have also reported increases in cerebral blow flow as measured with PET in the frontal brain regions of cocaine dependent subjects following cocaine administration (Mathew et al., 1996). Taken together, imaging studies demonstrate that addiction to cocaine disrupts prefrontal brain function, a region considered critical for cognitive function, which may compromise control over drug use.

Functional changes in brain activation following cocaine administration have also been demonstrated in nonhuman primates. Cocaine induced dose-dependent changes in functional brain activity in the prefrontal cortex determined by cerebral blood flow

and PET imaging in drug naïve rhesus monkeys (Howell et al., 2001; Howell et al., 2002). Significant increases in blood flow to the prefrontal cortex and anterior cingulate were also induced when cocaine was self-administered during imaging (Howell et at., 2008, submitted). In another set of experiments utilizing fMRI, changes in relative cerebral blood volume (rCBV) were determined in cynomolgus monkeys following an infusion of amphetamine (Jenkins et al., 2004). Amphetamine caused large increases in rCBV in the anterior cingulate, substantia nigra, ventral tegmental area, caudate, putamen, and nucleus accumbens. Collectively, results from imaging studies demonstrate that stimulants induce a unique pattern of brain activity within regions innervated by dopamine.

In the present study, drug naïve rhesus monkeys were given increasing access to cocaine self-administration under a fixed-ratio schedule. The goal was to use this model of increasing drug intake to determine the progression of changes in brain metabolic activity in response to acute stimulant activation as a function of cocaine self-administration history. The advantage of using nonhuman primates is the ability to carefully control cocaine access which allows for better interpretation of metabolic changes associated with chronic cocaine administration. PET imaging with the glucose analog FDG, was used to measure drug-induced changes in brain metabolism first in the drug naïve state, then again following 60 sessions of limited cocaine access (1hr/day) and following 60 sessions of extended cocaine access (6hrs/day). Results highlight progressive increases in metabolic activity as a result of self-administration history throughout the prefrontal cortex, the cingulate cortex, and within the striatum.

B. Materials and Methods

Subjects

Four male and two female adult (6-8 years old) rhesus monkeys (Macaca mulatta) weighing between 9 and 15 kg were used. All monkeys were experimentally naïve at the start of the present experiments. Between daily experimental sessions, monkeys were individually housed, provided access to food daily (Purina Monkey Chow, fresh fruit and vegetables), and ad libitum access to water. Animal use procedures were in strict accordance with the National Institutes of Heatlh's "Guide for the Care and Use of Laboratory Animals" and were approved by the Institutional Animal Care and Use Committee of Emory University.

Self-Administration

Monkeys were surgically implanted with chronic indwelling intravenous catheters. Implantation was done under a combination of Telazol and isoflurane (1.0-2.0%) anesthesia using aseptic techniques. One end of a silicone catheter was inserted into either the femoral or jugular vein and advanced into the vena cava. The distal end of the catheter was routed subcutaneously and attached to a vascular access port (Access Technologies, Skokie, IL) in the interscapular region. Preoperative antibiotic (ceftriaxone) and postoperative analgesic (flunixin meglumine) were administered according to veterinary staff direction. Catheters were flushed daily with 1.0 ml of heparinized (100 U/ml) saline to maintain patency.

During self-administration sessions, each monkey was seated in a commercially available primate chair (Primate Products, Miami, FL). A response panel equipped with

a lever and stimulus lights was attached to the front of the chair. The skin over the vascular access port was cleaned with 95% ethanol and betadine, and then a special right-angle Huber needle (Access Technologies, Skokie, IL) was inserted into the port. The chair was then enclosed in a ventilated, sound-attenuating chamber (Med Associates, St. Albans, VT). Polyvinyl-chloride (PVC) tubing connected the Huber needle to a motor-driven syringe pump (Model PhD 2000; Harvard Apparatus, Holliston, MA) located outside the test chamber containing the drug solution. When activated, the pump delivered a unit dose of 0.1 mg/kg/infusion (-) cocaine HCl (National Institute on Drug Abuse, Bethesda, MD) in a volume of 2.0 ml during a 7.0 second infusion. Med-PC (Med Associates, St. Albans, VT) software systems controlled all experimental events and data collection.

Each monkey was trained on a fixed-ratio (FR) schedule of drug self-administration. Each test session began with the presentation of a red stimulus light. The completion of a FR 20 response requirement changed the stimulus light from red to white for 15 seconds and initiated a 0.1mg/kg infusion of cocaine. The infusion was followed by a 60-second timeout during which stimulus lights were extinguished and responding had no scheduled consequences. At the end of the timeout, the red light was presented again to initiate the next FR component. FR self-administration training began with an FR 1 and increased sequentially when at least 3 infusions were earned in three consecutive testing days at each FR until FR 20 was reached. Only 5 infusions were available for each session during FR self-administration training. It took on average about 30 sessions to complete FR self-administration training. Once the

terminal schedule was reached, the sessions were limited to 1 hour each day, 6 days/week, but no limit was placed on the number of available infusions. Subjects were maintained on this limited access schedule for 60 sessions. The limited access condition was then followed by 60 sessions on an extended access schedule during which the session length was increased to 4 hours each day with a limit of 60 available infusions to prevent adverse effects. Four weeks of withdrawal from cocaine self-administration followed the extended access condition.

Imaging Procedures

[Fluorine 18]-fluorodeoxyglucose (18-FDG) was synthesized from fluorine 18 fluoride produced in a biomedical cyclotron by the proton, neutron reaction on 98% [oxygen 18] enriched water. 18-FDG was used to measure the effects of acute cocaine administration on cerebral glucose metabolism in subjects with a long-term history of cocaine use. Each subject received five PET scans throughout the cocaine self-administration history – two before self-administration in the drug naïve state, one following limited and extended access conditions, and one after 4 weeks of withdrawal. Subjects received an i.m. injection of 18-FDG (15 mCi) immediately followed by an injection of cocaine (1.0 mg/kg) in their home cages and remained undisturbed during the uptake phase. In the drug naïve state, subjects also underwent an additional scan during which they received an i.m. injection of saline instead of cocaine as a baseline measure to which all cocaine-induced brain activation scans were compared. Immediately following the 45-min uptake phase, subjects were taken to the PET scanner for imaging under a combination of Telazol and isoflurane anesthesia. Whole brain, 3D

imaging data were collected with a Siemens Focus 220 microPET scanner located at the Yerkes Imaging Center, a facility dedicated exclusively to nonhuman primate research. The microPET scanner has a 26 cm transaxial field of view and an 8 cm axial field of view. The reconstructed resolution is 1.7 mm in all directions. The acquired data was corrected for randoms and dead-time. Registered PET images were normalized to mean whole brain activity using whole brain ratios of glucose metabolism.

Data Analysis

All analyses were conducted within the Statistical Parametric Mapping (SPM5) toolbox (Wellcome Trust Centre for Neuroimaging, London, UK) within MATLAB (version 7.3; MathWorks, Natick, MA). Within-subject PET images were coregistered using a normalized mutual information algorithm and a six rigid body transformation. We used a standard MRI rhesus monkey brain template that had been previously generated by non-biased averaging of T1-weighted magnetic resonance images (MRIs) with a spatial resolution of 0.6 X 0.6 X1.0mm of sixteen subjects. Coregistered PET images were then normalized to this template using the same algorithm. Spatial smoothing was applied using a 4mm kernel and generating a final pixel size of 1.2 X 1.2 X 2mm. Contrasts were generated between saline treatment and administration of cocaine at each stage of cocaine access using a paired t-test. Analysis and uptake normalization was limited to voxels within gray matter. Scans were normalized for gain differences by proportional scaling. Between-subject differences in global cerebral blood flow and tracer uptake were accounted for using analysis of covariance (ANCOVA) by subject. Effects for each voxel were estimated using a general linear model with a minimum statistical threshold

of $p<0.0167$ and a cluster size of 10 contiguous voxels. The t-maps are from all six subjects for drug naïve, limited, and extended access. Only five subjects were scanned following withdrawal, so the t-maps were recreated comparing drug naïve to withdrawal using only the remaining five subjects.

C. Results

Drug Naïve State

In the drug naïve state cocaine-induced increases in metabolism were localized only in the prefrontal cortex (Figure 5.1). Specifically, the medial prefrontal cortex was activated compared to glucose utilization following an injection of saline (Figure 5.2). There was no significant activation in the striatum (Figure 5.3).

Limited Access Condition

Self-administration training sessions allowed for a maximum of 5 cocaine infusions per session (0.1 mg/kg/infusion). Training generally took about 30 sessions and total cocaine intake for all subjects averaged 9.3±1.3 mg/kg. Following acquisition of self-administration, cocaine became available under limited access conditions. Self-administration sessions were performed 6 days per week for a total of 60 sessions and during limited access, cocaine intake averaged 82.4±13.3 mg/kg for the group. During this early stage of cocaine self-administration under limited access conditions, acute administration of cocaine markedly increased metabolic activity throughout the prefrontal cortex (Figure 5.1). In addition, there was robust activation of the orbitofrontal cortex (Figure 5.2). Recruitment of activity was also noted in the striatum, particularly bilateral activation of the putamen and more lateral regions of the frontal cortex including primary areas of sensorimotor processing (Figures 5.3).

Figure 5.1 Cocaine-induced (1.0 mg/kg, i.m.) metabolic effects along midline sagittal brain sections following each cocaine access condition. Imagines for each condition represent 2mm lateral sections from right (A) midline (B) and left (C). In the drug naïve state (n=6), cocaine-induced increases in metabolic activity was primarily within the medial regions of the prefrontal cortex. As cocaine self-administration history increased from limited to extended access (n=6), increases in metabolic activity became more robust leading to widespread, lateral activity throughout frontal cortex. Following 4 weeks of withdrawal (n=5), metabolic activity in the frontal cortex was far less robust, with only a small region of increased activity within sensorimotor cortex.

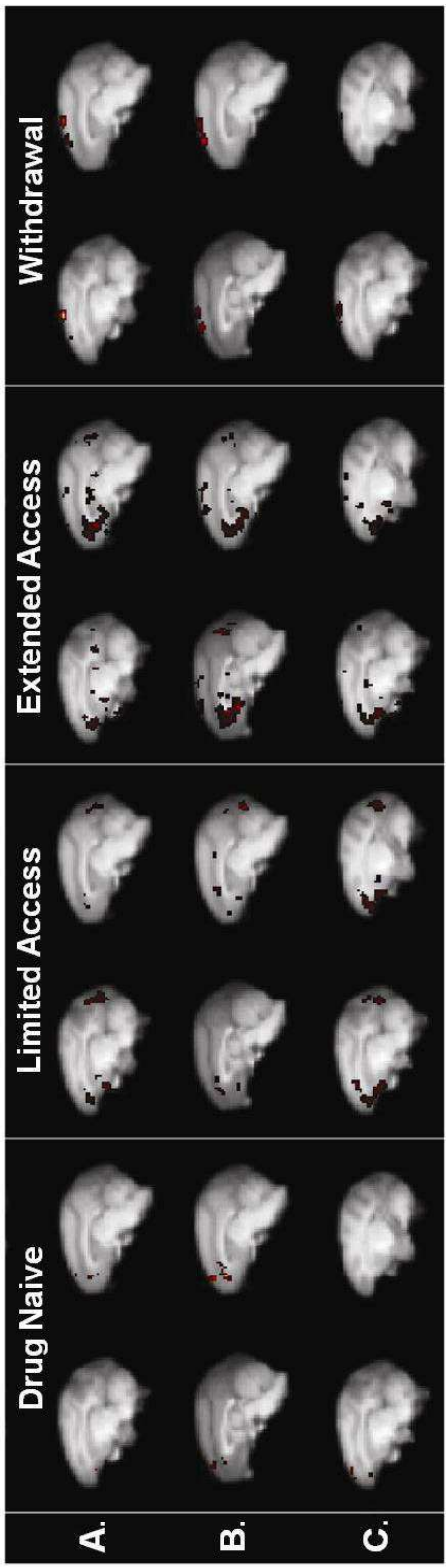

Drug Naive
Limited Access
Extended Access
Withdrawal
A.
B.
C.

Figure 5.2 Cocaine-induced (1.0 mg/kg, i.m.) activation within the prefrontal cortex following each cocaine access condition. Images for each condition represent 2mm coronal sections anterior (left to right) from the first appearance of the orbitofrontal cortex. In the drug naïve state (n=6), cocaine-induced increases in metabolic activity was primarily within the medial regions of the prefrontal cortex. During the limited access condition, increased metabolic activity encompassed large portions of the orbitofrontal cortex. During extended access (n=6), areas of increased metabolic activity included not only the orbitofrontal cortex, but also a large portion of ventral medial cortex and anterior cingulate cortex. Following 4 weeks of withdrawal (n=5), metabolic activity in the frontal cortex was far less robust, with only a small dorsal region of increased activity.

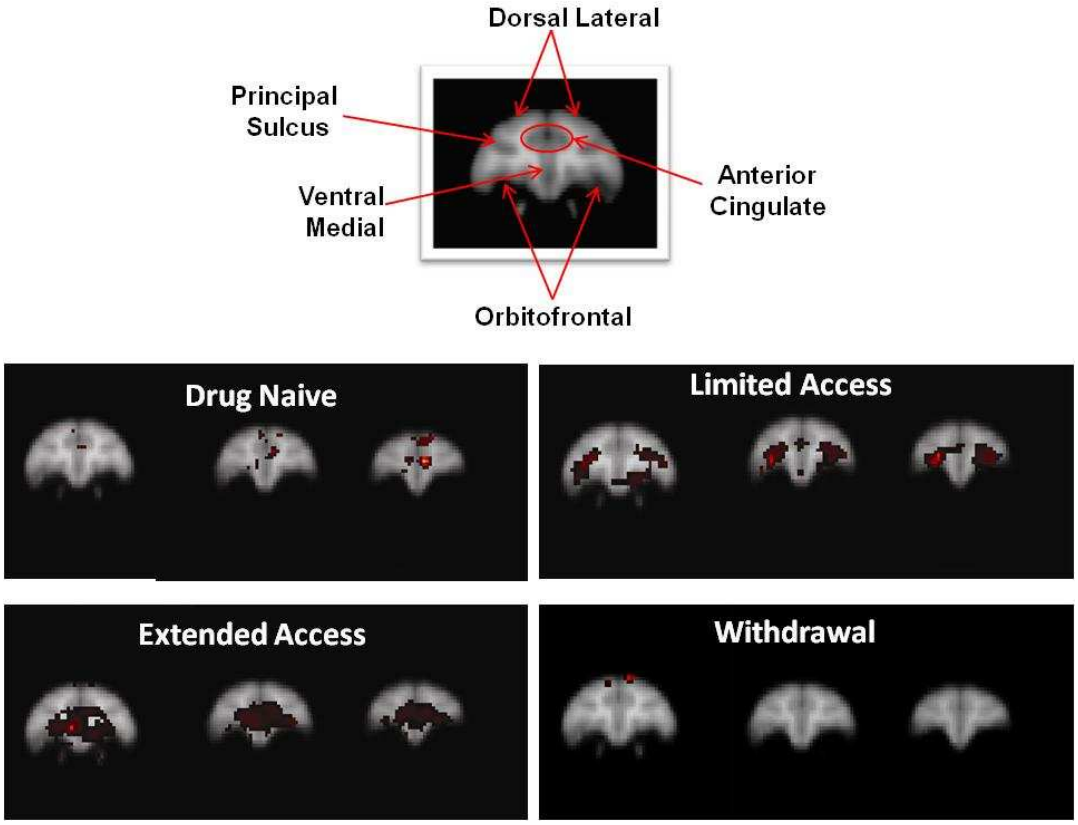
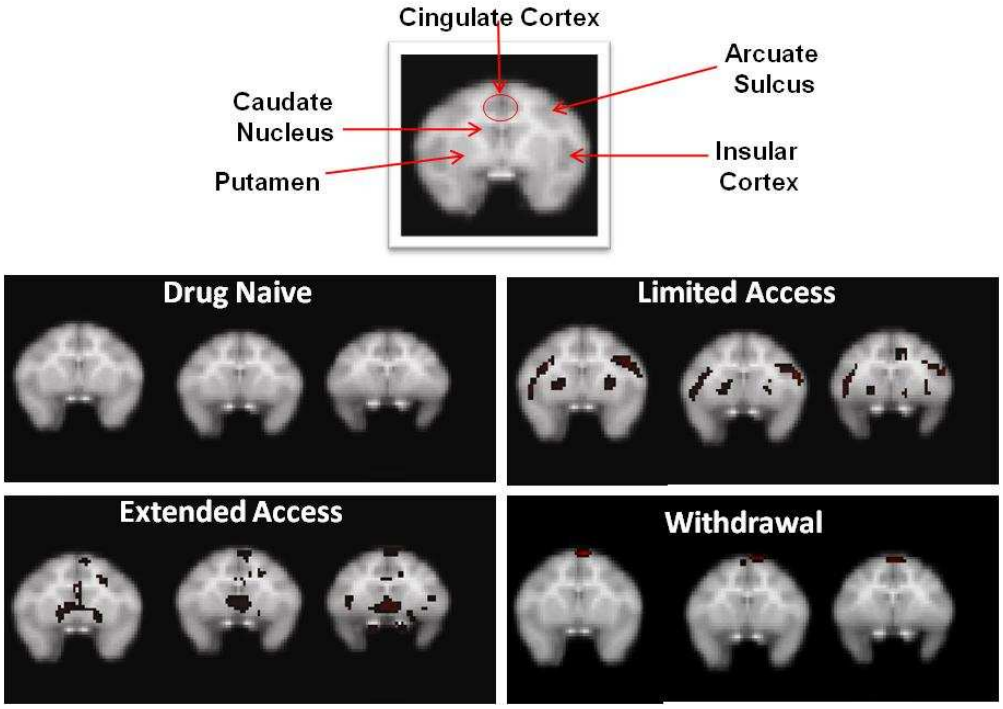

Dorsal Lateral
Principal Sulcus
Anterior Cingulate
Ventral Medial
Orbitofrontal
Drug Naive
Limited Access
Extended Access
Withdrawal

Figure 5.3 Cocaine-induced (1.0 mg/kg, i.m.) activation within the striatum following each cocaine access condition. Images for each condition represent 2mm coronal sections anterior (left to right) from the first appearance of the head of the caudate nucleus. In the drug naïve state (n=6), there was no increase in cocaine-induced metabolic activity within the striatum. During the limited access condition, bilateral increases in metabolic activity were observed in the putamen and along regions of motor cortex ventral to the arcuate sulcus. During extended access (n=6), areas of increased metabolic activity were found to be more medial, with the largest area of activity encompassing more ventral regions of the striatum. Finally, following 4 weeks of withdrawal (n=5), metabolic activity within the striatum was far less robust, with only a small dorsal region of increased activity seen above the cingulate cortex.


Cingulate Cortex
Arcuate Sulcus
Caudate Nucleus
Insular Cortex
Putamen
Drug Naive
Limited Access
Extended Access
Withdrawal

Extended Access Conditions

Total cocaine intake increased considerably above limited access levels when cocaine access was increased to 4hr/day during the extended access condition, averaging 245.0±27.8 mg/kg for the entire group. During the longer duration of cocaine self-administration under extended access conditions, the pattern of metabolic activity following acute administration of cocaine resembled that seen during limited access conditions except activity was now more extensive (Figure 5.1). Cocaine induced even broader activation of the prefrontal cortex to encompass most of the orbitofrontal and medial cortices, as well as, the anterior cingulate cortex. Cocaine also induced a small area of metabolic activity in a more superior area of the frontal cortex involved in primary motor processing (Figures 5.1 & 5.2). In the striatum, a concentrated area of enhanced metabolic activity was now more medial to the putamen, encompassing ventral portions of the striatum including the nucleus accumbens (Figure 5.3).

Drug Withdrawal

Finally, after 4 weeks of drug withdrawal during which subjects remained undisturbed in their homecages, a final set of FDG-PET scans revealed that cocaine no longer induced the same robust pattern of metabolic activity. Surprisingly, there was no activation of the prefrontal cortex or within the striatum (Figure 5.2 & 5.3). Only the small area of superior activation of the frontal cortex first seen during extended access remained activated during withdrawal (Figure 5.1).

D. Discussion

Function changes in glucose metabolism were characterized in six rhesus monkeys with the positron emitting tracer 18-FDG following acute administration of cocaine at various phases of cocaine self-administration history. In a series of within-subject, repeated-measures studies, the FDG-PET method allowed for the visualization of the metabolic effects of cocaine under conditions in which duration of cocaine exposure had been systematically varied. The present metabolic mapping demonstrates that acute administration of cocaine to drug naïve rhesus monkeys induced a discrete pattern of enhanced metabolic activity that was restricted to areas within the medial prefrontal cortex. As cocaine exposure increased from limited to extended access, cocaine-induced activation expanded throughout the cortex. It appears that cocaine exposure resulted in recruitment of cortical regions beyond the prefrontal cortex to encompass regions of sensorimotor processing and the anterior cingulate cortex. Enhanced metabolic activity also emerged in the striatum, first in the putamen then in more ventral medial regions. This array of gradual increases in metabolic activity suggests that the initial subjective effects of cocaine may be mediated by activity in the prefrontal cortex but as history with cocaine self-administration increases more higher-order processes may become affected, such as compulsivity, attentiveness, impulsivity, and emotion as cortical regions become activated in response to acute cocaine administration. Similarly, cortical regions of increased metabolic activity also send projections within the striatum to regions that demonstrated enhanced cocaine-induced metabolic activity following cocaine self-administration. Finally, 4 weeks of drug

withdrawal resulted in recovery of cocaine-induced metabolic activation in both cortical and subcortical regions. Areas of previous enhanced activity were no longer activated in response to cocaine.

A corresponding pattern of metabolic activity has likewise been associated with cocaine dependence in diagnosed cocaine abusers measured for regional brain metabolism with FDG-PET (Volkow et al., 1991). Within a week of cocaine withdrawal, metabolism in the basal ganglia and orbitofrontal cortex was markedly higher in subjects with a history of cocaine abuse than levels measured in normal comparison subjects. This increased activity in the striatum and prefrontal cortex parallels the enhanced metabolic activity seen following limited and extended access conditions in the present study. Similarly, subjects measured within 2-4 weeks of withdrawal did not exhibit significantly different metabolic effects in either the cortex or the striatum when compared to controls as was also noted in the rhesus monkeys following a 4 week withdrawal from cocaine self-administration. Imaging in the withdrawal state is, however, a major caveat when comparing regional metabolic effects in cocaine abusers and the direct metabolic effects of cocaine as determined in this study. The acute effects of cocaine on brain activity have otherwise been investigated using functional MRI imaging methods in cocaine-dependent subjects, and cocaine does in fact induce activation in several regions including the nuclueus accumbens, caudate, putamen, as well as cingulate and prefrontal cortices when compared to infusions of saline in those same subjects (Breiter et al., 1997). Cocaine-dependent subjects also show increases in metabolism in the medial and orbitofrontal cortices following an infusion of

methylphenidate (a stimulant pharmacologically similar to cocaine) compared to normal control subjects (Volkow et al., 2005) when determined with FDG-PET. In both the MRI and FDG-PET studies, administration of drug and subsequent changes in functional activation was positively correlated with drug craving (Breiter et al., 1997; Volkow et al., 2005). Here, we compared cocaine-induced metabolic effects following limited and extended cocaine self-administration to a saline baseline in the drug naïve state. As the history of cocaine self-administration increased, an acute infusion of cocaine induced progressively enhanced metabolic activity within both the prefrontal cortex and striatum. This was in contrast to the more restricted regions of activation in response to cocaine that was noted when the animals were drug naïve.

Additionally, this progressive involvement of cortical and striatal domains as a function of cocaine self-administration history has also been previously demonstrated in cynomolgus monkeys utilizing the 2-[^{14}C]deoxyglucose (2-DG) method. In a series of studies, groups of subjects self-administered cocaine under different access conditions (drug naïve, 5 days, and 3.3 months) and were then evaluated for changes in functional responses to cocaine as assessed by autoradiography (Lyons et al., 1996; Porrino et al., 2002; Porrino et al., 2004). Initial exposures to cocaine resulted in metabolic effects of cocaine contained primarily in the ventral medial regions of the prefrontal cortex compared to saline treated subjects. Changes in activity were also noted in the ventral striatum and small areas of the dorsal striatum. Following chronic exposure to cocaine self-administration, activity intensified within the striatum to encompass both dorsal and ventral regions. The gradual expansion of the metabolic effects of cocaine is similar

to findings from the current study which also showed recruitment of metabolic activity in the cortex and striatum in response to cocaine following a history of cocaine self-administration. The main difference between these studies is that with the 2-DG method, changes in glucose utilization represented a decrease in activity rather than the increases in metabolic activity demonstrated in the current study. This discrepancy may be attributed to differences between postmortem analysis of brain tissue with autoradiography and acquiring images of glucose utilization with PET. Despite the difference in the direction of cocaine-induced effects on brain activity, there is an obvious pattern in the recruitment of cortical and subcortical regions as drug history progresses from the drug naïve state to chronic drug use and withdrawal.

Functional changes in cerebral blood flood determined with PET imaging and 15-O water provides an additional measure to characterize drug-induced changes in brain activity (Howell et al., 2001). Brain activation normalized to global blood flow showed prominent drug-induced activation in the prefrontal cortex of drug naïve rhesus monkeys who were conscious during image acquisition (Howell et al., 2002). Subsequent experiments characterized the effect of cocaine during active self-administration under a fixed-ratio schedule during imaging acquisition (Howell et al., 2008, submitted). The pattern of activation induced by cocaine self-administration differed qualitatively compared to non-continent cocaine administration with the majority of activation localized primarily in the medial region of the prefrontal cortex including the anterior cingulate cortex. In the present study, a history of cocaine self-administration also contributed to qualitative differences observed following acute

cocaine administration compared to the drug naive state. Limited and extended access conditions lead to more intense and widespread metabolic effects in the prefrontal cortex that also included anterior cingulate cortex. Cocaine-induced increases in metabolic activity within the prefrontal cortex of subjects with a history of drug use are relevant to the compulsive and obsessive behaviors that are key symptoms of cocaine addiction in humans.

In summary, the present study is the first to use functional brain imaging to document acute cocaine-induced changes in metabolic activity throughout the cocaine self-administration history of nonhuman primates. The longitudinal design of this study incorporated carefully controlled drug access conditions that were uniform across subjects which allowed for the characterization of progressive metabolic changes within the same subjects. In the drug naïve state, cocaine induced robust activation that was restricted to the prefrontal cortex. Following limited and extended access conditions, the pattern of metabolic activity expanded to include more regions of the frontal cortex and induced activity within both the dorsal and ventral striatum. The metabolic effects of cocaine were far less robust following just a brief period of drug withdrawal. The results obtained are consistent with the effects of cocaine history on brain activity in diagnosed cocaine abusers (Volkow et al., 1999). Moreover, there is recent evidence that imaging active drug use in rhesus monkeys produces a similar pattern of cortical activation (Howell et al., 2008, submitted). Taken together, it is apparent that a history of cocaine self-administration increases sensitivity to cocaine-induced metabolic effects in brain regions that are innervated by dopamine terminals. These brain regions create

a network of connectivity that is associated with the reinforcing and subjective effects of cocaine. The pattern of activity characterized here in the rhesus monkey could lead to the creation of metabolic profiles of cocaine abuse that can be identified with FDG-PET and possibly lead to individualized treatment for human cocaine addicts.

CHAPTER VI

Conclusions

The development of cocaine addiction in humans involves a progressive increase in cocaine intake which can result in altered dopamine system function. Changes in the responsiveness of the dopamine system may underlie the undesirable behavioral effects associated with compulsive drug use and dependence. Abstinent cocaine-dependent subjects experience intense craving and desire to procure drug but experience diminished cocaine-induced euphoria and high with continued drug use which results in even greater consumption. Undeniably, the inability to control drug intake and susceptibility to relapse during periods of abstinence makes treatment of cocaine addiction difficult. In this study, rhesus monkeys were provided increasing access to cocaine self-administration then evaluated for changes in drug-seeking behavior, dopamine neurochemistry, acoustic startle, and global brain activation as function of drug history. The objective of this study was to determine the consequences of cocaine self-administration history on subsequent behavioral and neurochemical effects of cocaine. A major strength of the study was the longitudinal, within-subject design that allowed for repeated measures throughout the cocaine self-administration history with multiple dependent measures. After evaluating cocaine-induced reinstatement, microdialysis, acoustic startle, and FDG-PET were used to identify neuroadaptive mechanisms that underlie altered sensitivity to cocaine as a function of drug history.

Taken together, these experiments indicated that a history of long-term cocaine self-administration produced substantial effects on dopamine neurochemistry and cerebral metabolism. These effects, however, were not associated with changes in drug-induced reinstatement or behavioral reactivity.

For the first group of subjects, the aim was to determine how drug-induced reinstatement as a model of relapse changed as cocaine self-administration progressed from limited (recreational) to extended (compulsive) drug access. Specifically, the goal was to determine if the propensity to relapse increased with cocaine exposure. Subjects received extensive self-administration training on a second-order schedule for over 5-6 months in order to establish stable behavioral baselines. Once responding stabilized, subjects self-administered cocaine under a second-order schedule for three months before supplemental access under a fixed-ratio schedule was added to increase cocaine exposure for an additional three months. Each month of limited access resulted in a similar number of infusions and consistent rates of responding. Even following supplemental drug intake during the extended access condition, the number of infusions and rates of responding during the preceding second-order schedule remained consistent and comparable to that seen during the limited access condition. The addition of the FR schedule allowed subjects to increase their cocaine intake without the limitations imposed by the second-order schedule. The increase in intake, however, did not result in an escalated pattern of responding. There was instead a progressive reduction in response rates during the FR schedule which did not in turn affect daily cocaine intake. Reinstatement determinations conducted once a month during each

access condition measured magnitude and persistence of the reinstatement effect. Robust, consistent peak reinstatement effects indicated that the behavior maintained under the second-order schedule was remarkably stable regardless of drug intake. Even with increased cocaine intake during the additional FR component, reinstatement effects remained comparable to previous determinations. Hence, the long history of operant conditioning and the stability of behavior maintained by the second-order schedule likely contributed to the consistency of subsequent drug-induced reinstatement.

Retrospective clinical evidence suggests that stimuli used to induce reinstatement in animals (drug reexposure, drug cues, and stress) are relevant to drug relapse in humans (Epstein et al. 2006). It was therefore hypothesized that, as reported in humans, there would be an enhancement of the reinstatement effect in rhesus monkeys as a result of increased access to cocaine self-administration. It is important to note, however, that there are differences in the mechanisms that underlie reinstatement in the animal model and criteria that induce relapse in the human condition. First, the reasons human drug abusers choose or are forced into abstinence result from a number of motivational or environmental circumstances, such as health, family, or legal issues. With the reinstatement model employed in the rhesus monkeys cocaine self-administration behavior was actually extinguished by eliminating response-contingent drug delivery. Secondly, relapse typically involves a conscious decision to resume drug taking regardless of the factors that may have initiated the behavior. In the laboratory, drug priming injections were given non-contingently to reinstate drug

seeking behavior. The ability to control and manipulate extinction and reinstatement variables is what makes the paradigm such a reliable tool in which to evaluate the abuse liability of drugs in animals. The caveat, however, is that the reinstatement effect was found to be insensitive to increases in cocaine intake. It was concluded that the stability of this effect may be attributed to the extensive behavioral history of the subjects before the initiation of reinstatement determinations. Consequently, rather than characterizing reinstatement in terms of cocaine self-administration history as originally intended for this aim of the study, the utility of reinstatement has instead been established as a consistent, stable behavioral model for which future within-subject, repeated measures studies in nonhuman primates may be an invaluable asset.

The second-order schedule of the extended access condition that was used with the first group of subjects was not designed to promote an escalating pattern of cocaine intake, so only the simple fixed-ratio schedule was used with the second group of subjects to provide an opportunity to examine the effect of cocaine self-administration history on drug intake. Following acquisition of self-administration, cocaine was available for 1 hr/day for 10 weeks. As expected, responding under this limited access condition was steady and consistent. When cocaine became available for 4 hr/day under the extended access condition, total daily drug intake increased markedly, however, cocaine intake did not escalate over the course of the 10 week access period. Instead, cocaine intake was stable with a similar number of infusions earned during each hour of access.

Despite the fact that an escalating pattern of cocaine intake did not emerge with extended cocaine access, several interesting observations were made while evaluating the effect this extensive cocaine history had on dopamine system function. The first measure was to determine the effects of cocaine self-administration history on drug-induced increases in striatal dopamine. Measures of dopamine with *in vivo* microdialysis demonstrated that acute administration of the indirect dopamine agonists, cocaine and amphetamine, resulted in diminished increases in neurotransmitter levels following long-term cocaine exposure. Tolerance to drug-induced increases in dopamine as a function of cocaine history is consistent with the human literature that proposes a hypofunctional dopamine system that develops in cocaine-dependent subjects. In fact, a downward trend in basal dopamine levels was also observed. The second measure was to characterize the acoustic startle response at each stage of cocaine access. Acoustic startle is an innate reflex elicited by a sudden and intense auditory stimulus. The reflex is mediated by a simple 3-synapse circuit and is sensitive to dopaminergic modulation by both direct and indirect dopamine agonists. Moreover, chronic cocaine users have been shown to have diminished responses to startle stimuli presumably as a consequence of an extensive drug taking history (Efferen et al., 2000). It was therefore hypothesized that the amplitude of the startle response would be similarly decreased as drug naïve rhesus monkeys were subsequently allowed to self-administer cocaine. Additionally, stimulant-induced enhancement of the startle response would also be diminished as a function of cocaine self-administration history and this effect would parallel the decreases in drug-induced dopamine levels

determined with microdialysis. However, there was no effect of drug history on subsequent measures of acoustic startle. Rather, robust yet consistent acoustic startle responses were evident across all determinations. Finally, the second group of subjects underwent repeated FDG-PET imaging studies to characterize progressive changes in cocaine-induced brain metabolic effects as a function of previous cocaine self-administration history. In the drug naïve state, significant increases in brain activity were restricted to the ventral medial regions of the prefrontal cortex. As cocaine exposure increased from limited to extended access, cocaine-induced activation expanded throughout the cortex and emerged in the striatum. It appears that cocaine exposure resulted in recruitment of cortical regions beyond the prefrontal region to encompass the entire frontal cortex including nearly the entire anterior cingulate cortex and induced activation within the dorsal and ventral striatum. Conversely, cocaine-induced activation was far less robust following withdrawal with no increases in activity within the prefrontal cortex or the striatum. Taken together, the *in vivo* microdialysis, acoustic startle, and FDG-PET imaging experiments were designed to evaluate changes in the tone and responsiveness of the dopamine system as a result of cocaine self-administration history. We found that extensive cocaine exposure decreases basal dopamine and metabolite levels and diminishes drug-induced increases in dopamine levels, as well as, enhances the metabolic effects of cocaine throughout the cortex. These changes were not associated with an escalated pattern of cocaine intake or a change in behavioral reactivity as determined by acoustic startle.

With cocaine dependence, tolerance develops to the cocaine high presumably due to neuroadaptions within the dopamine system. Cocaine abusers have reported increasing their dose in an attempt to recapture, intensify, and prolong euphoria. Throughout the present study, only one dose of cocaine was used to maintain cocaine self-administration to allow for uniformity of drug self-administration across subjects. Although the dose provided in the present study has been shown to maintain reliable self-administration in rhesus monkeys, the dose may have been too low to produce an escalated pattern of intake. In 2004, Mantsch et al. compared rodents with extended access to high versus low doses of cocaine. During self-administration, rodents provided access to the lower dose of cocaine maintained a consistent level of daily cocaine intake (mg/day) whereas rodents that had access to the higher drug dose escalated drug intake within five days of the two week access period. An increase in drug dose may have resulted in the progressively increased cocaine intake seen in the rodent model. Measures of dopamine system function with microdialysis and FDG-PET imaging throughout each period of cocaine access have shown that regardless of the presence of escalation in drug intake that long-term cocaine exposure has a profound effect on the ability of cocaine to modulate dopamine neurotransmission and brain metabolic activity. Similarly, during the reinstatement paradigm, the maintenance dose of cocaine was the only dose tested during repeated determinations. Creating a dose-response curve may have better shown shifts in the magnitude of the reinstatement effect. Repeated determinations with one drug dose in this study were designed to clearly demonstrate the ability of cocaine to reinstate extinguished responding.

Tolerance or potentiation of responding would indicate a shift in the effectiveness of that dose. Since there was no change in responding to the maintenance dose during repeated reinstatement determinations, it is possible that the dose may have been at the peak of the dose-response curve for cocaine-induced reinstatement and could not be enhanced further. This however has been shown not to be the case in cocaine-induced reinstatement studies in nonhuman primates (Banks et al. 2007; Platt et al. 2007). Higher doses of cocaine will produce enhanced reinstatement effects in nonhuman primates beyond that observed for the 0.1 mg/kg dose of cocaine used in this study.

In summary, the present study demonstrates that although self-administration and stimulant-induced behavioral effects of cocaine are unchanged with increasing access to cocaine, tolerance to cocaine-induced enhancement of dopamine developed over time. This decrease in dopaminergic response to drug was complemented by a substantial increase in cocaine-induced brain metabolic effects. These studies therefore created a profile of brain function in rhesus monkeys with a prolonged history of drug self-administration. The characteristics described here may extend findings from the animal model to a better understanding of the human condition and lead to more individualized pharmacological treatment for cocaine abusers.

-- *Future Directions*

Measures of cocaine-induced reinstatement were determined under a second-order schedule of drug reinforcement in the first group of rhesus monkeys. Although the reinstatement effect was robust, the behavior maintained on this schedule proved to be too stable as reinstatement effects were unchanged as cocaine self-administration history progressed. Reevaluating reinstatement under the fixed ratio schedule of drug reinforcement that was used with the second group of subjects may be better for determining changes in reinstatement behavior as a function of drug history. Reinstatement under the fixed ratio schedule would also be consistent with methods used in the rodent literature in which persistent, dose-dependent increases in drug-seeking behavior were obtained in rodents provided extended access to cocaine under a similar reinforcement schedule (Mantsch et al., 2004; Ahmed and Cador, 2006; Knackstedt and Kalivas, 2007). I hypothesize that cocaine-induced reinstatement effects would be enhanced as cocaine self-administration history progressed from limited and extended access under the fixed ratio schedule.

The acoustic startle reflex has been shown to be diminished in cocaine abusers as compared to control subjects possibly as a consequence to changes in dopamine system function (Efferen et al., 2000). However, the acoustic startle reflex in rhesus monkeys was ineffective as a measure of dopamine system responsiveness over time. Both baseline and drug-induced increases in the startle response were consistent even following extended access to cocaine self-administration. Other behavioral processes that are more related to changes noted in cocaine abusers such as obsessive,

compulsive drug preoccupation or increased impulsive actions could also be measured in rhesus monkeys as a function of drug history. For example, the delay discounting task is a measure of impulsive choice used in human and animal subjects (Ho et al., 1999). Subjects are presented with two choices that have different reward values and the delay task evaluates the manner in which small immediate rewards are chosen over larger rewards that follow a delay of varying length. In most delay tasks using animal models, food pellets are used as the reward. Training rhesus monkeys to lever press for food rewards would be relatively easy which would make the delay discounting task a quick and simple measure of changes in impulsivity as a function of cocaine self-administration history in the current model. Rodent studies have shown that previous cocaine exposure increases the number of impulsive choices and increases sensitivity to changes in the size and timing of the reward (Roesch et al., 2007; Simon et al., 2007). Therefore, I would hypothesis an increase in impulsive behavior in the rhesus monkeys as function of cocaine self-administration history. This hypothesis would corroborate with the imaging studies that showed an enhancement of cocaine-induced metabolic activity in the frontal brain regions as cocaine self-administration history progressed. The delay discounting task would also provide an additional behavioral profile that can be directly correlated to the human condition.

Finally, I would maximize the utility of PET imaging to further identify changes in dopamine system function at each phase of cocaine access. Data from PET studies have shown that cocaine abusers have decreased dopamine release in the striatum, reductions in D_2 receptor availability, and disruptions in activity in frontal brain regions

(Volkow et al., 2007). Additional PET imaging studies in the current rhesus monkey study could use [^{11}C] raclopride, a dopamine D_2 receptor agonist that is sensitive to competition with endogenous dopamine (Volkow et al., 1997). [^{11}C] raclopride binding would be used to determine the level of dopamine receptor availability under normal conditions and be used to measure cocaine-induced increases in dopamine as a function of drug history. Dopamine levels would then be compared to saline levels determined in the drug naïve state. Because cocaine addiction is believed to result from decreased dopamine system function in the brain, I would hypothesize that both dopamine receptor levels and competition at the receptor following acute cocaine administration would be progressively diminished as a function of cocaine self-administration history. This hypothesis would be consistent with microdialysis data from this study that showed tolerance to cocaine-induced increases in dopamine in the striatum with increased cocaine exposure.

Altogether, these proposed studies would continue to evaluate the progressive behavioral and neurobiological effects of cocaine on the brain of nonhuman primates throughout their drug history and provide insight into the human process from recreation drug use to cocaine addiction.

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