The Effects of Chronic Cocaine Self-Administration on Cognition and Brain Metabolism

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Cocaine users display a wide range of cognitive impairments and dysfunction in brain regions important for cognition. Treatment outcome is dependent on cognitive ability. It is important to understand these deficits and the underlying neurobiology. The first aim was to determine whether cocaine is sufficient to cause cognitive deficits, and if so, to determine the specificity of these cognitive deficits. Secondly, we assessed cerebral metabolic function after a drug free period. We used rhesus monkeys in a longitudinal study in which 14 animals were characterized prior to assignment to matched control (n=6) and cocaine self-administration (n=8) groups. Self-administration took place daily over nine months during which, visual and auditory contextual cues were presented. Weekly cognitive assessments were conducted following a 72 hour drug free period. We employed a stimulus discrimination/reversal task to evaluate associative learning and cognitive flexibility and the delayed match-to-sample task to assess visual working memory. In the cocaine group, we observed significant impairments in reversal performance and visual working memory after self-administration compared to controls. We examined distractibility in both groups, using brief novel distractors. Subsequently, an identical approach was used for exposure to a distractor previously associated with cocaine (experimental group), or water in the control group. In the cocaine group, stimulus discrimination was unaffected by either distractor, whereas reversal performance was disrupted by both the novel and appetitive distractors relative to baseline. Visual working memory was impaired in the cocaine group in the presence of the novel distractor. The control group's performance was unaffected by the presentation of either

distractor. Monkeys were drug free for 20 months prior to assessment of metabolic function using ^{18}F-2-deoxyfluoro-D-glucose positron emission tomography. The cocaine group showed greater cerebellar activity than the control group while performing a visual working memory task (relative to control task). This work confirms that cocaine self-administration is sufficient to cause long lasting cognitive impairments in cognitive control, visual working memory and attention. These data also suggest that cocaine exposure alters cerebellar function, but future studies will need to be conducted to confirm that cocaine exposure is the direct cause of the metabolic differences observed.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

First, I would like to thank my parents April and Herbert Pruitt for their continued support and unconditional love. I would like to thank my Pittsburgh friends Margurite Matthews, Camille Linton, Damon Young for keeping me sane and offering a balance. Thank you Travis Carless for your support and friendship at a time when I needed it most. You will forever be special in my life. I would like to thank my labmates Kate Gurnsey, Jessica Nicolazzo, and Carol Ernhard for their support and help with conducting the experiments. Without you all none of these projects would have been completed. Thank you Andreea Bostan for always taking the time to help me edit my work, give constructive feedback and for being such a wonderful and supporting friend. A special thanks to Dr. Hank Jedema, PhD for having the patience to train me in statistics, pushing me to try different methods of analysis and offering constructive criticism. Lastly, I would like to thank my wonderful advisor Dr. Charles Bradberry, PhD for believing in me, supporting me and aiding in my growth as a scientist.

1.0 INTRODUCTION

Cocaine-related biomedical and psychosocial problems remain a public health problem in the United States. Cocaine addiction is a chronically relapsing disorder characterized by compulsive drug seeking despite the adverse consequences (Volkow and Li 2004); (Mendelson, Mello et al. 1990). Criteria for cocaine dependence include persistent drug use at a cost of other important life activities (work, school, family), inability to control or cut down on drug use despite an explicit desire to do so, and using larger amounts of drugs over longer periods than intended (for complete list, see section 1.1.2 DSM IV criteria for drug abuse). Recently, much of what we knew about cocaine addiction was centered on the rewarding and reinforcing properties of cocaine (see section 1.2 for discussion of pharmacological effects and mechanisms of drug action). Over the last 20 years, there has been a growing shift in the addiction field from studying the rewarding properties of drugs of abuse to cultivating a better understanding of the cognitive deficits associated with drug dependence and the underlying mechanisms that may be contributing to the deficits. Cocaine users suffer from a wide range of cognitive deficits and these deficits predict treatment outcome (see section 1.2.5.1 on cognitive impairments). Functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) have been used to explore alteration in function, structure and metabolism in cocaine abusers, specifically in brain regions that underlie executive function (see section 1.2.5.2 on cortical alterations in cocaine users). What remains unclear is whether these impairments in cognition

and alterations in brain metabolism are a result of cocaine exposure alone as opposed to predispositions, genetic components or environmental compenents. The overall aim of this thesis dissertation was to conduct a longitudinal study in cocaine chronically self-administering non-human primates to better understand the direct effects of cocaine on specific cognitive domains and brain metabolic function.

This introduction begins by giving an overview of cocaine abuse and treatment options. Dopamine and the dopaminergic pathways are discussed, as they are thought to underlie not only the rewarding properties of cocaine, but also the impairments in cognition and cortical function. Following this, there is an overview of the cognitive deficits and cortical impairments observed in cocaine users. This discussion ends with an outline of limitations found in the human literature and proposes an argument for using non-human primates to better understand cocaine addiction. This introduction is not only meant to provide the background information necessary to understand the importance of my thesis work, but also illustrate advances in the addiction field.

1.1 COCAINE ADDICTION OVERVIEW

1.1.1 DSM Criteria for Drugs of Abuse

Cocaine abuse and dependence are defined as follows (Taken directly from American Psychiatric Association. 1994. Diagnostic and Statistical Manual of Mental Disorders: DSM-IV. Washington D.C.: American Psychiatric Association. pp. 181-183):

Substance abuse is defined as a maladaptive pattern of substance use leading to clinically significant impairment or distress as manifested by one (or more) of the following, occurring within a 12-month period:

1. Recurrent substance use resulting in a failure to fulfill major role obligations at work, school, or home (such as repeated absences or poor work performance related to substance use; substance-related absences, suspensions, or expulsions from school; or neglect of children or household).

2. Recurrent substance use in situations in which it is physically hazardous (such as driving an automobile or operating a machine when impaired by substance use).

3. Recurrent substance-related legal problems (such as arrests for substance related disorderly conduct).

4. Continued substance use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of the substance (for example, arguments with spouse about consequences of intoxication and physical fights).

1.1.2 The Significance of Studying Cocaine Addiction and Treatment Options

Cocaine abuse is a major public health problem. In 2009, 4.8 million Americans age 12 and older had abused cocaine in any form and 1.0 million had abused crack at least once in the year prior to being surveyed. *Source:* National Survey on Drug Use and Health *(Substance Abuse and Mental Health Administration Web Site).* In 2009, almost one million visits to the ER involved an illicit drug, either alone or in combination with other types of drugs. Cocaine use resulted in the highest number of visits among all illicit drugs

(http://www.drugabuse.gov/publications/drugfacts/drug-related-hospital-emergency-room-visits).

Moreover, there is no adequate therapy for treating cocaine addiction. Attrition rates in clinical trials of treatment for cocaine dependence are high and often in the 75% range (Sayre, Schmitz et al. 2002). Pharmacological interventions can be classified as those that interfere with the reinforcing effects of drugs of abuse or those that compensate for the adaptations that predate or develop after long-term use. Most studies looking to discover medication for cocaine addiction are preliminary. Many of the new promising drugs to treat cocaine addiction target GABA (Kampman, Pettinati et al. 2004); (Raby and Coomaraswamy 2004); (Gonzalez, Sevarino et al. 2003). Currently, there are no FDA-approved medications for treating cocaine addiction and so finding targets (neurotransmitter systems or brain circuitry) for developing medication to treat cocaine is of great importance. Importantly, cognitive function is a factor in treatment retention in cocaine dependence (Aharonovich, Hasin et al. 2006); (Schmitz, Mooney et al. 2009). Aharonovich et al. (2006) examined the relationship between baseline performance on a cognitive assessment battery and subsequent treatment response in cocaine abusing subjects (Aharonovich, Hasin et al. 2006). Subjects who dropped from the treatment performed significantly worse on cognitive assessment than those that were able to complete the 12 week treatment. In a similar study, Green and colleagues (2009) examined the relationship between treatment outcome and baseline performance on a measure of decision making in cocaine using subjects. They report that cocaine abusing subjects who had better decision making as measured by the Iowa Gambling Task were more likely to show a reduction in cocaine positive urine, a measure of cocaine use, when treated with citalopram (Green, Moeller et al. 2009). The latter study suggests that both behavioral-cognitive and pharmacological treatments are best for

treating drug addiction. Behavioral interventions, particularly, cognitive-behavioral therapy, have been shown to be effective for decreasing cocaine use and preventing relapse. These therapies have been aimed at strengthening inhibitory control, providing alternative reinforcers and strengthening executive functions. Treatments must be tailored to the individual patient's needs in order to optimize outcomes. This often involves a combination of treatment, social supports, and other services. Understanding the effects of chronic cocaine exposure and the neurobiology of cognitive deficits resulting from chronic cocaine use may have significant clinical relevance because it may provide insight for developing pharmacological treatments as well as behavioral therapy for treating cocaine addiction. Dual approaches that pair cognitive behavioral strategies with medications to compensate or counteract the neurobiological changes induced by chronic drug exposure might provide more robust and longer lasting treatment options for cocaine addiction..

1.2 COCAINE

1.2.1 Mechanisms of Action

Cocaine is a psychomotor stimulant characterized by its ability to increase alertness, heighten arousal and cause behavioral excitement. Most of the behavioral and physiological actions of cocaine can be explained by its ability to block reuptake of dopamine, serotonin and norepinephrine. Cocaine binds to the transporters of these neurotransmitters, thus blocking reuptake and increasing their concentrations in the synaptic cleft. The blockade of the dopamine transporter appears to be the most important for cocaine's stimulating, reinforcing, and addictive

properties. It is now widely accepted that the rewarding effects of drugs of abuse are due to its ability to bind to the dopamine transporter in the ventral tegmentum and increase dopamine particularly in the nucleus accumbens (Di Chiara and Imperato 1988); (Ritz et al., 1987); (Madras, Fahey et al. 1989). Outside of dopamine's involvement in reward, dopamine is important for executive cognitive functions that are disrupted in cocaine abusers (Aalto, Bruck et al. 2005); (Sawaguchi and Goldman-Rakic 1991); (Bolla, Cadet et al. 1998); (Nieoullon 2002); (Floresco and Magyar 2006).

1.2.2 Dopamine's involvement in reward processing and cognition

Dopamine is manufactured in nerve cell bodies located within the ventral tegmental area (VTA) and is released in the nucleus accumbens and the prefrontal cortex. Dopamine is important not only in mediating the immediate pleasurable aspects of natural rewards, but also in mediating the arousal effects that are predictive of impending rewards. Much of what we initially knew about dopamine came from recordings of single dopaminergic neurons in alert monkeys while they received rewards, performed by Wolfram Schultz and his colleagues. When the monkeys were presented with various appetitive stimuli such as juice, dopaminergic neurons responded with short bursts of activity. After repeated pairing of visual and auditory cues followed by reward, the time of phasic activation of the dopaminergic neurons switched from changing after the reward to changing in the presence of a cue. These data make the argument that dopaminergic neurons encode expectations about the external environment. Dopamine encodes the prediction of reward, learning of reward associations and incentive value of approach behavior (for review see Schultz, Tremblay et al. 2000).

Dopamine signals through five receptor subtypes: D1, D2, D3, D4, and D5. D1 and D2 are the most common types found in the brain. Both types are found in large numbers in the striatum (ventral and dorsal). D1 and D2 have opposite effects on second-messenger substance cyclic adenosine monophosphate (cAMP) (Kebabian and Calne 1979). D1 receptors stimulate, whereas D2 receptors inhibit postsynaptic activity. The D2 receptor functions not only post-synaptically, but pre-synaptically as an autoreceptor (removing DA from the synapse). In cocaine exposed subjects, the behavioral effects of dopamine are proposed to be mediated by D1 and D2 receptors, which are present in the nucleus accumbens, caudate and putamen, amygdala and prefrontal cortex (Nader, Daunais et al. 2002). Differential adaptation in the D1 versus D2 signaling pathway may underlie neuroplastic changes resulting from chronic cocaine exposure.

Dopamine has also been shown to be important in cognition. Via pharmacological manipulations, scientists have been able to assess which receptors play important roles in executive function (decision making, cognitive flexibility, working memory, attention, inhibitory control). Dopamine is important for reversal learning (Mehta, Swainson et al. 2001); (Kruzich and Grandy 2004) and working memory (Sawaguchi and Goldman-Rakic 1991). Local administration of D1 receptor antagonist into the dorsolateral prefrontal cortex induced pronounced deficits on a working memory task (Sawaguchi and Goldman-Rakic 1991). Reversal learning is impaired following infusions of a D2 agonist into the rat medial striatum (Goto and Grace 2005). Systemic infusions of D2 antagonist impaired reversal learning in drug free monkeys (Lee, Groman et al. 2007). From these studies, we can postulate that the dopamine function is important for cognitive flexibility and working memory. Cocaine alters dopamine and this may result in the impaired cognitive function observed in cocaine users.

1.2.3 Dopaminergic Pathways

Dopamine (DA) modulates both cortical and subcortical areas via the mesolimbic and mesocortical pathways. Initial work on DA's role in addiction and drug reward focused on the mesolimbic DA pathway. The influence of cocaine on DA levels within the mesolimbic system has been demonstrated to be responsible for the powerful reinforcing effects of the drug (For review see (Volkow, Fowler et al. 2002). The mesolimbic dopamine pathway originates in the VTA and projects to striatum, specifically the nucleus accumbens (NAc) where it terminates on medium spiny cells that contain GABA. The mesolimbic pathway, which includes the NAc, amygdala, and hippocampus, is relevant for drug reward and for drug-related memories and conditioned responses (Koob and Bloom 1988). Specifically, dopamine in this particular pathway has been implicated in the motivation costs required to complete a task that demands high-level effort; goal seeking and cost benefit evaluation of behavior decision making (Flagel, Clark et al. 2011). Recent work recognizes the importance of cognition in treating addiction and involvement of the mesocortical DA pathway. The mesocortical pathway originates in the VTA and projects to frontal lobe regions. Dopamine axons project to prefrontal cortex in primates and other animals (Williams and Goldman-Rakic 1993); (Smiley and Goldman-Rakic 1993). The mesocortical pathway, which includes the cingulate gyrus (CG) and orbitofrontal cortex (OFC) may be relevant for the compulsive drug administration and poor inhibitory control. Functional connectivity studies conducted in cocaine users show a decrease in connectivity between midbrain and cortical regions (Gu, Salmeron et al. 2010); (Tomasi, Volkow et al. 2010). This suggests that alterations in mesocortical and mesolimbic pathways may be involved in the impairments in cognition observed in cocaine users. There is also evidence suggesting neuroadaptations following cocaine exposure in these regions (for review see (Volkow, Fowler

et al. 2007); (Volkow, Fowler et al. 2009)). These neuroadaptations in the mesocortical and mesolimbic pathways are thought to contribute to addiction.

1.2.4 Effects of Cocaine on Dopamine Transmission

Chronic cocaine use among human addicts has been associated with neuroadaptations in dopaminegic pathways (Malison, Best et al. 1998); (Volkow, Wang et al. 1997); (White and Kalivas 1998). Changes in D2 receptor density have drawn the most attention. Cocaine abusers show significant reductions in DA D2 receptors in the striatum compared with controls (Volkow, Fowler et al. 1993); (Martinez, Broft et al. 2004). This reduction in DA D2 receptors has been reported to last for 3-4 months after cessation of drug use (Volkow, Fowler et al. 1993). Reductions in DA D2 receptors in the striatum are associated with reduced cortical metabolism (Volkow, Fowler et al. 1993). Similar results have been found in non-human primates. Chronic cocaine self-administration in non-human primates resulted in decreased D2 receptors (Moore, Vinsant et al. 1998); (Czoty, Morgan et al. 2004). Nader and colleagues (2006) examined baseline DA D2 receptors in non-human primates prior to self-administration, after 3 months of self-administration and after 12 months of being drug free. D2 receptor availability decreased by 15-20% within 1 week of initiating self-administration and remained reduced by approximately 20% during the 1 year of exposure (Nader, Morgan et al. 2006). Long-term reductions in D2 receptor binding were observed, with decreases persisting for up to 1 year of abstinence in some monkeys. Three out of five monkeys had 100% recovery of their D2 receptors and two did not see any recovery even after 12 months of being drug free. Taken together, these data suggest that chronic cocaine exposure alters DA D2 receptor expression in striatal regions. This alteration in striatal DA may result in altered DA neurotransmission in cortical areas that are important for

executive functions. The alteration in cortical DA may also underlie the cognitive deficits observed in cocaine users.

Because cocaine binds to the dopamine transporter (DAT), regulatory changes in the dopaminergic system may occur within the brains of cocaine addicts. During acute abstinence, DAT availability as measured by PET is elevated in the striatum (Malison, Best et al. 1998). In vivo single-photon emission computed tomography (SPECT) measurements of DAT densities in living cocaine users vary depending on the time since the last cocaine administration. For example, when the DAT was imaged in vivo within 96 h of drug abstinence, [^{123}I]]β-CIT (radiotracer that measures DAT binding) uptake in the striatum was approximately 20% higher in the cocaine users compared to age-matched drug-free control subjects, suggesting elevated DAT levels. After a prolonged period of drug abstinence (3–18 months), [^{123}I]β-CIT measures were still elevated but to a lesser extent and demonstrated a trend toward a return to baseline values measured in drug-free control subjects. In autopsied cocaine users, DAT levels in the striatum were normal compared to controls (Wilson, Levey et al. 1996). These studies collectively suggest that during acute abstinence, DAT is elevated, but during long-term detoxification, DAT levels normalize.

Work conducted in non-human primates shows similar evidence of elevation in DAT with increased cocaine exposure. While initial cocaine exposure in non-human primates resulted in a significant decrease in DAT-binding sites, longer exposures of 3 months to 1 year resulted in elevated densities within the limbic sectors of the striatum (Letchworth, Nader et al. 2001). The densities of dopamine D_1 receptor binding sites, dopamine transporter binding sites and β adrenergic receptor binding sites were significantly decreased in the caudate nucleus to 51%, 17% and 61% of control, respectively, two weeks after repeated cocaine injections (Farfel,

Kleven et al. 1992). Beveridge examined D1, D2, and DAT in monkeys that were previously exposed to cocaine (Beveridge, Smith et al. 2009). After 30 days of being drug free, D1 and DAT levels were increased in the striatum, whereas D2 levels showed a decrease. After 90 days, there was no difference in D1, D2 or DAT from control monkeys. Chronic cocaine exposure increases DAT levels. Following periods of being drug free, DAT levels are reduced and normalized to levels similar to that of controls.

1.2.5 Acute Behavioral and Physiological Effects of Cocaine

Cocaine is abused for its ability to give the user a "high" and "rush." The high is characterized as a sense of well being, enhanced alertness, heightened energy and profound euphoria. Acutely, cocaine increases heart rate, blood pressure, stereotypy and locomotion, increases arousal, decreases fatigue and produces a profound euphoria (Johanson and Fischman 1989).

Cocaine, like many stimulants, causes profound behavioral activation in rodents. At low doses, rodents exhibit increases in locomotion, rearing and mild sniffing behaviors. However, psychomotor stimulants do no increase gross locomotion (activities refer to movements of the entire body or major segments of the body) in primates (Bradberry 2007). Humans using large amounts of cocaine occasionally exhibit motor stereotypies in the form of repetitive picking and scratching, which is not observed in monkeys.

1.3 THE EFFECTS OF CHRONIC COCAINE EXPOSURE

1.3.1 Cognitive Impairments Observed in Cocaine Abusers

1.3.1.1 Impairments in Cognitive Flexibility

Reversal performance or cognitive flexibility is defined as the ability to adapt one's behavior once a stimulus-reward outcome has been switched. A growing number of studies demonstrate that reversal performance is impaired in cocaine users (Ersche, Roiser et al. 2008); (Fillmore and Rush 2006); (Jentsch, Olausson et al. 2002). Impaired cognitive flexibility may account for the maladaptive patterns of behavior observed in chronic drug users (Anderson, Bechara et al. 1999). Ersche et al. (2008) were the first to show impaired reversal performance in cocaine users performing a probabilistic reversal-learning task relative to healthy controls, amphetamine and opiate users. They showed marked preservative responding to the previously rewarded stimuli even after reward contingencies had been switched (Ersche, Roiser et al. 2008), while the acquisition of the initial reward-stimuli was unimpaired. Fillmore and Rush (2006) observed reversal impairments in polydrug (cocaine and heavy alcohol) users performing a cued Go/No go task (Fillmore and Rush 2006).

Consistent with human studies, Jentsch, Taylor and colleagues (2002) were the first to show impaired reversal learning in cocaine exposed non-human primates. Monkeys were experimenter-administered cocaine for 14 days and then tested in a 3 object discrimination and reversal task, 9 and 30 days after withdrawal from cocaine (Jentsch, Olausson et al. 2002). These monkeys acquired the discrimination normally but made more errors that controls in acquiring the reversal-learning component of the task. This study lacks baseline performance. Animals learned to perform the task after cocaine exposure. As a result, they were looking more at

learning than actual reversal performance. Reversal impairments have also been observed in rats and mice. Rats tested on an odor discrimination-reversal task after withdrawal from either non-contingent or cocaine self-administration showed deficits in reversal performance (Calu, Stalnaker et al. 2007); (Schoenbaum, Saddoris et al. 2004). Evidence in the literature strongly points to or suggests reversal learning deficits in cocaine exposure subjects, but do not show that cocaine results in the observed impairments. While these preclinical studies suggest that cocaine exposure results in impaired reversal performance, they fail to account for baseline performance. Collectively, both the human and preclinical studies show that cocaine users have impaired cognitive flexibility, but what remains unclear is whether these impairments result from cocaine exposure.

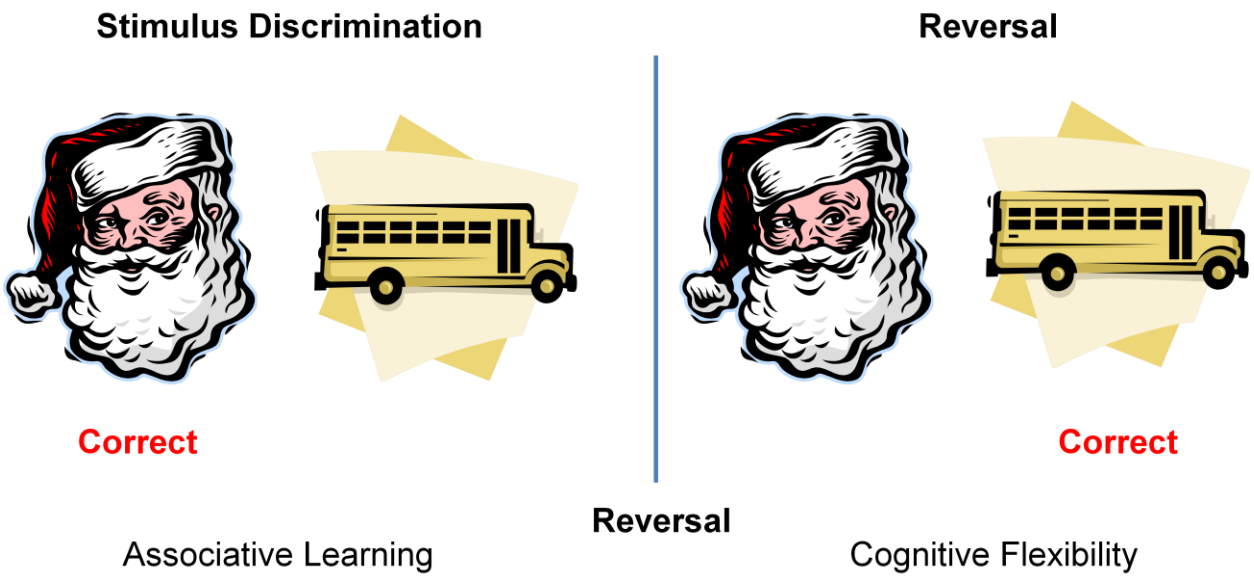

Figure 1 Diagram of Stimulus Discrimination/Reversal Task

The stimulus discrimination task in broken up into two trial types: stimulus discrimination and reversal. Stimulus discrimination trials tests associative learning or the ability to associate a reward with a particular stimuli. Once the animal learns the reward contingencies, the reward contingencies are switched (reversal). Reversal trials assess cognitive flexibility or the ability to adapt behavior to changes in the environment

1.3.1.2 Stimulus Discrimination/Reversal Task: A Probe for Orbitofrontal Dysfunction

Historically the stimulus discrimination/reversal task has been used to assess cognitive flexibility. In this task, subjects learn to associate different objects (ranging from 2-3 objects) with a reward amount. After learning the association (stimulus discrimination), the reward contingencies are switched (reversal) so that each cue now predicts the other outcome (Figure 3). Lesion studies were the first to shine light on the role of the orbitofrontal cortex (OFC) in reversal performance. Butter (1969) was the first to suggest a role for the OFC in reversal learning (Butter 1969). Systemic studies in monkeys with discrete cortical lesions have demonstrated that associated learning (stimulus discrimination) depends largely on inferior and mesial temporal lobes (Murray, 1993), while reversal learning is sensitive to orbitofrontal functions (Butter 1969); (Dias, Robbins et al., 1996) and medial striatum (Clarke, Robbins et al. 2008). Performance on the reversal-learning task is impaired (Fellows and Farah 2003); (Dias, Robbins et al. 1996); (Freedman, Black et al. 1998). Neurophysiological studies in monkeys and neuroimaging studies in humans suggest that the OFC is involved in representing the reward value of stimuli, rapid learning, and relearning of associations between visual stimuli and rewarding or punishing outcomes (Rolls 2000); (Dias, Robbins et al. 1996); (Freedman, Black et al. 1998). FMRI studies show OFC activation while performing the reversal-learning task (Kringelbach and Rolls 2003); (O'Doherty, Critchley et al. 2003). Patients with OFC lesions are impaired on reversal trials (Fellow and Farah, 2003). Patients repeatedly chose the previously rewrded stimulus after the reward contingencies have been switched. This data together suggest that the stimulus discrimination/reversal task is a great probe for OFC dysfunction. Damage or alterations in this region result in impaired performance on this task.

Much evidence supports the role of the OFC in mediating reversal learning and cognitive flexibility. In essence, the reversal-learning task provides a reliable and useful tool for probing OFC dysfunction in clinical and preclinical models of addiction. Patterns of reversal impairments seen in cocaine users closely mirror that seen with OFC lesions or inactivations. What remains unclear is whether cocaine exposure is sufficient to cause impairments in reversal performance. Because the literature consistently shows impairments in cognitive flexibility as measured by reversal performance, we hypothesized that we would observe strong deficits in cognitive flexibility following chronic cocaine self-administration. Consistent with this hypothesis, some clinical studies have shown a direct relationship between cognitive impairments and drug use (Bolla, Rothman et al. 1999). Greater use of cocaine (grams per week) was associated with larger decrements on tests measuring executive functioning, visuoperception, psychomotor speed, and manual dexterity. This suggests that cocaine may be sufficient to cause impairments, but a more direct assessment, such as the one proposed for this thesis project, is necessary to test this hypothesis.

1.3.1.3 Impairments in Working Memory

Working memory is the process of maintaining, updating and storing information over a short delay. There have been inconsistencies in the literature concerning the effects of cocaine exposure on working memory. Some clinical studies show working memory deficits in cocaine users compared to controls (Hoff, Riordan et al. 1996); (Bechara and Martin 2004); (Kubler, Murphy et al. 2005); (Beatty, Katzung et al. 1995); (Bolla, Funderburk et al. 2000); (Goldstein, Leskovjan et al. 2004); (Tomasi, Goldstein et al. 2007) However, other studies show no deficits in working memory (Bolla, Rothman et al. 1999); (Colzato, Huizinga et al. 2009). Jovanovski et al. (2005) reported in a recent comprehensive quantitative literature review that among the many

cocaine-related neurocognitive deficits revealed in a range of studies, tests of working memory were moderately impaired (Jovanovski, Erb et al. 2005).

Working memory assessments have also been conducted in pre-clinical studies of cocaine addiction. Adult male rhesus monkeys with an extensive cocaine self-administration history (~5 years), were trained to perform a delayed match-to-sample task (Gould, Gage et al. 2012). There were no differences in baseline working memory performance between the cocaine and the control groups, when animals received low doses of cocaine (0.1 mg/kg/injection). High-dose cocaine self-administration disrupted delayed match-to-sample performance but tolerance developed after 4 days at the higher dose. Acute abstinence from cocaine did not affect working memory performance. After 30 days of abstinence, accuracy increased significantly relative to baseline, while performance of cocaine-naive monkeys was unchanged. Rats that are allowed extended access to cocaine self-administration have impaired working memory (George, Mandyam et al. 2008). This observation was not observed in rats that were given limited access to cocaine. The literature from both a clinical and preclinical level is inconsistent. The inconsistencies within the studies may be due to a lack of a true drug free baseline and differences in drug history. We hypothesized that we would see marginal impairments in working memory, as the cortical areas that underlie working memory are altered by cocaine use.

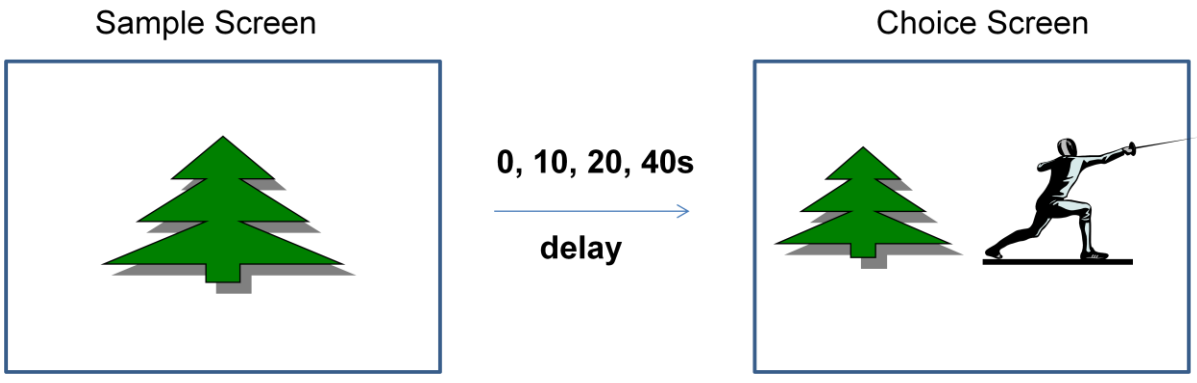

Figure 2 Diagram of Delayed Match-to-Sample Task

The delayed match-to-sample task is a task used to assess visual working memory. On any given trial, a novel stimuli appears on the screen. The animal must hold the stimuli for 1s in order to start the delay period. A random delay of 0,10,20, or 40s is followed by a choice screen. The choice screen has one match image and a distractor image. The animal much choose the image that matches the image from the sample screen.

1.3.1.4 Delayed Match-to-Sample: A Probe for Working Memory Function

The delayed matched to sample task is used to tax visual working memory. Many studies probe working memory function using a delayed match to sample (DMS) or delayed non-match to sample (DNMS) tasks. A stimulus is presented as a sample, and after a random delay period, the effective maintenance of the sample information is evaluated by presenting a match stimulus and a distractor(s) stimulus and requiring subjects identify the match stimulus (Figure 2). Several studies have established that the use of a variety of delay task procedures provides a valid measure of working memory (D'Esposito, Detre et al. 1995); (Fuster 1997); (Goldman-Rakic 1992); (McCarthy, Blamire et al. 1994). Using the DMS task, we probed working memory function prior to self-administration and during administration to determine whether working memory is directly impaired by chronic cocaine self-administration. We hypothesized that we would observe impairments in working memory following cocaine self-administration.

1.3.1.5 Dorsolateral Prefrontal Involvement in Working Memory

Studies in nonhuman primates have shown that lesions of the dorsolateral prefrontal cortex (DLPC) give rise to severe impairments in working memory (Goldman-Rakic, 1992). Visual working memory is associated with ventrolateral prefrontal cortex (Wilson et al., 1993). The orbitofrontal cortex has also been implicated in working memory (Otto and Eichenbaum 1992); (Wallis 2007). In single-unit recording studies, neurons have been identified in the

DLPFC that remain active during the delay periods in the tasks (Goldman-Rakic 1987). Functional neuroimaging studies show increased DLPFC activity during working memory tasks (Jonides, Smith et al. 1993); (Petrides, Alivisatos et al. 1993); (McCarthy, Blamire et al. 1994). In the current study, impairments in working memory would suggest alteration in the lateral prefrontal cortex.

1.3.1.6 Cerebellar Involvement in Working Memory

The cerebellum has long been considered important for control of movement. Recently, neuroimaging studies have shown that the cerebellum may also be involved in executive functions (Parkins 1997); (Schmahmann and Sherman 1998); (Owen, McMillan et al. 2005), Strick PL, 2009;). A recent review by Strick (2009) suggests that the cerebellum may play a role in human cognition (for review see (Strick, Dum et al. 2009). The cerebellum is involved in both verbal and non-verbal working memory tasks, such as the paced auditory serial addition task (PASAT), the n-back task and the Sternberg paradigm (e.g., (Beneventi, Barndon et al. 2007); (Chen and Desmond 2005); (Chen and Desmond 2005); (Tomasi, Caparelli et al. 2005). Patients with cerebellar damage are impaired in verbal working memory (Ravizza, McCormick et al. 2006); (Desmond, Chen et al. 2005); Burkk et al., 2003; (Silveri, Di Betta et al. 1998)). Patients with lesions in the cerebellum show impairments in working memory (Ravizza, McCormick et al. 2006). While lesions of the cerebellum do not produce the same degree of impairment as seen in frontal lesion patients (Ravizza, McCormick et al. 2006), it remains clear that there is a role for the cerebellum in working memory performance.

There were two regions within the cerebellum that are specific to executive functions (emotional processing, language, working memory and spatial processing): one cluster in lobule VI/Crus I, and another in lobule VIIB. Anatomical studies in monkeys show

interconnections between the prefrontal cortex, specifically in the DLPFC and lobule VII, Cruz I, Cruz II, and lobule VIIB (Kelly and Strick 2003). These findings are consistent with the concept of cerebro-cerebellar circuits that may underlie the involvement of the cerebellum in executive functions (for review see Bellebaum and Daum 2007) . Bilateral cerebellar activation have been reported in lobules VI and Cruz I during the n-back task and is consistent with other studies showing cerebellar posterior lobe activation during working memory tasks (e.g., (Honey, Bullmore et al. 2000); (LaBar, Gitelman et al. 1999); (Tomasi, Caparelli et al. 2005); (Valera, Faraone et al. 2005). Given the functional loop between the cerebellum and cortical regions and clear involvement of cerebellum in working memory, we focused our regions of interest analysis in the Cruz I and lobule VI region of the cerebellum.

1.3.1.7 Attentional Impairments

Attention is not a unitary phenomenon and has been theoretically broken down into 3 categories: (a) orienting, (b) alerting and (c) executive attention (Posner and Rothbart 2007). Executive attention is the type of attention most studied in cocaine users as it involves sustaining/controlling attention during conflict. There are inconsistencies as to whether attention is affected by cocaine exposure. Since attention is not a single construct, it is hard to determine whether attention as a whole is affected or just specific types of attention. Horner's (1999) review of 17 studies of cocaine abusers (some samples also abused alcohol) found attention largely unimpaired in individuals abstinent several days or weeks to several months (Horner 1999). However, a more recent review conducted by Jovanovski and colleagues (2005) found that drug use had the largest effect on several measures of attention (Jovanovski, Erb et al. 2005). Examples of tests of attention with the largest effect size are the: Stroop interference, Stroop color, Paced Auditory Serial Addition Test, Numerical Attention Test and WMS Digit Span. The

Stroop task, a task most often used in the clinical population is a task designed to examine executive attention or attention that involves conflict. In the Stroop task, subjects must respond to the color of the ink while ignoring the color of the word. A drug-related version of the Stroop task has been used to measure the degree of involuntary attention, or attentional bias toward drug-related words compared with stimuli (Goldstein, Tomasi et al. 2007); (Hester, Dixon et al. 2006); Carpenter KM, 2006, Copersino ML, 2004). Stimulant-dependent individuals have a significantly greater attentional bias in favor of drug related cues (Hester, Dixon et al. 2006); (Carpenter, Schreiber et al. 2006); (Copersino, Serper et al. 2004); (Hester and Garavan 2009); (Ersche, Bullmore et al. 2010) as measured by the drug Stroop task. Cocaine users show a decrease in performance on trials with irrelevant drug stimuli, whereas control participants only show performance decreases with inherently evocative stimuli. The increased salience of drug-related cues is thought to contribute to the maintenance of drug taking behavior (Goldstein and Volkow 2002); (Lubman, Yucel et al. 2004). Using the Stroop task to measure attentional bias in cocaine users is important because it has been shown to predict relapse (Streeter, Terhune et al. 2008); (Carpenter, Schreiber et al. 2006). Clinical research indicates that environmental cues associated with substance use increase reports of craving, motivation to take drugs and likelihood of relapse (Childress, McLellan et al. 1988); (O'Brien, Childress et al. 1992); (Droungas, Ehrman et al. 1995). It is important to consider the effects of cocaine on attention, given the relationship between attention and executive functions, such as working memory. We are able to directly probe the interaction between attention, cognitive flexibility and working memory by presenting attentional distractors during a stimulus discrimination/reversal task and DMS task. Given the literature on attentional biases towards cocaine related cues in cocaine users, we hypothesized

that cognitive flexibility and visual working memory would be impaired during the presentation of the attentional distractors that were previously paired with cocaine availability.

1.3.1.8 Cortical Involvement in Attention

Executive attention is thought to rely on regions like the anterior cingulate, lateral ventral cortex, prefrontal cortex and basal ganglia, with dopamine being the main modulator. The neural mechanism underlying these attentional biases in human cocaine users has been documented using neuroimaging techniques. Similar brain regions are activated when drug cues are presented to cocaine abusers. Studies where drug-related stimuli are presented to either active or abstinent users have demonstrated significant activation in regions such as the amygdala, nucleus accumbens and hippocampus (Grant, London et al. 1996); (Maas, Lukas et al. 1998); (Childress, Mozley et al. 1999); (Garavan, Pankiewicz et al. 2000); (Kilts, Gross et al. 2004); (Kilts, Schweitzer et al. 2001); (Wexler, Gottschalk et al. 2001); (Bonson, Grant et al. 2002). Mesocortical regions such as anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (DLPFC) and orbitofrontal cortex (OFC) show significant increases in activity following drug cue exposure (For review see Wilson, Sayette et al. 2004). In addition, significant physiology reactivity of users to cocaine related material (Carter and Tiffany 1999); (Johnson, Overton et al. 1998); (Modesto-Lowe and Kranzler 1999) has been observed. Garavan and colleagues (2009) were the first to examine the neural mechanisms underlying the attentional bias for drug-related stimuli under differing working memory (WM) demands in active cocaine-users (without psychiatric disorders) (Hester and Garavan 2009). Activation in response to cocaine-related words relative to neutral words differed in the rostral cingulate and medial OFC, regions that have been shown to be hypoactive in cocaine users. Because cue reactivity causes increased activation in cortical regions that are important for cognitive function, we hypothesized that

cognitive flexibility would be altered due to limited capacity of function within these cortical brain areas.

1.3.2 Cortical Alterations

1.3.2.1 Functional Abnormalities: Positron Emission Tomography

Using the FDG-PET to measure changes in brain glucose metabolism (an indirect measure of brain function), Volkow and colleagues were the first to observe changes in the OFC and basal ganglia in cocaine dependent subjects compared to controls (Volkow, Hitzemann et al. 1992). Using PET, Volkow and colleagues (1991) showed that chronic cocaine users who were within 1 week of cocaine withdrawal had higher rates of regional brain metabolism in the basal ganglia and OFC than normal control subjects had. During more protracted withdrawal periods (1-6 weeks abstinence), metabolic activity in the OFC was decreased relative to controls. In 25-day abstinent cocaine abusers, Bolla and colleagues showed alterations in cerebral blood flow in the OFC using PET with (^{15})O during a decision making task. Cocaine abusers showed increased activation in the OFC and a decrease in activation in the DLPFC and medial PFC relative to healthy drug free controls (Bolla, Eldreth et al. 2003). The same group performed a similar study in 23-day abstinent cocaine abusers performing a modified version of the Stroop task (attention task) and found decreases in the anterior cingulate (ACC) and lateral PFC relative to healthy controls performing the task (Bolla, Ernst et al. 2004). PET imaging studies in humans consistently show hypoactivation of cortical regions in abstinent cocaine users compared to controls.

Consistent with human studies, a FDG-PET study done in chronically cocaine self-administering non-human primates showed a decrease in metabolic function in the OFC

(Beveridge, Smith et al. 2006); (Porrino, Lyons et al. 2002); (Lyons, Friedman et al. 1996). In a review, Porrino et al., (2004) outlined functional metabolic activity of acute cocaine self-administration (5 days) and chronic cocaine self-administration (100 days) (Porrino, Daunais et al. 2004). Acute cocaine self-administration resulted in significant dose-dependent changes in glucose utilization throughout all levels of the ventral striatum, as well as in the caudate rostral to the anterior commissure. The decreases in activity broadened across the orbital surface to not only include area 13 but also area 11 and some of area 12 with chronic self-administration. In the last aim of this thesis project, FDG-PET was employed to assess metabolic rates of glucose utilization during a visual working memory task. This allowed us to assess long-term changes in metabolic activity following a 20-month drug free period. I hypothesize that DLPFC will show reduced activation relative to controls performing a working memory task (relative to control task) following an extended period of abstinence.

1.3.2.2 Functional Abnormalities: Functional Magnetic Resonance

Studies using functional magnetic resonance imaging (fMRI) have also shown differences in brain function in cocaine users who were in early abstinence. FMRI activation in the cingulate, pre-supplementary motor cortex, and insula was significantly reduced in cocaine users while performing a go–nogo task compared with controls (Kaufman, Ross et al. 2003). Hester and Garavan (2004) used an inhibitory GO-NO/GO WM task and found lower PFC activation in abstinent cocaine abusers compared to control subjects (Hester and Garavan 2004). They also found an over-reliance on the cerebellum in active cocaine users compared to controls. This was the first time there was a report of increased cerebellar activation in cocaine users performing a working memory task. They hypothesize a compensatory role for the cerebellum in working memory. More recently, using a sustained visual attention task, Tomasi et al. (2007) showed that

cocaine users in early abstinence had hypoactivation in the thalamus and hyperactivation in occipital and frontal cortices compared with controls. One of the few studies to examine brain function during working memory in cocaine users showed that cocaine users in early abstinence had lower activation in the mesencephalon and thalamus compared with controls (Tomasi, Goldstein et al. 2007). Moeller et al., (2010) performed a similar study and showed decreased frontal activation during a working memory task correlated with treatment outcome (Moeller, Maloney et al. 2010).

1.3.2.3 Structural Abnormalities: Voxel Based Morphometry

In addition to functional abnormalities, cocaine abuse has been associated with structural abnormalities. Voxel based morphometry (VBM) is an automated technique that is used to assess tissue concentration or tissue volume based on high-resolution anatomical MRI. Franklin et al (2002) first reported that cocaine-dependent subjects had lower gray matter density in the orbitofrontal, anterior cingulate, insular, and superior temporal cortex relative to comparison subjects using VBM (Franklin, Acton et al. 2002). Sim et al., (2007) recently reported lower white matter density in the right cerebellum, which correlated with duration of use and lower gray matter density in premotor cortex, temporal cortex, the left thalamus, the cerebellum and frontal cortex of active users (Sim, Lyoo et al. 2007). There is also evidence of reduced frontal white matter volumes (Lim, Wozniak et al. 2008). Matochik et al. (2003) investigated gray and white matter densities in individuals abstinent from cocaine for 20 days (Matochik, London et al. 2003). Short-term abstainers had lower gray matter in the cingulate gyrus, lateral PFC and the OFC compared to controls. Hanlon et al., (2011) was the first to show a relationship between cognitive performance and neurostructural integrity in cocaine users compared to abstinent individuals (Hanlon et al. 2011). Cortical gray matter density was correlated with performance

on an array of cognitive tests. Specifically, there was a negative correlation between performance on the delayed match to sample task (errors at the long delay) and gray matter density in the medial prefrontal cortex within the cocaine abstainers and users. In addition, abstainers showed an increase in gray matter and performed better than cocaine users on tests of executive function. Decreases in gray matter in cocaine users relative to controls in cortical areas are of particular interest given the cognitive deficits in users that are thought to rely on functions subserved by these regions.

1.4 VALIDITY OF USING FDG-PET AS A MEASURE OF BRAIN FUNCTION IN NON-HUMAN PRIMATES

Quantification of glucose utilization rate with FDG-PET in the rat was developed by Sokoloff et al., (1977). His work laid out a solid foundation for us the of C-14-labeled deoxyglucose and autoradiography for the quantification of cerebral metabolic rate of glucose. This work has since been conducted in humans and in non-human primates. FDG-PET has been used for over 30 years to assess cerebral metabolic function. FDG-PET is a minimally invasive imaging technique that makes it possible to quantify cerebral metabolic rates of glucose, during rest or following a cognitive task. PET used in conjunction with FDG, an analog of glucose, has been used in both humans (Mosconi 2005) and non-human primates (Kalin, Shelton et al. 2005); (Porrino, Daunais et al. 2005) as a means of directly visualizing regional differences in metabolic demand. FDG is administered to a subject where it is taken up by high glucose using cells, phosphorylated and retained in tissue with high metabolic demand. FDG uptake occurs over a 30-minute window allowing for an easy assessment of what brain regions are active during a

given period. A PET scanner is used to form images of the distribution of FDG around the body. FDG is trapped in the body. The limitation of using FDG-PET is that changes in metabolic demand cannot be captured at a trial-by-trial level like in fMRI studies. The advantage of this technique is that it provides detailed anatomical resolution of differences in metabolic demand between sessions that have differing levels of cognitive difficulty.

The validity of using this approach in non-human primates to assess changes in metabolic demand during a cognitive task has been demonstrated in published studies. In sleep-deprived monkeys, Porrino et al., (2005) showed increased metabolic activity during a working memory task in the DLPFC, medial temporal lobe (MTL) and dorsal striatum (DStr) during increased working memory compared to baseline (no working memory) in healthy, drug free monkeys. Similar regions have been shown to be active using fMRI in humans performing a working memory task (Jonides, Smith et al. 1993); (Petrides, Alivisatos et al. 1993); (McCarthy, Blamire et al. 1994). We previously used this approach to characterize brain regions that underlie inhibitory control in monkeys performing the Stop task. In a preliminary study, we were able to replicate brain activation in the inferior frontal cortex, a region also shown to be active in human fMRI studies (Rubia, Smith et al. 2003); (Garavan, Ross et al. 2002) (Garavan, Ross et al. 1999); (Konishi, Nakajima et al. 1999). Since we were able to replicate findings observed in fMRI using PET, we decided to use FDG-PET as a reliable method to assess metabolic function in monkeys that previously self-administered cocaine.

1.5 LIMITATIONS IN CLINICAL STUDIES OF COCAINE ADDICTION

There are limitations in human studies that make it harder to draw conclusions about the direct effects of cocaine on cognition and brain function. There are also considerable differences across studies in the characteristics of the stimulant abusers included in terms of the duration of use, the pattern of use, the number of abstinence episodes and the degree of drug use other than stimulants. This is compounded further by similar incongruities in control populations as well as difficulties in matching user populations to controls on characteristics such as age, IQ, education and socioeconomic status. Finally, the influence of potential premorbid deficits on the cognitive performance of chronic cocaine users remains a significant confound that cannot be easily ruled out. Many of the investigations into the cognitive and neurobiological consequences of cocaine use often imply or assume that cocaine exposure is the cause of these deficits. However, the influence of factors such as concomitant psychiatric illness, lifestyle differences and the use of multiple licit and illicit substances can be significant confounds in the interpretation of these data. Perhaps most difficult is assessing whether any of these impairments actually occur because of drug exposure itself or predate any drug experiences. The limitations in human studies suggest a need for longitudinal studies to be run in preclinical settings where the environment is controlled and a temporal assessment of cocaine on cognition can be examined. Our goal was to use cognitive tasks that are used in clinical settings to assess whether cocaine might result in impaired cognition, in order to better understand what may be happening in human cocaine users.

1.6 VALIDITY OF USING NON-HUMAN PRIMATES AS A MODEL OF ADDICTION

Non-human primates (NHPs) can be trained to perform tasks probing specific cognitive domains known to be impaired in human cocaine users. They are able to perform complex tasks making them a better model than the rodent model and easier to compare human cognitive studies to non-human primate studies. NHPs have been used in self-administration studies for close to 60 years and this has proven to be a valid and reliable model of human drug abuse. Monkeys share cytoarchitectural, neurochemical and ultrastructural similarities with humans, particularly with respect to the prefrontal cortex (Carmichael and Price 1994); (Porrino and Lyons 2000); (Hardman, Henderson et al. 2002). Given the importance of the prefrontal cortex in cognition, the similarity in cortical structure between human and monkeys is very important for probing cognitive function. Connectivity patterns of the non-human primate prefrontal cortex are highly homologous to those of humans (Ongur and Price 2000). Organizational structure and connections between areas such as the striatum and prefrontal cortex are similar in primates (Haber and McFarland 1999); (Haber and Knutson 2010). Other advantages of non-human primates include similarities in the anatomy and physiology of dopaminergic (Sanchez-Gonzalez, Garcia-Cabezas et al. 2005) and noradrenergic systems (Smith, Beveridge et al. 2006). Using initially drug naive subjects in a longitudinal design to characterize within-subject changes with respect to neurobiology associated with chronic drug use can shed light on the temporal course of drug exposure on cognition and neuroadaptations in the brain. The main benefit of this study is that we are able to assess the direct effects of cocaine on cognition in a preclinical model that parallels human addiction. Using non-human primates to model aspects of addiction

significantly adds to the value of this thesis project because it allows us to probe deeper into what changes may be resulting from addiction so that better treatments can be designed.

1.7 SUMMARY AND AIMS

Cocaine users suffer from a wide range of cognitive deficits, primarily in executive functions of decision making, cognitive flexibility, working memory and inhibitory control. Of these deficits, attentional impairments are the most consistent deficits found in cocaine addicts, suggesting attentional impairments may be contributing to the impairments observed in other cognitive domains. Greater deficits in cognitive function predicts poor treatment outcome. While there is some evidence showing a correlation between drug use and cognitive impairments, it is still unclear whether cocaine is sufficient to cause cognitive impairments. The mesolimbic and mesocortical dopmainergic pathways are thought to play an important role in the rewarding effects of cocaine and alterations in these pathways are thought to contribute to cognitive and cortical dysfunction observed in cocaine abusers. Limitations in human studies, such as drug history, poly drug use and pre-morbitites make it hard to draw conclusions from clinical studies. Presently, it is still unclear whether cocaine self-administration is sufficient to cause cognitive deficits and if so, the specificity of these deficits.

Neuroimaging studies conducted in current users and abstinent populations show decreases in cortical gray matter and hypoactivation of cortical areas necessary for efficient cognitive function. Metabolism in the OFC is reduced up to 3 months post cessation of drug use. Consistent with impaired cortical regions, cognitive deficits have been shown to last months after cessation. Few studies have been able to examine long term changes in metabolic function.

Here we will determine whether chronic cocaine self-administration is sufficient to cause impairments in associative learning, reversal performance and visual working memory. We will

also assess the impact of attentional distractors on these cognitive domains as a way of assessing the long term effects of cocaine during extended periods of abstinence. Lastly, we will assess long term metabolic differences between the cocaine and control groups.

2.0 CHRONIC COCAINE SELF-ADMINISTRATION IN RHESUS MONKEYS: IMPACT ON ASSOCIATIVE LEARNING, COGNITIVE CONTROL, AND WORKING MEMORY *PUBLISHED PORTER ET AL., 2011

There is increasing interest in the nature and etiology of cognitive deficits associated with drug dependence (Rogers and Robbins, 2001), arising from the likelihood that decisions to continue use despite negative outcomes reflect impairments in higher cognitive processing. Clinically, this is important because of evidence that therapeutic outcome is related to cognitive abilities at treatment onset (Teichner et al., 2002; Patkar et al., 2004; Aharonovich et al., 2006). However, the extent to which those deficits result from drug use, rather than pre-existing traits is unclear, making controlled animal studies necessary (Rogers and Robbins, 2001). Such studies avoid the confounds of pre-existing differences, poly-drug use, varying length and amounts of drug use, and lifestyle stressors unique to drug using populations. Determining if there is a causal relationship between cocaine self-administration and specific cognitive deficits was the primary aim of this study.

In addition to etiology, the specificity of cognitive impairments associated with cocaine use are unclear. One domain consistently impaired is cognitive control/flexibility (Garavan and Hester, 2007), reflected by increased perseverative responding subsequent to the reversal of reward contingencies (Fillmore and Rush, 2006; Ersche et al., 2008). The inability to adapt to altered reward contingencies does not appear to result from impaired associative learning in users who understand task contingencies from verbal instructions (Ersche et al., 2008), though previous animal studies indicate impaired acquisition of an unfamiliar task in which stimulus discriminations had to be learned by trial and error (Liu et al., 2008).

Impaired working memory has been reported (O'Malley et al., 1992; Hoff et al., 1996; Bechara and Martin, 2004; Kubler et al., 2005; Verdejo-Garcia and Perez-Garcia, 2007), though the literature is surprisingly mixed (Bolla et al., 1999; Pace-Schott et al., 2008). We have examined the issue of whether specific cognitive domains are affected by cocaine self-administration through the employment of tasks that evaluate associative learning, cognitive control/flexibility, and visual working memory. This study complements previous rodent studies examining effects of cocaine on reversal learning (Schoenbaum et al., 2004) and working memory (George et al., 2008).

In this study, rhesus monkeys were extensively characterized on cognitive tasks prior to initiating an extended period of cocaine self-administration. After initiating self-administration, cognitive assessments were conducted weekly in drug-free animals to evaluate the impact of cocaine exposure on distinct cognitive domains. We used a stimulus discrimination/reversal task to assess associative learning and cognitive control/flexibility. The reversal component requires orbitofrontal cortex (Dias et al., 1996; Fellows and Farah, 2003; Hornak et al., 2004; Izquierdo et al., 2004; Murray and Izquierdo, 2007). A delayed match-to-sample task was used to assess visual working memory. Delayed match-to-sample performance has been linked to the ventrolateral prefrontal cortex (Wilson et al., 1993; Elliott and Dolan, 1999). We hypothesized that chronic cocaine exposure would impair reversal learning and working memory thus implicating orbitofrontal cortex and ventrolateral PFC dysfunction. Using an array of tasks associated with distinct cognitive domains allowed us to address the question of whether cocaine use has a selective impact on particular aspects of cognitive function.

2.1 MATERIALS AND METHODS

2.1.1 Subjects

Young adult (43-57 months at beginning of study) male rhesus macaque monkeys (n=14) with no previous non-clinical drug exposure or behavioral training were used for the present study. Upon acquisition, animals were initially trained to target for food reinforcement, then habituated to pole and collar handling and placement in a behavioral primate chair (Primate Products, Redwood City, CA). Water regulation was then established, and subjects were taught to use a sipper tube attached to the chair for water reinforcement. Animals were water regulated 5 days per week and were supplemented (weekly avg. 25ml/kg/day) at the end of each day following training and testing. Animals received *ad libitum* access to water over the weekend. Water regulation started at least 24 hours prior to cognitive testing. Initially all monkeys were pair housed in standard stainless steel primate cages. Over time, incompatibility emerged among some pairs. When attempts at re-pairing were unsuccessful, monkeys were subsequently singly housed to avoid conflict injuries. At the end of the experiment, 9 animals continued to be pair housed, while 3 controls and 2 cocaine monkeys had to be individually housed.

2.1.2 Surgery

Prior to baseline cognitive assessments, all animals had a vascular access port placed midscapula from which a catheter extended subcutaneously to an internal jugular vein. The vascular access port allows percutaneous non-stressful access to vasculature for cocaine self-administration without the need for a protective jacket and with reduced risk of infection because nothing is external to the skin (Wojnicki et al., 1994).

2.1.3 Self-Administration

Both groups self-administered (either water or cocaine) in the chambers used for cognitive testing. Animals self-administered by touching an abstract shape on the touch screen for the required number of touches. Once the response requirement was met, a green border around the white screen would appear and either cocaine (cocaine group) was administered intravenously via the vascular access port or water (control group) via a sipper tube. Contextual cues were also associated with reinforcement for later determination of their impact on behavior. These cues, consisting of a specific auditory sequence of tones and distinct visual border to the screen, were present throughout the entire session except during the time of reward delivery. During the cocaine self-administration sessions, animals were allowed to self-administer up to 6 infusions of cocaine. After the first week, animals typically administered all 6 infusions. The cocaine group began self-administering an average daily dose 0.6mg/kg (0.1mg/kg/infusion, 6 infusions) of cocaine daily under a fixed ratio-3 (FR-3) schedule and 5 minute time out. Response requirements were gradually increased to a terminal FR-30, a 10 minute time out interval and 3.0mg/kg cumulative session dose (six 0.5mg/kg/infusions of 5 mg/ml, delivered at a rate of 0.15 ml/sec). All animals reached the 3.0 mg/kg session dose over 15 days of gradually increasing the unit dose.

The control group animals began self-administering an average of 1.8ml/kg water under a FR-3 schedule (6 infusions, 0.3ml/kg/infusion). After 23 days, the water amount was increased to 11.8ml/kg of water (18 infusions, 0.66ml/kg/infusion) and the schedule was switched to a random ratio of 5-15 with a time out of 3.3 minutes in order to maintain cumulative amounts of lever pressing and chamber time similar to the cocaine group.

2.1.4 Cognitive Assessment

Table 1 Daily schedule for self-administration and cognitive assessment procedures

Monday	Cognitive Testing (SD/REV or DMS)
Tuesday	1. Progressive Ratio 2. Self -Administration
Wednesday	Self -Administration
Thursday	Self -Administration
Friday	Self -Administration
Saturday	No Self - administration, ad lib water
Sunday	No self -administration, water lines removed

Daily schedule for self-administration and cognitive assessment procedures. Animals performed either the stimulus discrimination/reversal (SD/REV) or delayed match-to-sample (DMS) task every Monday and self-administered Tuesday-Friday. The Progressive Ratio evaluation of water reward efficacy on Tuesday took place in a morning session separate from the self-administration session in the afternoon. Animals had ad-lib water starting Friday after testing until water lines were removed on Sunday morning

All cognitive assessments took place in a sound attenuated chamber (Eckel Industries, Ontario, Canada, model AB4240) fitted with a 40 W house light and white noise generator. The E-prime software suite (Psychology Software Tools, Pittsburgh, PA), coupled with a 15" touch screen (Elo systems CarrollTouch), was used for all stimulus presentation, response recording, and data processing. Using baseline measures for each cognitive task described below, performance and age-matched control (n=6) and experimental (n=8) groups were established. Self-administration and cognitive assessments were organized as indicated in Fig. 3 and Table 1. The animals self-administered Tuesday through Friday. Cognitive assessments were conducted on Mondays, when the animals had been drug free for approximately 72 hours. A stimulus discrimination/reversal task was used to assess associative learning and cognitive control/flexibility, resp., and a delayed match-to-sample task was used to assess visual working memory. Testing on the delayed match-to- sample task or the stimulus discrimination/reversal task alternated each week. On Tuesday mornings, a progressive ratio assessment of water reward efficacy

took place, with self-administration beginning in a separate session Tues afternoon. Both groups self-administered and performed cognitive tasks contemporaneously.

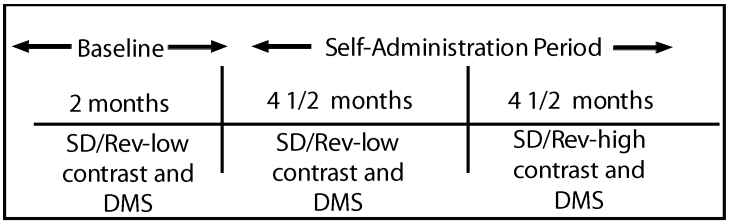

Figure 3 Timeline of self-administration and cognitive testing

Timeline of self-administration and cognitive testing. Note the change in reward contrast for the stimulus discrimination/reversal task.

2.1.5 Stimulus Discrimination/Reversal Task

On each cognitive assessment day, 3 novel, visually distinct stimuli were employed, thus requiring the subjects to learn a new operational stimulus-reward association at the beginning of each session. Each trial of the task began with presentation of a blue square, which the animal had to touch, thereby indicating he was attending to the task. Touching the blue square led to presentation of two of the three stimuli, randomly presented left and right of midline. The stimuli were associated with a high, medium, or low water reward. On any given trial, an animal had a choice between a high or low, high or medium, or medium and low reward contingency. A correct response was recorded when the monkey touched the stimulus with the relatively greater reward. Once a criterion of 27/30 correct responses on consecutive trials were made, the contingency was reversed in that the high and low stimuli were switched, with the middle stimulus unchanged. Reaching the same performance criterion after the reversal resulted in presentation of a new set of three stimuli for a discrimination block. Animals were allowed to do as many discrimination and reversal trials as possible within a 200 trial session.

Two different reward levels were used to assess association learning (stimulus discrimination) and the cognitive flexibility needed to adapt to altered contingencies (reversal performance). The initial stimulus discrimination/reversal task had a low contrast in reward levels between stimuli (stimulus discrimination/reversal-low reward contrast). The stimuli were associated with a high (0.1 ml/kg), medium (0.05 ml/kg) or low (0.02 ml/kg) water reward. The stimulus discrimination/reversal-low reward contrast was used to assess baseline performance (pre-admin low contrast) across 10 daily sessions prior to initiating self-administration. The same reward values were used for nine sessions after initiation of self-administration (post-admin low contrast) over a period of 4-5 months. During the post-admin low contrast period, all control animals continued to reach the stimulus discrimination criterion, but only five of the eight cocaine monkeys met the stimulus discrimination criterion. In order to increase incentive, we increased the reward contrast between stimuli by changing the reward values to 0.12ml/kg, 0.03ml/kg, and 0.001ml/kg for the high, medium, and low reward values (post-admin high contrast). All animals subsequently met the discrimination criterion, and reversal performance could then be evaluated in 7 of the 8 animals (there were insufficient remaining trials in one animal). The high reward contrast was used for the subsequent duration of self-administration (9 sessions, over 4-5 months).

2.1.5.1 Stimulus Discrimination/Reversal Task Analysis

Associative learning was evaluated in two ways. In order to employ a comparable analysis as used for the reversal task, the primary approach was comparison of accuracy over the first 15 trials of the discrimination component, binning every three trials to reduce noise inherent in averaging binary data. We limited our analysis to the first 15 trials presented for each stimulus set in order to focus on the subject's adaptation to a new stimulus set or the reversal of the reward contingencies. To analyze discrimination performance using the stimulus discrimination/reversal low reward contrast task (for which baseline and post self-administration performance was available), a three way repeated measures ANOVA was used to make a between groups comparison of stimulus discrimination performance with bin number, and exposure period (baseline vs post self-administration) being the repeated factors. For

stimulus discrimination/reversal high reward contrast, only a between groups comparison of performance in the post-self-administration period was possible. A two way repeated measures ANOVA was used with performance across bins being the repeated factor. An additional approach was to calculate trials to criterion in order to assess how well animals were able to sustain high levels of accuracy needed to reach criterion performance on the stimulus discrimination blocks (27/30 trials correct). If an animal did not meet the stimulus discrimination criteria, a conservative score of 200 trials (the session limit) was assigned. A two way repeated measures analysis was used with performance across each period (pre-admin low contrast, post-admin low contrast, and post-admin high contrast) being the repeated factor. We followed this with a Holm-Sidak-corrected multiple comparison procedure to evaluate the effect of period within each group.

The measure of cognitive control/flexibility was performance over the first 15 trials post reversal of the reward contingencies, also binned three trials at a time. A two way repeated measures ANOVA was used with performance across bins (during the post-admin high reward contrast period) being the repeated factor. It was not possible to compare trials to criterion on the reversal blocks, because the 200 trial sessions always began with a discrimination block, and by the time the stimulus discrimination criterion was reached, thereby initiating a reversal block, there were only a limited number of trials remaining.

2.1.6 Delayed Match-to-Sample Task

At the start of each trial (total of 160 trials/session), a sample stimulus (an image randomly selected from a 400 image pool) would appear on the touch screen. Pressing the sample stimulus accurately and holding it for 1 s led to its offset. Following a specified delay interval (randomly selected from 0, 10, 20, or 40s,), the sample and a novel stimulus (randomly selected from the image pool) appeared, randomly assigned to the left and right side of the screen. Choosing the sample stimulus within 10 s following the presentation of the two stimuli led to a water reward (0.075 ml/kg). No reward was delivered for choosing the wrong

stimulus or pressing the area outside of the choice stimuli. The inter-trial intervals for a correct response and incorrect response were 2 s and 7 s, resp. Baseline performance was determined from ten daily sessions prior to initiating self-administration, after which animals performed the task once every two weeks to assess visual working memory.

2.1.6.1 Delayed Match-to-Sample Task Analysis

Accuracy was evaluated only using trials in which one of the two stimuli was chosen. Thus, any trials with a non-choice error (omission or touching the screen outside of a stimulus) did not contribute to the measure of choice accuracy. Only sessions in which animals completed at least half of the 160 trials and met a side bias criterion (responses on each side were $\geq$ 25% and $\leq$ 75% of the trials completed) were included in the analysis. In the cocaine group 33 out of the 152 possible sessions were omitted and in the control group 16 of the 114 possible sessions were removed. Data were analyzed over 19 sessions during the self-administration period. Baseline data were averaged across the 10 sessions prior to the start of self-administration. The measures used for group matching were accuracy at the 40 second delay and the area under the curve of accuracy across all delays.

A three way repeated measures ANOVA was used to carry out a between group comparison of accuracy, with delay interval, and exposure period (baseline vs. post self-administration) being the repeated factors. To follow up any interaction observed in the three way repeated measures ANOVA, a two way repeated measures ANOVA was used for a within group comparison with period (baseline and post self-administration periods) and delay interval being the repeated measures.

2.1.7 Progressive Ratio Assessment of Water Reward Efficacy

A progressive ratio procedure was used for a weekly assessment of efficacy of the water reward used to motivate task performance. Animals touched a stimulus on the screen once for the first water reinforcement (1.0 ml/kg). The number of responses required for each subsequent water reinforcement

doubled, until the animal failed to complete the response requirement within 15 minutes or the animal failed to touch the screen for 10 minutes.

2.1.7.1 Progressive Ratio Analysis

The measure of water reward efficacy was the breakpoint, defined as the sequence number of the last reinforcement received (also the power of two equaling the number of responses required) before the session was terminated because responding ceased. Progressive ratio data were collected throughout the entire self-administration period (30 weekly sessions) and the baseline was collected a week before the start of self-administration.

2.2 RESULTS

2.2.1 Baseline performance matching of groups and comparability of daily vs intermittent assessments

A. B. C.

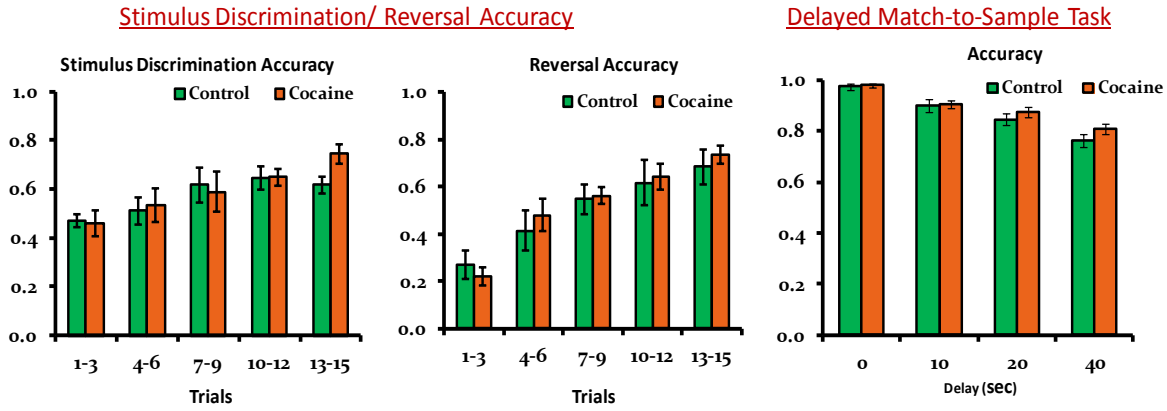

Figure 4 Performance matching of groups before cocaine exposure

Performance matching of groups prior to cocaine exposure. Baseline performance on A) stimulus discrimination, B) reversal, C) delayed match-to-sample task is shown. There was no significant difference in performance between groups on any task prior to initiating self-administration.

Baseline performance of the control and cocaine groups on the stimulus discrimination/reversal-low reward contrast and delayed match-to-sample tasks are shown in Fig. 4. There were no differences between the groups during the baseline period. There was also not a noticeable drop off in performance on any of the cognitive tasks as a result of going from the daily assessment used to establish baseline performance to assessments once every two weeks. This is demonstrated in the comparison of the control

group baseline performance and post-administration performance for the stimulus discrimination/reversal task shown in Fig. 5, and the delayed match-to-sample task shown in Fig. 10b.

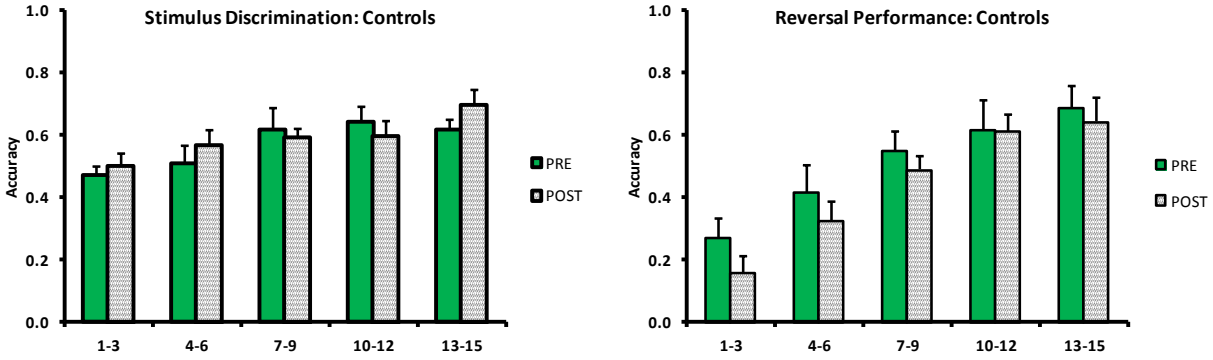

Figure 5 Validity of intermittent cognitive assessment

Validity of intermittent cognitive assessments. Comparison of baseline performance based on 10 consecutive daily sessions with subsequent assessments conducted at two week intervals, control group only. By two way repeated measures ANOVA, there was no effect of pre- vs post-administration for stimulus discrimination, $F_{(1,5)}$=0.35 p=0.58, or reversal performance, $F_{(1,5)}$= 1.11 p=0.34.

2.2.2 Stimulus Discrimination Performance

A. **B.**

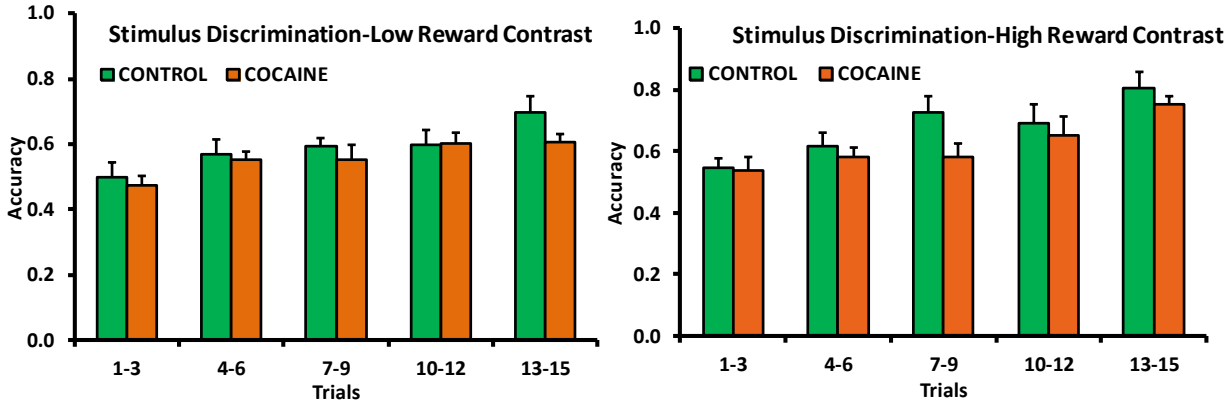

Figure 6 Between group comparison of stimulus discrimination performance on low reward contrast task and high reward contrast

Between groups comparison of stimulus discrimination performance on the low reward contrast task and high reward task. Over the first 15 trials of 9 sessions conducted over approximately 5 months of self administration, there was no significant between group difference in stimulus discrimination on either version of the task.

During the first 4-5 months of self-administration, there was no significant effect on accuracy by group or exposure period (baseline, post-admin low reward contrast). In addition, there was no significant group X exposure period X trial bin interaction. Associative learning is clearly occurring over those fifteen trials, given the main effect of bin number ($F_{(4,\ 9)}$ = 28.9, $p < 0.0001$). Fig. 6A shows the comparison between groups in the post self-admin low reward contrast period. Two-way repeated measures on just this exposure period also shows a main effect of trial bin number ($F_{(4,9)}$ = 8.85, $p < 0.001$) without a group by bin number interaction ($p = 0.53$). However, despite this indication of equivalent associative learning, only five of the eight cocaine monkeys were able to meet the stimulus discrimination criterion of 27/30 correct trials. Thus, there was an apparent difficulty in the cocaine group

to sustain high levels of accuracy across 30 consecutive trials used for criterion evaluation. This is reflected in a marginal interaction on trials to criterion between group and period (pre-admin low contrast, post-admin low contrast, post-admin high contrast), group X period $F_{(1,24)} = 3.283$, $p = 0.055$, Fig. 7. In the control group, there was no significant difference in trials to criterion between any of the periods. However, it took the cocaine group more trials to reach criterion during the post-admin low contrast compared to the baseline period ($t_{(1,7)} = 2.607$; $p = 0.015$). Thus, while maintaining the low reward contrast, there is not an effect of self-administration on trials to criterion in the control group, but there is an impairment in the cocaine group. Increasing the reward contrast, significantly decreased trials to criterion in the cocaine group (post admin low-contrast vs post admin high contrast, ($t_{(1,7)} = 2.70$ $p = 0.012$). However, increasing reward contrast in the control group had no effect on trials to criterion.

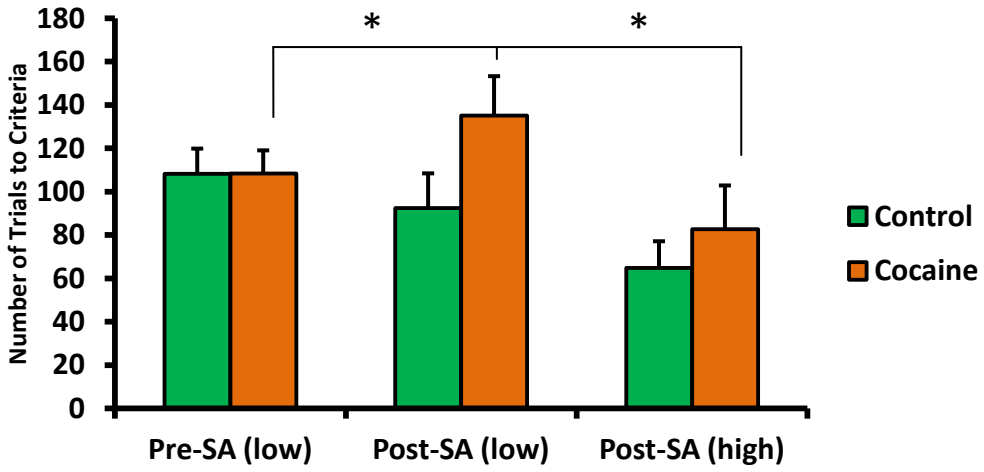

Figure 7 Between-groups comparison of trials to criterion on the stimulus discrimination task across conditions

Between group analysis of stimulus discrimination trials to criteria a) Between groups comparison of trials to criterion on the stimulus discrimination task across conditions. Pre-SA (low) = baseline prior to self-administration, low reward contrast; Post-SA (low) = sessions during self-administration over the first 4-5

months, low reward contrast; Post-SA (high) = sessions during self-administration months 5-9, high reward contrast. Group X period interaction: $F_{(1,24)}=3.28$, p=0.055.

To explore the time course of the increase in trials to criterion, we evaluated performance in 3 session epochs. Although we lacked the statistical power (<0.1) to confirm a group by block interaction, the time course of the change in trials to criterion is shown in Fig 8. That change is significantly different between groups when evaluated over the entire post-admin low contrast period (Fig. 7).

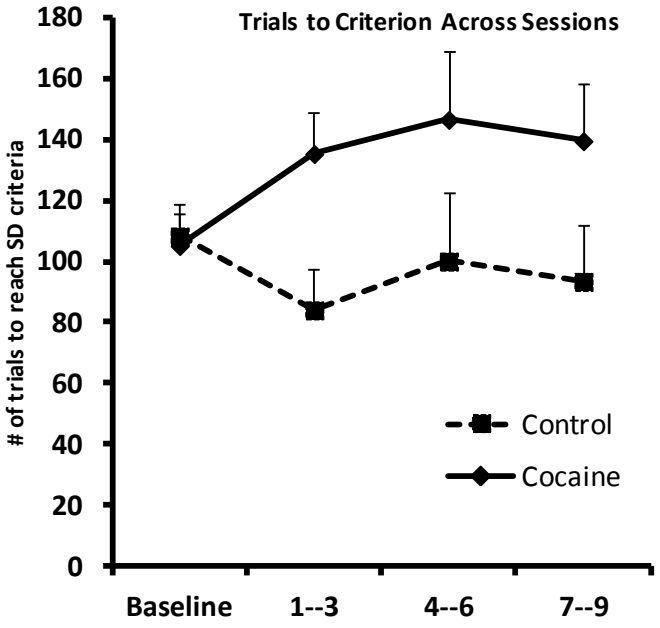

Figure 8 Stimulus discrimination performance across all sessions of the stimulus discrimination low bias task

Stimulus discrimination performance across all sessions of the stimulus discrimination low bias task. Sessions were binned by 3.

2.2.3 Stimulus Reversal Performance

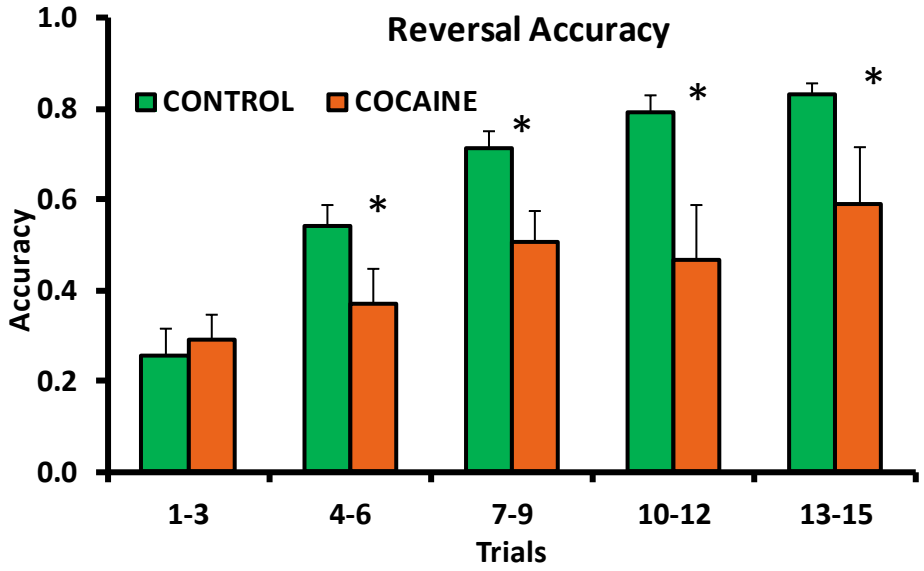

Figure 9 Between-group comparison of reversal performance on the high reward contrast stimulus discrimination/reversal task during months 5-9 of self-administration.

Between groups comparison of reversal performance on the high reward contrast stimulus discrimination/reversal task during months 5-9 of self-administration. The cocaine group shows a decrease in accuracy compared to controls. Two way repeated measures ANOVA shows a main effect of group ($F_{(1,11)}$=5.131; p= 0.045), and a group X bin interaction ($F_{(1,4)}$=3.49, p=0.015). * p< 0.05

Because not all cocaine animals were meeting discrimination criterion during the post-admin low contrast period, reversal performance could not be evaluated, necessitating the increase in reward contrast between stimuli. All animals subsequently reached criterion on the discrimination component of the stimulus discrimination/reversal task (though 1 of the 8 never reached criterion with enough (15) trials remaining in the fixed 200 limit to allow evaluation of reversal performance). In contrast to the lack of difference in discrimination accuracy between groups (Fig. 6), accuracy on the reversal learning component during the first 15 trials following the reversal was significantly poorer in the cocaine group,

with a two way repeated measures ANOVA indicating a main effect of group ($F_{(1,11)} = 5.13$, $p = 0.045$), and a group X bin interaction ($F_{(1,4)} = 3.49$, $p = 0.015$, Fig. 9).

Table 2 Number of reversal encountered per session

Period	Control	Cocaine
Baseline	1.00 ± 0.10	1.14 ± 0.16
Post-admin low contrast	1.22 ± 0.17	0.67 ± 0.20
Post-admin high contrast	1.51 ± 0.26	1.12 ± 0.27

The number of times each groups encounters the reversal trial period over all three blocks (baseline, post-admin low contrast, post-admin high contrast).

It is possible that less frequent encounters with reversals contributed to the deficits on reversal performance in the cocaine group. A two-way repeated measures ANOVA of reversals encountered per session did not reveal a group X period interaction ($F_{(1,11)} = 2.98$, $p = 0.072$) or a significant main effect. There was also not a significant difference between the two groups in the post-admin high contrast block (independent t-test $t_{(1,11)} = 1.08$, $p = 0.30$). Thus, reversal impairments in the cocaine group seen during the post-self-admin high contrast condition are not accompanied by less frequent encounters of reversals. Table 2 lists the number of reversals encountered by each group in the different periods, excluding the cocaine animal for which reversal accuracy was not available.

2.2.4 The Effects of Cocaine on Visual Working Memory

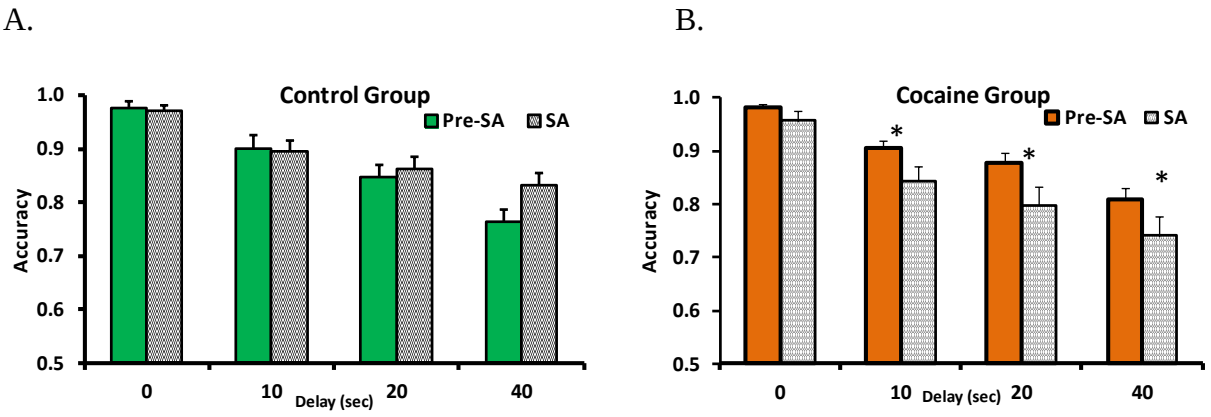

Figure 10 Within-group comparison of delayed match-to-sample performance

Within group evaluation of cocaine self-administration on the delayed match-to-sample performance. A) The control group did not show a significant difference in choice accuracy, when comparing baseline accuracy to the self-administration period. B) In the cocaine group, there was a main effect of period ($F_{(1,7)}$=8.62; p=0.02) and a significant interaction between period and delay ($F_{(3,36)}$=4.61; p=0.012), with accuracy decreased after cocaine self-administration compared to the baseline period.

A 3-way repeated measures ANOVA of delayed match-to-sample accuracy revealed a delay X period X group interaction ($F_{(3,36)}$ = 8.88, p<0.001). A Period X Group interaction ($F_{(1,12)}$=5.98; p=0.031) and Delay X Period interaction ($F_{(3,12)}$=3.61; p=0.022) was also revealed. A follow up two-way repeated measures ANOVA indicated that in the cocaine group, there was a main effect of period ($F_{(1,7)}$ = 8.62, p=0.02) and a significant interaction between period and delay ($F_{(3,36)}$ = 4.61, p=0.012), with accuracy decreased after cocaine self-administration compared to the baseline period (Fig. 10A). The delay dependency of the decreased accuracy is consistent with specific impairments in working memory following cocaine self-administration. The control group did not show a significant difference in accuracy (Fig. 10B), when comparing baseline performance to that during the self-administration period ($F_{(1,5)}$ = 0.56, p = 0.49) nor an interaction. To explore the time course of the decrease in working memory, we compared accuracy at the 40 s delay, averaged over four session epochs to baseline. A one-way repeated measures ANOVA

revealed that the first post admin epoch was significantly different from baseline. Increased variability associated with fewer sessions obscured differences from baseline in the later epochs. The time course for this metric is shown for both cocaine and control groups in Fig. 11.

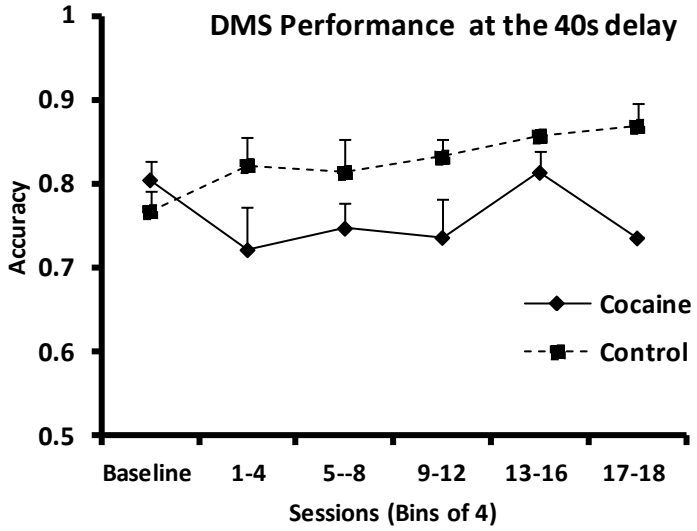

Figure 11 Time course of changes in accuracy at the 40s delay of the delayed match-to-sample task

A within group comparison analyzing accuracy at the 40 s delay of the delayed match-to-sample task across all sessions. Sessions were binned by 4. A post hoc analysis (Holm-Sidak) indicated the cocaine group had impaired working memory during sessions 1-4 post self-administration compared to baseline.

2.2.5 Comparison of Impairments across Cognitive Domains

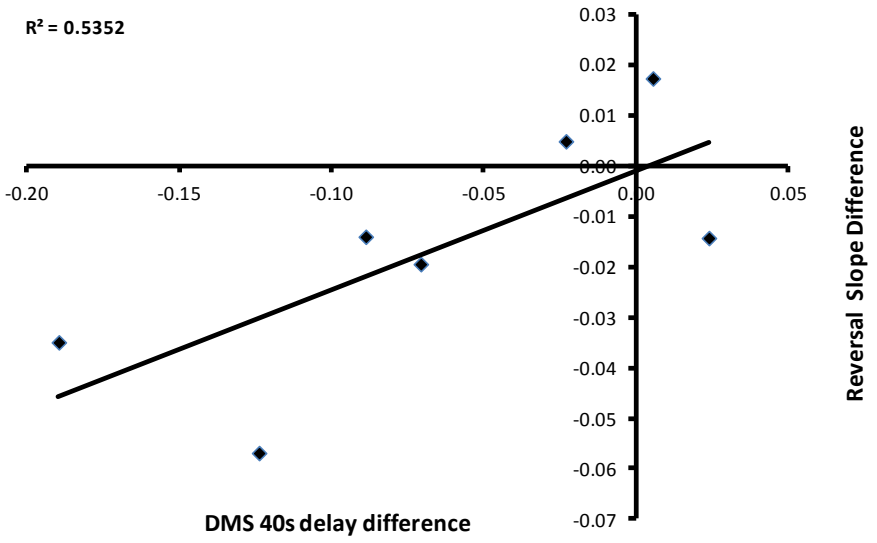

Figure 12 Regression of memory deficits with reversal deficits in the cocaine group

Regression of memory deficits with reversal deficits in the cocaine group. X axis is the difference in accuracy at the 40 second delay of the delayed match-to-sample task. Y axis is the difference in the reversal accuracy slope across the first 15 trials of each session. There was a marginally significant correlation between change in working memory and reversal performance. R^2=0.535 p=0.062

The extent to which impairments in one cognitive domain extend to another can help to address the possibility of a common underlying substrate or mechanism. To examine this, we evaluated the regression of changes in working memory performance pre and post self-administration with those of reversal performance. The metrics employed were accuracy at the 40 s delay interval and the slope of reversal accuracy across the first five (three trial) bins following stimulus reversal. There was a marginally significant regression ($F_{(1,6)}$=5.76p = 0.062, Fig. 12), consistent with the possibility of a common mechanism of impairment on the two tasks.

2.2.6 Impact of Cocaine Self-Administration on Water Reward Efficacy

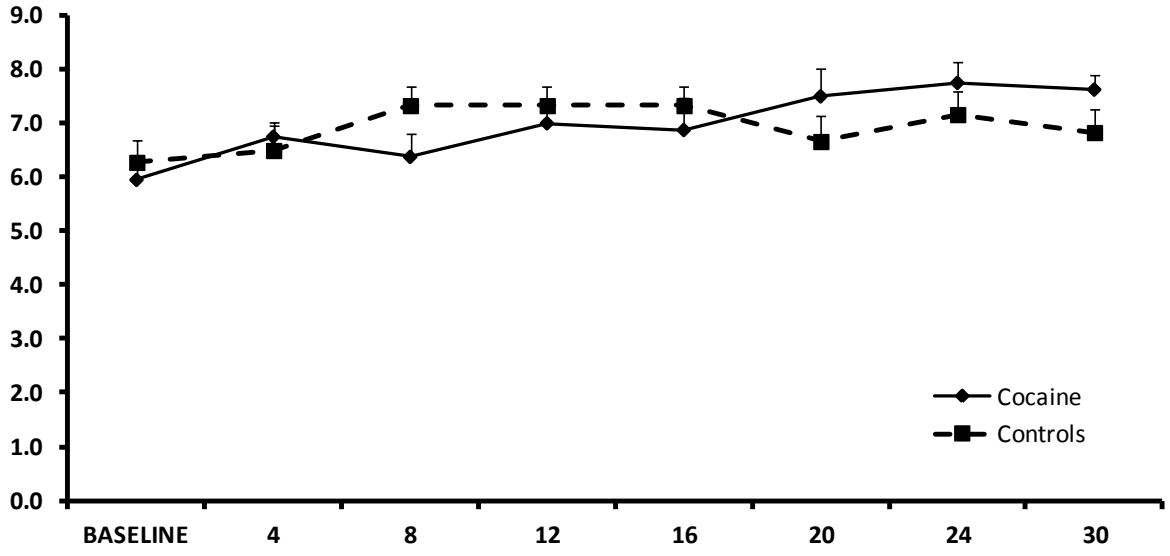

Figure 13 Between-groups comparison of water reward efficacy

Between groups comparison of water reward efficacy, measured by breakpoint on a progressive ratio schedule of self-administration. There was no difference between groups either before, or after self-administration.

Water reward efficacy (breakpoint on a progressive ratio task) was assessed weekly in both groups throughout the entire self-administration period (30 sessions). As can be seen in Fig. 13, there was no difference between groups in breakpoint over the self-administration period.

2.3 DISCUSSION

The nature and extent of cognitive deficits associated with cocaine use have been widely studied. However, whether they represent predisposing traits or consequences of drug use is difficult to assess in clinical investigations. We demonstrate by longitudinal assessment of cognitive function across multiple

domains in a non-human primate model that cocaine self-administration causes strong deficits in cognitive control/flexibility. Less pronounced deficits in visual working memory (delayed match-to-sample) and the ability to maintain a stimulus discrimination were also observed.

A stimulus discrimination/reversal task was used to assess associative learning and cognitive control/flexibility. During the first 4-5 months of self-administration, we did not observe a significant difference between the groups on stimulus discrimination accuracy, evaluated over the first 15 trials after presentation of a new set of stimuli. However, unlike all animals from both groups during baseline performance, and all control animals during self-administration, only 5 of the 8 cocaine animals were able to meet the stimulus discrimination criteria of 27/30 consecutively correct trials within a 200 trial session. This suggests intact associative learning, but difficulty in maintaining the high level of accuracy needed to reach criterion performance.

The improved discrimination performance after increasing the reward contrast between stimuli permitted a comparison of cognitive control/flexibility (reversal performance) between groups. The cocaine group was less able to adapt their responding to the reversal of the reward contingencies, consistent with the clinical literature indicating that chronic cocaine users show impaired reversal performance, while the acquisition of an initial stimulus-reward association was not impaired (Fillmore and Rush, 2006; Ersche et al., 2008). These results also extend previous findings with non-contingent cocaine exposure in vervet monkeys (Jentsch et al., 2002) and rodents (Schoenbaum et al., 2004). While a reduced frequency of encountering reversals during the post-admin low contrast period could have contributed to the impaired reversal performance, the task had been well learned prior to initiating self-administration, and there was no difference in frequency of reversals during the post-admin high contrast period. Though recent reports indicate that damage to medial striatum can impair reversal performance (Clarke et al., 2008), such effects are most frequently associated with orbitofrontal cortex dysfunction (Butter, 1969; Hornak et al., 2004; Izquierdo et al., 2004). This region shows structural (Franklin et al.,

2002) and functional (Volkow et al., 1993; Grant et al., 2000; London et al., 2000) abnormalities in drug using populations. Given its role in inhibitory control and decision-making, it plays a central role in heuristic conceptualizations of cortical systems implicated in addiction and other syndromes of impaired consumptive inhibitory control (Bechara, 2005; Schoenbaum et al., 2006; Schoenbaum and Shaham, 2008; Volkow et al., 2008).

There have been inconsistencies in the literature in regard to whether cocaine exposure affects working memory. Some clinical studies (Hoff et al., 1996; Bechara and Martin, 2004; Kubler et al., 2005), and a rodent self-administration study (George et al., 2008), have shown working memory deficits in cocaine using populations. In other cases no deficits have been reported (Bolla et al., 1999; Pace-Schott et al., 2008). That inconsistency may be a reflection of a modest effect size. We were able to match performance very carefully between our groups prior to any exposure. That, combined with a longitudinal approach enabled us to observe what appears to be a clear, though relatively modest, working memory impairment which was apparent soon after initiating exposure. While visual working memory is usually associated with ventrolateral prefrontal cortex (Wilson et al., 1993), orbitofrontal contributions are also apparent (Otto and Eichenbaum, 1992; Wallis, 2007), consistent with the marginally significant relationship we observed between working memory and reversal impairments.

It appears that deficits emerge relatively quickly after initiation of self-administration. All assessments were made three days after last exposure, thus a "short-term withdrawal" effect is possible. Withdrawal is a difficult concept to address with cocaine use. Preliminary results indicate no difference in total sleep and average sleep epoch duration between groups over the weekend prior to testing (Gomez et al., 2010). Thus, dysregulation of cocaine's pharmacological targets is more likely than a generalized fatigue or sleep deprivation.

It has been noted that performance on a wide range of cognitive tasks is often inextricably linked to attention and reward (Maunsell, 2004; Sarter et al., 2006; Kennerley and Wallis, 2009). We believe the progressive ratio evaluation of water reward efficacy indicates no difference between our groups in motivation. However, increasing the reward contrast between stimuli on the discrimination task improved performance in the cocaine, but not the control group. Thus, it appears that an interaction between reward incentive and task difficulty (Maunsell, 2004) distinguishes between our groups. For very simple tasks, such as the progressive ratio task in which only repetitive touching to a non-moving stimulus is needed, the incentive of water reward is unchanged. However for a more difficult task such as the stimulus discrimination, or perhaps the working memory task, greater rewards are needed to support the focused attention required by the task (Maunsell, 2004). Given the importance of dopamine in signaling rewards (Schultz, 2007), and the clear dysregulation by cocaine of dopamine systems (Weiss et al., 1992; Letchworth et al., 2001; Nader et al., 2002; Nader et al., 2006), and reward circuitry in general (Kalivas and Volkow, 2005), it is also possible that reward incentives are communicated across systems less effectively, requiring a greater contrast between different rewards to effectively engage cognitive networks.

Attentional impairments are possible in the cocaine exposed animals, given their difficulty in maintaining high levels of accuracy on the discrimination task needed to achieve criterion. We have also presented preliminary results of increased intra-individual variability in response times in the cocaine animals (Olsen et al., 2009), an observation consistent with greater attentional lapses (Castellanos et al., 2005), and increased distractibility in them as well (Porter et al., 2010). Thus, to the extent that working memory and attention are overlapping, increased distractibility or attentional lapses could have contributed to errors on the delayed match-to-sample task (Duncan, 2001). A common substrate, such as impaired attention, as a contributor to the general pattern of deficits is also suggested by the trend toward a correlation of impairments in working memory and reversal performance across individuals,

In contrast to the current findings in which a well-learned discrimination task did not reveal impairments in accuracy over the first 15 trials following cocaine self-administration, we previously reported (Liu et al., 2008) that initial acquisition of a stimulus discrimination task and a spatial working memory task was significantly impaired in a group of monkeys with a history of self-administration. Following extensive training, animals that showed impaired stimulus discrimination task acquisition eventually became equally adept as the control group (unpublished results). This pattern of acquisition impairment is similar to the effects of orbitofrontal lesions (Otto and Eichenbaum, 1992). We believe the more pronounced discrimination impairment seen previously (Liu et al., 2008) reflects an inability to focus on the relevant structure of a new task to be acquired in the animals with a history of drug exposure. In contrast, if a learning set has already been established by experience with the task (Harlow, 1949), performance is much less impaired by experimental manipulations, as the present study indicates.

The contrast between effects of cocaine on novel task acquisition versus performance of a familiar one, and the somewhat generalized effects observed, is consistent with the adaptive coding model of prefrontal cortex function proposed by Duncan. That model posits a less compartmentalized and regionally specific distribution of function than often presumed, evidenced by the high level of adaptability of broad areas of prefrontal cortex to accommodate specific task contingencies. It is proposed that part of the function by which the prefrontal cortex mediates acquisition of task contingencies is also by minimizing distractibility, or increasing focused attention. To quote Duncan: "In this model, working memory, selective attention and control are simply three different perspectives on the same underlying processing function" (Duncan, 2001). We also feel that Sarter's description of "attentional effort" (Sarter et al., 2006) needed for task performance is also an intuitively appealing description of the impairment in the cocaine-exposed animals. Thus, the overall nature of the impairments appears to be a mixture of

highly selective effects on orbitofrontal cortex associated reversal performance along with a more generalized impairment of prefrontal dependent working memory/attentional focus.

These results represent a unique study in which a broad range of cognitive domains were studied longitudinally in non-human primates to determine the effects of chronic cocaine self-administration. The results strongly suggest that, in addition to the substantial literature indicating the contribution of inherent differences between individuals for risk of addiction (Deroche-Gamonet et al., 2004; Tarter et al., 2007; Belin et al., 2008), cocaine use by itself causes cognitive deficits. Understanding the neurobiological basis of these deficits may help in the development of therapeutic approaches to address them, potentially increasing the likelihood of abstinence based on the links between cognitive performance and treatment outcome.

3.0 LATENT VULNERABILITY IN COGNITIVE PERFORMANCE FOLLOWING CHRONIC COCAINE SELF-ADMINISTRATION *PUBLISHED PORTER ET AL., 2012

Cocaine dependent individuals display selective cognitive deficits (Beatty et al. 1995; Bolla et al. 1998; Bolla et al. 2003; Ersche et al. 2010; Ersche and Sahakian 2007; Kaufman et al. 2003; O'Malley et al. 1992). Some are relatively specific, such as impairments in reversal performance, a measure of cognitive control, whereas others are more general, such as attentional impairments (Jovanovski et al. 2005). It might be predicted that there would be a selective interaction between attentional and other domain-specific cognitive deficits, however, this has been little studied outside of one report employing a working memory task (Hester et al. 2006). We previously demonstrated large impairments in cognitive control/flexibility, assessed using stimulus reversal performance, and more modest impairments in visual working memory in drug-free rhesus monkeys after extended chronic cocaine self-administration (Porter et al. 2011). Stimulus discrimination was not affected. Reversal impairments likely reflect damage to the orbitofrontal cortex, an area repeatedly shown to be abnormal in cocaine dependence (Bolla et al. 1998; Lucantonio et al. 2012; Volkow and Fowler 2000; Volkow et al. 1991) and addiction in general (Schoenbaum and Shaham 2008). The orbitofrontal cortex is implicated in the valuation of rewards and stimuli that represent them (Haber and Knutson 2010; Padoa-Schioppa and Assad 2006; Roesch and Olson 2004; Rolls 1996; Schoenbaum et al. 2003; Schultz 2000), but is also implicated in decision making and inhibitory control (Rolls and Grabenhorst 2008). Given the

importance of these functions in maintenance of sobriety, it is important to evaluate whether environmental events, associated with drug use or otherwise, could selectively impair the proper function of a key brain region whose integrity is necessary for good decisions and self-control.

In the current study, following extended cessation from cocaine in a subset of the previous group we reported on (Porter et al. 2011), we again evaluated domain-specific cognitive performance. Unlike our previous work, animals were tested with and without two types of attentional distractors. One distractor was an appetitive compound contextual cue previously paired with either cocaine (in the experimental group), or water (in the control group). The other distractor was a novel compound stimulus. Given the importance of attention to cognitive function in general (Maunsell 2004), we predicted that an attentional challenge, such as the presentation of a distractor, would produce a selective pattern of impairment that would recapitulate the pattern of impairments seen during active cocaine self-administration, despite the fact that performance between the control and cocaine groups was equivalent following the extended cessation. Selective impairments by the distractors, despite equivalent performance in their absence, would represent a latent vulnerability consistent with long term dysfunction. Because of reports of greater attentional bias in favor of drug-related cues (Carpenter et al. 2006; Copersino et al. 2004; Ersche et al. 2010; Hester et al. 2006; Hester and Garavan 2009), we also predicted that a cocaine associated distractor would produce a greater impairment than the novel distractor in the cocaine experienced animals.

3.1 METHODS AND MATERIALS

3.1.1 Subjects

Young adult (6-7 years old at the time of testing) male rhesus macaque monkeys (n=11) with previous drug exposure and extensive behavioral training were used for the present study. These monkeys participated in a previous study, which looked at the effects of chronic cocaine self-administration on cognition (Porter et al. 2011). We were only able to use a subset of animals for this study due to unexpected illness and loss of motivation to work. From the original group of 8 cocaine and 6 control animals, one cocaine animal was deceased due to lymphoma, and another was removed from the study due to behavioral problems. For the discrimination/reversal task, one cocaine animal failed to reach criteria during the discrimination blocks, leaving 6 control and 5 cocaine animals. For the DMS task, one of the controls was not cooperative, leaving 6 cocaine and 5 control monkeys. Animals were water-regulated 5 days a week and were supplemented to meet physiological needs at the end of each day following training and testing. Animal use conformed to the "Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research" (National Research Council 2003).

3.1.2 Apparatus

All cognitive assessments took place in a sound attenuated chamber (Eckel Industries, Ontario, Canada, model AB4240) fitted with a 40 W house light and background white noise. The E-prime software suite (Psychology Software Tools, Pittsburgh, PA), coupled with a 15" touch

screen (Elo systems CarrolTouch), was used for all stimulus presentation, recording of responses, and initial data processing.

3.1.3 Surgery

Prior to self-administration, all animals had a vascular access port placed midscapula from which a catheter extended subcutaneously to an internal jugular vein. The vascular access port allows percutaneous non-stressful access to vasculature for cocaine self-administration without the need for a protective jacket and with reduced risk of infection because nothing is external to the skin (Wojnicki et al. 1994).

3.1.4 Self-Administration

The self-administration protocol and cognitive characterization of animals has been previously published (Porter et al. 2011). Animals self-administered by touching an abstract shape on the touch screen for the required number of touches. Once the response requirement was met, either cocaine (cocaine group) was administered intravenously via the vascular access port or water (control group) via a sipper tube. During the cocaine self-administration sessions, animals were allowed to self-administer up to 6 infusions of cocaine at a unit dose of 0.5 mg/kg, which they typically did. The cumulative amount of cocaine self-administered over a 12-month period ranged from 528-546 mg/kg.

3.1.5 Stimuli used as distractors

During the chronic cocaine or water self-administration, an audiovisual compound stimulus had been present for the entirety of each self-administration session, except during the time of reward delivery. This stimulus consisted of a distinct sequence of tones (rising or falling) and a distinct visual border around the screen. Because the stimulus was always present in the background during the self-administration session, it is considered to be a contextual cue. In that regard, it is important to note as well, that all self-administration and cognitive testing took place in identical chambers. Two compound stimuli used as distractors were counterbalanced across groups: half of the cocaine group and half of the control group saw an abstract "blue sun" border with descending tones (stimulus set 1). The other half of the each group saw a red "Navajo blanket" border and heard ascending tones (stimulus set 2). The stimulus set present during self-administration was the appetitive distractor, the other was the novel distractor.

3.1.6 Experimental timeline: counterbalancing of distractors across cognitive tasks

Animals had not self-administered cocaine for 3 months before we assessed whether novel or appetitive distractors would interfere with associative learning, reversal performance and/or working memory. Because the aim of the study was to assess selective attentional vulnerability across cognitive domains, we counterbalanced stimulus presentation across the different cognitive tasks. As a conservative approach, we chose to use an ascending order of predicted influence to avoid possible accelerated habituation of a novel distractor by an appetitive one. As a result, we did not counterbalance across cue types. We first evaluated the novel distractor across all domains, then repeated that with the appetitive distractor. Six baseline sessions for

both cognitive tasks (no distractor presentation) were collected. Then, the novel distractor was presented during the stimulus discrimination/reversal and delayed match-to-sample (DMS) tasks. Table 3 shows a daily schedule for how the distractors were distributed across the cognitive tasks. We followed the same daily cue presentation schedule during cognitive assessment for both novel stimuli and appetitive distractors (Table 3). Establishing the post-cessation baseline, the novel distractor effects, and the appetitive distractor effects each took approx. one month, beginning at three months after cocaine self-administration ceased.

3.1.7 Distractor presentation during stimulus discrimination/reversal task

We probed associative learning and cognitive control/flexibility using a stimulus discrimination/reversal task, similar to the task used previously to show reversal deficits resulting from cocaine exposure (Porter et al. 2011). For this experiment, we used a 2-object stimulus discrimination task instead of the 3-object stimulus discrimination task used previously because of motivational issues in some of the monkeys from both groups. Given inherent variability in cognitive performance data, we needed to ensure compliance in the small number of sessions used for distractor presentations. In brief, two stimuli were associated with a high or low water reward. A correct response was recorded when the monkey touched the stimulus associated with the high reward. Once a criterion of 27/30 correct responses on consecutive trials was reached, the reward contingency was reversed. Reaching the same performance criterion after the reversal resulted in presentation of a new set of stimuli for a discrimination block. Distractor presentations during the stimulus discrimination and reversal blocks were alternated across sessions. Animals performed 6 sessions per distractor type (novel or appetitive); 3 sessions with a distractor presented during the first 20 trials of stimulus discrimination

component and 3 sessions in which the cues were presented during the first 20 trials of the reversal component of the task. This allowed us to counterbalance distractor presentations across trial types and minimize habituation (Table 3).

Table 3 Schedule for counterbalancing stimulus presentations across tasks

Weekday:	Environmental Stimulus Presentation:
Monday	Stimulus Discrimination
Tuesday	Stimulus Reversal
Wednesday	DMS (Block 2; delay period only)
Thursday	Stimulus Reversal
Friday	Stimulus Discrimination

3.1.8 Distractor presentation during DMS task

We intermixed associative learning and reversal performance assessment with working memory assessment. The DMS task employed here is the same task used previously to assess working memory during self-administration (Porter et al. 2011). In brief, a sample stimulus would appear on the touch screen to start each trial. Pressing the sample stimulus accurately and holding it for 1s led to its offset and the start of a delay period (randomly selected from 0, 10, 20, or 40s). Following the delay period, the sample and a novel stimulus (randomly selected from the image pool) appeared, randomly assigned to the left or right side of the screen. Choosing the sample stimulus within 10 s following the presentation of the two stimuli led to a water reward (0.075 ml/kg). No reward was delivered for choosing the wrong stimulus or pressing the area outside of the choice stimuli. The inter-trial intervals for a correct response and incorrect response were two seconds and seven seconds, resp. Not responding within a 10 second window resulted in an omission. This task was performed once a week (Table 3), and was broken up into three blocks,

in order to limit distractor exposure time. The first 60 trials (block 1) were performed in the absence of a distractor, trials 61-100 (block 2) were performed with a distractor presented for 7s during the delay period) and trials 101-160 (block 3) were again performed in the absence of a distractor.

3.1.9 Behavioral Analysis

Stimulus discrimination and reversal task accuracy was averaged over the first 10 trials of the stimulus discrimination and reversal component, during which the distractor was presented. Because of the few number of sessions, we could not evaluate performance across the first fifteen trials in bins of three as previously reported in Porter et al., (2011), but for comparison purposes, data for the reversal task is shown in that format in Fig. 16. For both distractor types, performance over the three sessions with distractors was compared with baseline performance (mean of six consecutive sessions without distractor stimulus presentation). A main effect of distractor type on stimulus discrimination and reversal performance was determined using repeated measures analysis of variance (RM ANOVA; SigmaStat 3.5). Post-hoc analysis was used for comparing each distractor type to baseline. All statistical analyses were conducted using absolute numbers. Results for stimulus discrimination and reversal tasks are presented as percent of baseline performance to facilitate visualization of effects.

For the DMS task, baseline data were averaged over six consecutive sessions for comparison of performance between groups following cessation. Distractor effects on accuracy were determined over 3 sessions for each type (novel or appetitive). We conducted a within session analysis, comparing performance in the absence of distractor (block 1 and block 3) to

performance in the presence of environmental distractors (block 2). Accuracy was analyzed over the first 20 trials during each block. For each stimulus type, we conducted a two way-RM ANOVA with two factors: block and delay interval. Differences between blocks at given delays were evaluated with Tukey post-hoc tests.

3.2 RESULTS

3.2.1 Baseline cognitive Assessment

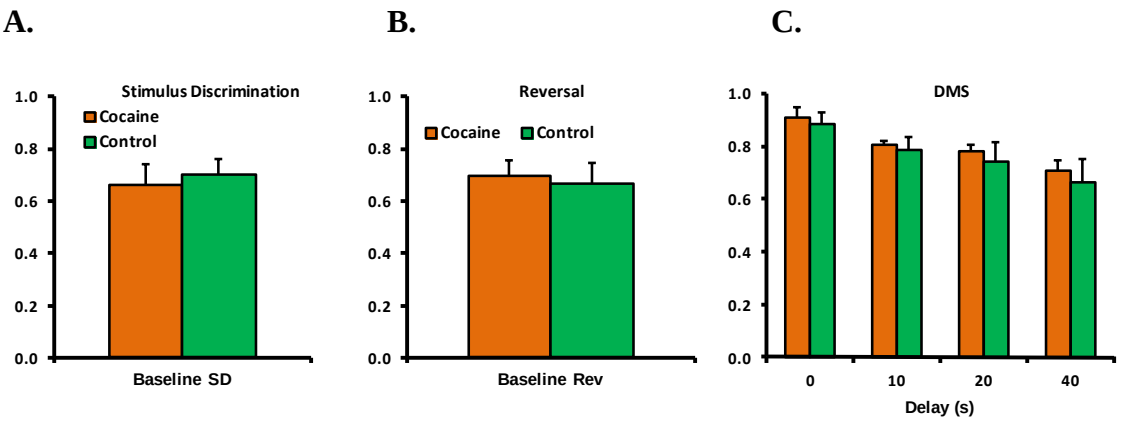

Figure 14 Post cessation performance following 3 months cessation from cocaine

Post cessation performance following 3 months cessation from cocaine on (A) stimulus discrimination, (B) reversal, and (C) DMS performance. There was no significant difference in performance between the groups on any task prior to distractor presentations.

There were no significant between group differences in accuracy on stimulus discrimination (Fig. 14A), reversal performance (Fig. 14B), or the DMS task (Fig. 14C). It should be noted that because we employed a two stimulus discrimination/reversal task, there is a quicker increase in

accuracy following reversal than previously observed with the three stimulus task used in Porter et al. (2011). Compare Fig. 4B from chapter 2 to baseline performance in Fig 14B herein.

3.2.2 The effect of novel and appetitive distractors or stimulus discrimination/reversal performance

There was no significant effect of either the novel or appetitive distractor on stimulus discrimination in either of the groups (Fig. 15A). However, there was a main effect of distractor on reversal performance (Fig. 15B) relative to baseline in the cocaine group ($F_{(4,2)}$=7.08; p=0.02). A follow up *post hoc* test revealed that performance during the novel (p<0.01) and the appetitive distractor (p=0.04) was decreased relative to baseline in the cocaine group. Fig. 16 illustrates the impact of each distractor across the first fifteen trials following stimulus reversal in the cocaine group relative to baseline performance, and shows the control group baseline performance. Neither distractor had an effect on reversal performance in the control group.

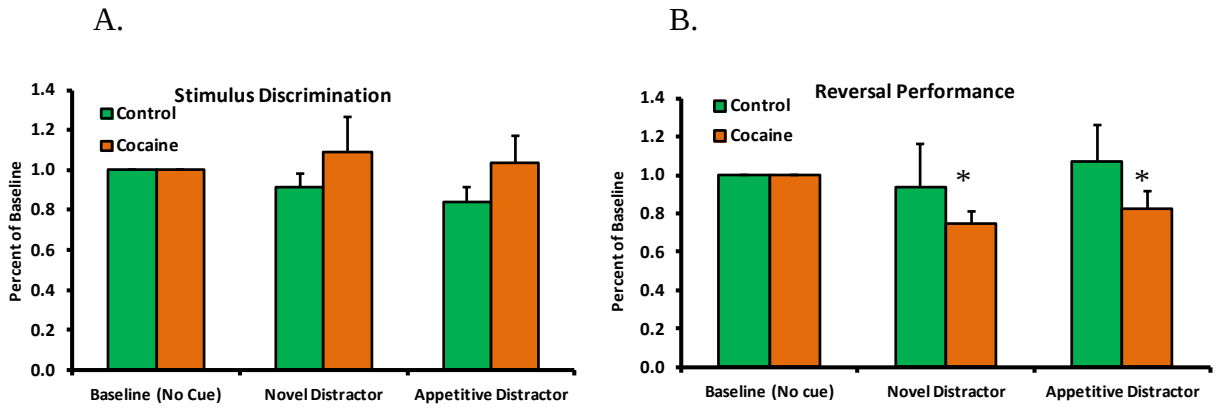

Figure 15 Accuracy on the stimulus discrimination and reversal task

Accuracy on the stimulus discrimination and reversal tasks in the presence of distractors. (A) Neither the cocaine group nor the control group showed an impairment in stimulus discrimination performance in

the presence of either distractor relative to baseline. (B) Within-group comparison of reversal performance during distractor presentation over the first 10 trials. *p<0.05 compared to baseline

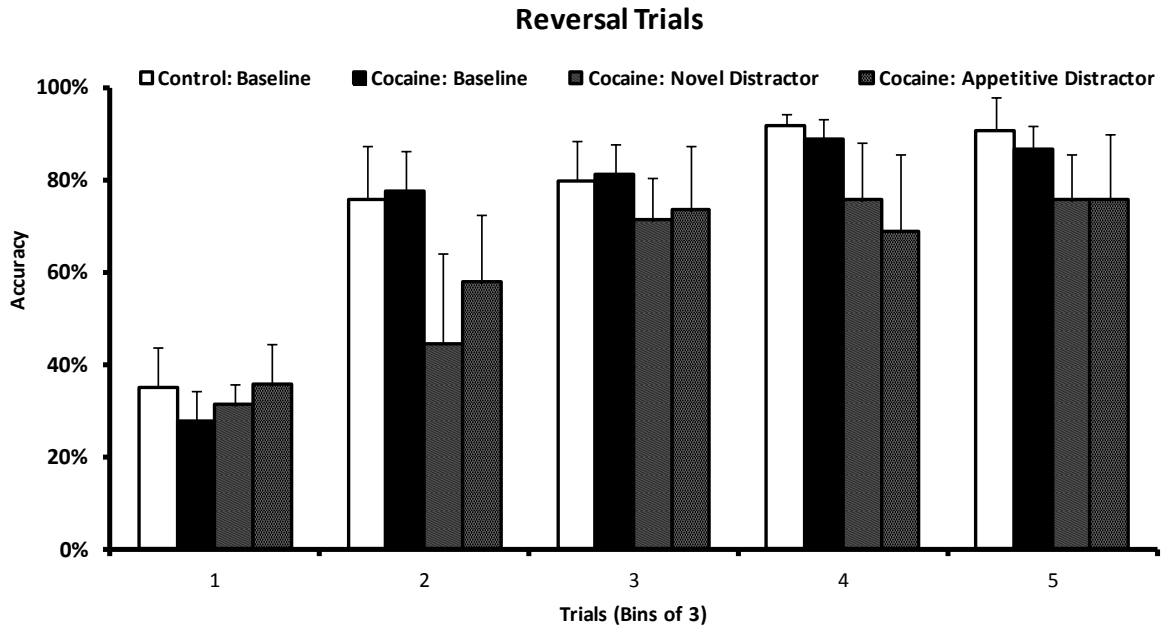

Figure 16 Progression of performance after reversal for both groups at baseline and in the presence of distractors within the cocaine group

Progression of performance after reversal for both groups at baseline, and in presence of distractors within the cocaine group. white = control baseline, black = cocaine baseline, diagonal = cocaine novel, horizontal = cocaine appetitive

3.2.3 The effect of novel and appetitive distractors on DMS performance

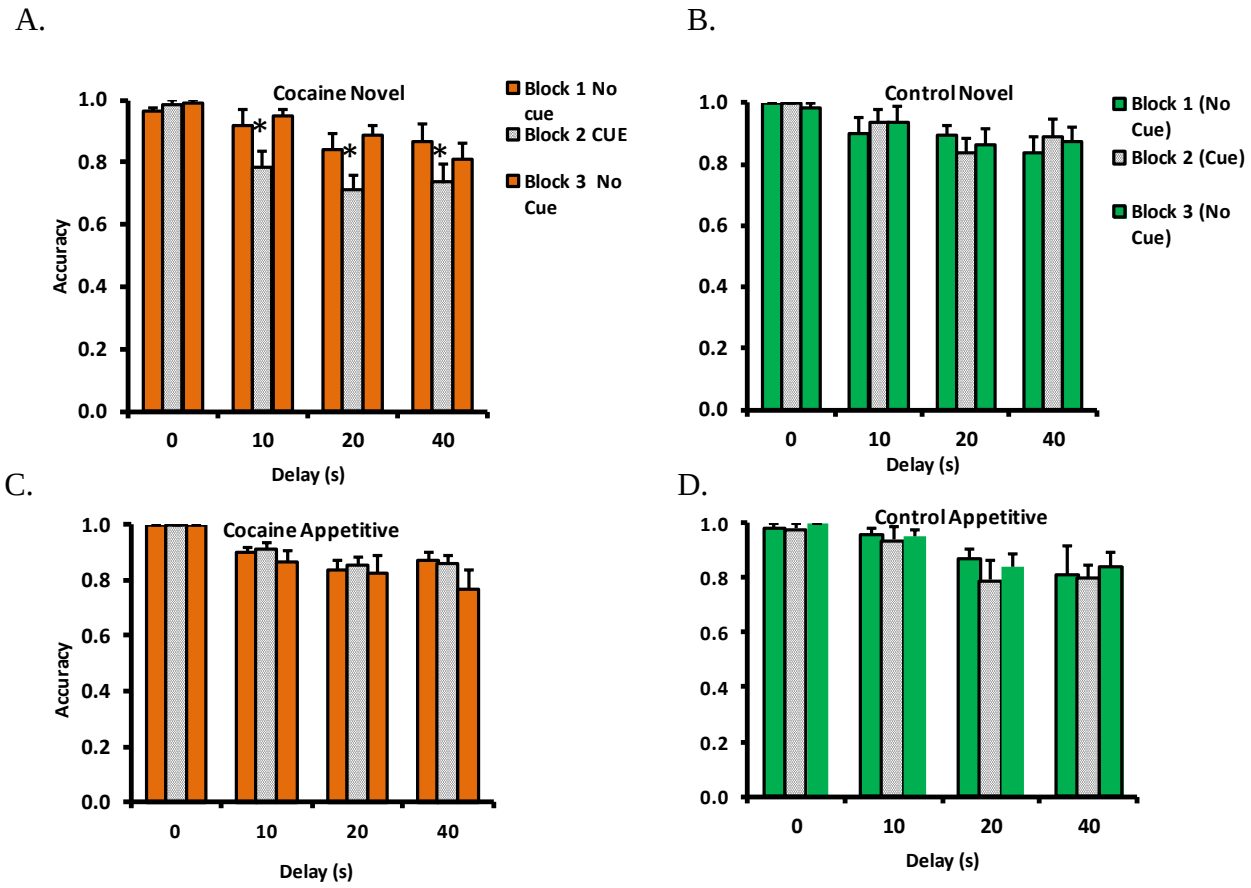

Figure 17 Within session effect of attentional distractors on DMS performance

Within session effect of attentional distractors on DMS performance. (A) In the cocaine group, there was a main effect of block ($F_{(5,2)}$=5.02; p=0.031), with a decrease in performance in the presence of the novel distractor (block 2) relative to the non-stimulus blocks 1 and 3. (B) In the control group, there was no effect of the novel distractor on working memory. In the presence of appetitive cues, neither the (C) cocaine group nor (D) control group showed impaired performance. *p< 0.05 relative to non-cue blocks. Solid bars are blocks of trials without distractors, cross-hatched bars are distractor blocks.

There was a main effect of the novel distractor (Fig. 17A) on working memory in the cocaine group ($F_{(5,2)}$=5.02; p=0.031) relative to non-distractor blocks. There was no effect of the novel distractor on the control's group performance (Fig. 17B). We did not observe an effect of the appetitive distractor on performance in either the cocaine group (Fig. 17C) or the control group (Fig. 17D).

3.3 DISCUSSION

The primary aim of this study was to examine the effects of attentional distractors on domain specific cognition in control and cocaine exposed monkeys following an extended cessation, after which there were no differences in performance. Secondly, we assessed whether the presentation of "appetitive" distractors previously paired with either drug availability in the cocaine group, or water availability in the control group would disrupt cognitive performance more than a novel, though similar distractor. Our data show a selective vulnerability to attentional challenge in the cocaine group across the same cognitive domains that were impaired even without distractors when tested (in a drug-free state) during chronic cocaine self-administration. This indicates continuing long-term dysfunction consistent with orbitofrontal cortex damage. Given the role of anterior cingulate cortex in attentional control, it is potentially implicated as well. We did not see greater impairments with the cocaine associated distractor compared to the novel stimulus.

3.3.1 Long-term domain specific impairments

Following the long term cessation, cognitive performance across the three tasks employed was equivalent between the cocaine and control groups. Thus, the pattern of selective impairments in which reversal performance was greatly impaired and DMS performance was modestly impaired was no longer evident. However, given clinical observations of cocaine-associated structural changes in the orbitofrontal and anterior cingulate cortex (Bartzokis et al. 2002; Ersche et al. 2011; Franklin et al. 2002; O'Neill et al. 2001; Sim et al. 2007), we hypothesized that an attentional challenge could reveal latent continuing dysfunction. Our main finding is that there remains a latent impairment in reversal performance that can be revealed by an attentional challenge. In the cocaine group, reversal performance was impaired by environmental distractors, whether they were novel or drug associated, whereas there was no effect of either on stimulus discrimination performance. Neither the novel, nor the appetitive distractor had an effect on stimulus discrimination or reversal performance in the control group. These data are in line with previous findings which showed that chronic cocaine resulted in impaired reversal performance, while having no effect on stimulus discrimination performance in monkeys (Gould et al. 2012; Jentsch et al. 2002; Porter et al. 2011). An important question also is whether baseline performance was equivalent due to cessation of cocaine, or whether continued practice helped the animals to overcome deficits previously seen during active self-administration using a three stimulus task (different from the two stimulus task used in the present report). The difference in task structure, and the lack of a comparison group that continued to self-administer cocaine to control for practice effects makes this impossible to answer.

We also examined working memory performance in the presence of novel and appetitive distractors. The presence of the novel distractor impaired working memory performance in the

cocaine group, relative to the non-distractor blocks. There was no effect of the novel distractor on working memory in the control group, and no effect of the appetitive distractor on working memory in either group. In our previous report examining the effect of chronic cocaine self-administration on working memory, the impairment was less robust than seen on reversal performance (Porter et al. 2011), similar to another recent report (Gould et al. 2012). In general, there are inconsistencies in the literature on whether working memory is impaired by cocaine exposure, with some reports indicating impairments in working memory (Bechara and Martin 2004; Hoff et al. 1996; Kubler et al. 2005; O'Malley et al. 1992; Verdejo-Garcia and Perez-Garcia 2007) whereas others do not (Bolla, 1999 #4540; Pace-Schott, 2008 #5884).

Thus, regardless of distractor type (novel or appetitive), we see a pattern of latent vulnerability in the cocaine group relative to the control group that recapitulates the frank dysfunction apparent previously, when chronically self-administering animals were tested drug free (72 hours post-cocaine). At that time, reversal performance was strongly impaired, DMS performance was weakly impaired, and stimulus discrimination performance was unimpaired.

3.3.2 Comparison of novel and appetitive distractors

We failed to see evidence of an attentional bias toward the appetitive distractor on any task. Despite the greater attention novel stimuli attract over habituated ones, we hypothesized that distractor stimuli repeatedly paired with drug exposure would be even more intrusive. Our intent to assess the impact of the distractors across multiple cognitive domains resulted in a design limitation in which the distractor order was not counterbalanced. Our conservative choice to initially expose animals to the novel distractor meant that evidence of greater impairment by the appetitive distractor in the cocaine group would have been strong evidence of attentional bias.

However, the pattern of results we observed does not permit strong interpretive statements. There is the possibility of generalization of the novel and appetitive distractors. The same frequency tones were used as audio components in both compound stimuli used as distractors, with the only difference being whether they were ascending or descending. Likewise, though the visual components were distinct, there was some similarity in that each formed a border around the touchscreen. The pattern of results seen in the DMS task in particular suggests a habituation in that the weak impairment seen with the novel distractor was lost when the studies progressed to the appetitive distractor presentation. The observation that an impairment in reversal performance remained during appetitive distractor presentation, whereas the DMS performance was unimpaired, suggests the appetitive distractor was more intrusive on cognitive control/flexibility (reversal performance) than on working memory (DMS). In a relevant clinical study, Hester et al., (Hester and Garavan 2009) conducted a study into neural mechanisms underlying attentional bias to cocaine cues in cocaine users, while varying working memory load. When cocaine stimuli were presented, active cocaine users showed a significant decrease in accuracy and increase in response time under high working memory load compared to controls. In addition to the impact of the design limitations discussed above, our experiment differed from that of Hester et al. in that our monkeys had not received cocaine for at least 3 months before we conducted the experiments, whereas Hester et al. examined active cocaine users (Hester and Garavan 2009). In another clinical study, when cocaine dependent subjects performed a color-word drug Stroop fMRI task, hypoactivations in the rostro-ventral anterior cingulate and medial orbitofrontal cortex were associated with a greater likelihood of errors (Goldstein et al. 2007). The pattern of hypoactivation of orbitofrontal cortex and anterior cingulate cortex has been seen in many types of tasks (Bolla et al. 2004; Hester and Garavan 2004; Li et al. 2008). The rostral

anterior cingulate has been implicated in attentional control and the orbitofrontal is critical for proper reversal performance. The enduring latent vulnerability to impairment by environmental distraction we observed in this controlled animal study is consistent with long lasting damage to these regions associated with drug exposure per se. Given that performance on cognitive tasks dependent on these areas is predictive of treatment outcome (Aharonovich et al. 2006; Aharonovich et al. 2003; Patkar et al. 2004; Streeter et al. 2008), understanding the neurobiological basis of the impairments we observed, and whether they respond to behavioral or pharmacotherapeutic approaches has the potential to improve clinical outcomes.

4.0 LONG LASTING CHANGES IN CEREBRAL METABOLIC FUNCTION IN DRUG FREE RHESUS MACAQUE MONKEYS THAT PREVIOUSLY SELF-ADMINISTERED COCAINE

Cocaine addiction is a disorder associated with chronic risk of relapse even after long-term abstinence. Abstinent cocaine users suffer from multiple cognitive deficits (Tomasi, Goldstein et al. 2007); (Hanlon et al., 2011); (Moeller, Maloney et al. 2010); (Kelley, Yeager et al. 2005); (Bolla, Ernst et al. 2004);(Bolla, Eldreth et al. 2003); (Beatty, Katzung et al. 1995). These cognitive deficits have been shown to be inversely correlated with retention and success of behavioral treatments (Moeller, Maloney et al. 2010); (Brewer, Worhunsky et al. 2008); (Aharonovich, Hasin et al. 2006); (Turner, LaRowe et al. 2009). It has been hypothesized that frontal abnormalities, whether functional (Moeller, Maloney et al. 2010); (Tomasi, Goldstein et al. 2007) or structural (Hanlon et al., 2011);(Matochik, London et al. 2003), may underlie the cognitive disruption observed in cocaine abusers during abstinence. While cortical areas have gained the most attention, there is some evidence suggesting that cerebellar activity is altered in cocaine abusers. Hester and Garavan (2004) using fMRI were the first to observe that the cerebellum may compensate for hypoactivity in cortical areas in active cocaine users. Active users showed increased cerebellar activity while performing a Go/No-Go task with increased working memory demand. Even though active cocaine abusers showed increased cerebellar activity, their performance was decreased relative to controls. Tomasi, et al (2007) conducted an

fMRI experiment in early abstinent cocaine users performing a verbal working memory task (Tomasi et al., 2007). Abstinent cocaine abusers showed widespread disruption in cortical areas, but also showed increased cerebellar activity, which correlated with better working memory performance in cocaine users only. While this may seem to contradict previous findings, it suggests the cerebellum may become more efficient over periods of abstinence via its interactions with cortical areas necessary for working memory performance. It is unclear whether the cerebellum continues to be altered in long-term cocaine abstainers and whether it is able to compensate in a manner that behavior is drastically improved. The purpose of this study was to look at cerebral metabolic function in rhesus macaque monkeys that were drug free for 20 months after self-administering cocaine chronically for 12 months. Using [^{18}F]-2deoxy-2-fluoro-D-glucose (FDG) with positron emission tomography (PET) during a visual working memory task (delayed match-to-sample task; DMS) allowed us to examine whether there were any long lasting effects of chronic cocaine self-administration on cerebral metabolic function. We hypothesized that the DLPFC, a region shown to be active during a visual working memory task (Porrino et al., 2005), would show a disruption in the cocaine group compared to matched controls. More specifically, we hypothesized a decrease in DLPFC activity as measured by FDG-PET. In light of work conducted by Hester and Garavan (2004) and Tomasi et al., 2007, we also hypothesized that there may be cerebellar differences between the groups. The cerebellum may have greater activity in the cocaine group relative to the control group. This study will allow us to better understand the long-term effects that chronic cocaine exposure has on metabolic brain function.

4.1 MATERIALS AND METHODS

4.1.1 Subjects

Young adult (age 8-9 years old at the age of testing) rhesus macaque males (n=11) that previously self-administered cocaine (n=6 cumulative dose: 528-546mg/kg) were compared to a control group (n=5) that self-administered water over a 12 month period. These monkeys participated in a previous study, which examined the effects of chronic cocaine self-administration on cognition (Porter, Olsen et al. 2011). Following this study, they were drug free for 20 months. We were only able to use a subset of animals from chapter 2 for this study due to unexpected illness and loss of motivation. We used 5 of the original control monkeys. One of the control monkeys was removed due to his unwillingness to work. We were only able to use 6 of the 8 original cocaine monkeys used in aim I due to illness. Animals were water-regulated 7 days a week and were supplemented to meet physiological needs at the end of each day following training and testing.

4.1.2 Experimental Design

The cocaine group was drug free for 20 months prior to the initial FDG-PET scan. Two months before the initial FDG-PET scans were conducted, animals were trained on a control task (details below) and the delay matched-to-sample task (details below) Animals previously performed a DMS task. We tried to make the current version more cognitively demanding by adding more distractor options. Briefly, the idea was to construct a pair of behavioral tasks that were similar in sensorimotor responses, and amount of reward received, but differed in that the experimental

task recruited working memory processes and the control task did not. We were able to achieve this by moving the intra-trial delay (experimental task) to an inter trial delay (control task). This was important for use of the subtraction method (described later) to isolate brain areas that were more active during the working memory task.

4.1.3 Training and acclimation procedures

Animals performed the control task daily until they reached 90% accuracy for three consecutive days. Animals met the performance criteria for the control task and were trained on the experimental task. Animals from both groups became proficient on the experimental task after 5 days, after which we alternated their training between the two tasks daily until the initial FDG-PET scan. The control task was always performed on the initial FDG-PET scan day. Following the initial scan, animals performed the delay task daily until the second FDG-PET scan. Typically, control and experimental task PET scans days were separated by 7-10 days.

During the training period, animals were habituated to the PET scan procedures. We accessed each animal's vascular access port daily and flushed 10 cc of saline through the line in order to habituate them to the FDG-PET procedure and minimize stress on PET scan days. This was done so that animals would acclimate to the FDG-PET scan procedures, thus minimizing disruption of performance on scan days.

4.1.4 FDG-PET procedures

On the day of the FDG-PET scan, animals were placed in their primate chair, accessed via their vascular port, and wheeled into a sound attenuating chamber where the cognitive tasks were

performed. Animals received an injection of 7-10 mCi of FDG after completing 5 trials of the cognitive task. We were able to inject the FDG from outside the chamber with minimal disruption to the animal. Animals performed each task for 35 minutes (over 70% of FDG uptake occurs within 30-40 min of injection; Price JC 2003) once the FDG was administered. After 35 minutes, animals were immediately anesthetized with Ketamine and quickly transported to the local PET center. PET scans were initiated exactly 60 minutes after the FDG injection.

4.1.5 Control task description

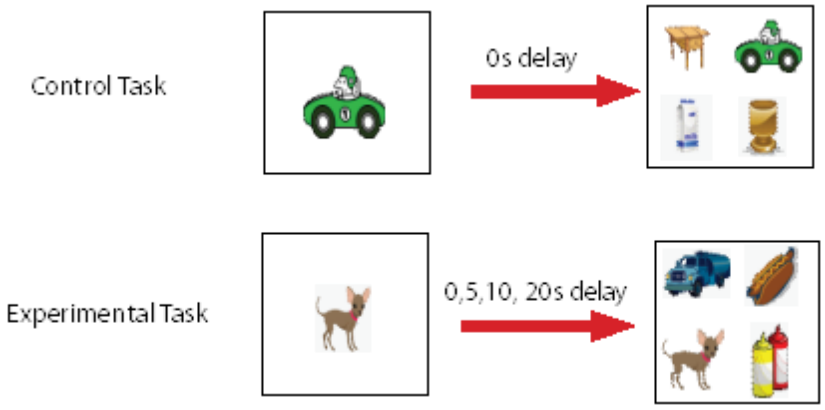

Figure 18 Diagram of control and experimental task

The control task consisted of a sample screen in which a single clip art picture was presented. Touching the picture resulted in the appearance of the match screen. The match screen has 4 stimuli; one a match and 3 novel distractors. The experimental task was designed the same as the control task, except a random delay interval (working memory component) was inserted between the sample screen and the match screen.

The control task was designed to be analogous to the experimental task with respect to sensorimotor demands and reward amount received. In order to control for animals performing

significantly more trials and receiving more water reward on the control task than the delay task, a 12-second inter-trial interval was imposed. Removing the intra-trial delay (experimental task) removed the working memory component and served as a control to isolate brain regions that were not specific to working memory processes.

Each trial starts with a sample image centered on the sample screen **(**see figure 1). Holding the sample image for 1-second led to the immediate presentation of the match screen. Four figures were presented on the match screen, only one that was the match image (previous sample image); the other 3 were non-match distractor images. Distractor images were included to increase task difficulty. This also made the task slightly harder than the previous version of the DMS task used in Aim I. We also wanted to avoid animals becoming over trained on the task. Match images was never placed in the same position on the match screen for two consecutive trials. Approximately 500-600 unique images with distinct features were used per session and so none of the clip-art images presented on a trial were repeated within a session. When the animal made a correct choice, a water reward amount of 0.07ml/kg was given via a sipper tube. For incorrect responses and omission (not responding within 5 sec), no water was given and the inter-trial interval commenced. Animals were allowed to perform as many trials as possible in a 35-minute window.

4.1.6 Experimental task: DMS task description

The experimental task is similar to the control task, but an intra-trial delay interval of 0, 5, 10, or 20 seconds was interposed between the sample screen and the match screen. Previously (aim I), at higher delays (40s), animals often would become frustrated and occasionally quit working. In order to reduce animal frustration and ensure good performance on the scan day, this version of

the DMS task was altered. We removed the 40s delay to minimize frustration on scan day. The water reward amount for a correct response was 0.07ml/kg and animals received no water for an incorrect response. There was an inter-trial interval of 2 seconds.

4.1.7 Scan processing and coregistration

PET images were acquired on a Siemens microPET P4 scanner (Siemens Medical Systems, Knoxville TN), which has a 7.8 cm axial field of view, a transaxial field of view of 19 cm, and a maximum intrinsic spatial resolution of 1.8 mm FWHM (Tai, Chatziioannou et al. 2001). CT images were acquired using a CereTom system (Neurologica, Inc., Danvers, MA), which is a clinical small-bore 8-slice (1.25 mm slice pitch) helical CT scanner designed for neuroimaging applications. The microPET and CereTom systems are aligned along a common isocenter, with a custom designed unified bed pallet servicing both PET and CT systems. This arrangement allows intrinsically co-registered PET and CT images to be acquired over a ~50 cm co-scan range. Prior to the acquisition of PET emission data, an axial CT scan was acquired (7 mAs, 120 kVp) over 15 cm (1 cm/sec). The resulting CT scan was used both for positioning the animal in the microPET gantry and for the attenuation correction of PET emission data.

PET emission data were acquired in list mode for 30 min commencing 60 min after the injection of [^{18}F] FDG and binned into a single 30 min static frame. The emission sinograms were reconstructed using filtered back projection with a 0.5/cm ramp filter and an image zoom of 1.5, resulting in PET image matrix of 256 x 256 x 63 voxels with dimensions of 0.63 mm x 0.63 mm (transaxial) x 1.21 mm (axial). Co-registered CT images were segmented into air, brain tissue, bone, and bed pallet segments and converted to appropriate mass attenuation coefficients (μ) for the respective segments at PET photon energies (511 keV). The resulting μ-maps were

forward projected into an attenuation sinogram for correction of PET emission data. Standard corrections for scatter, scanner deadtime, and random coincidences were applied to the PET emission data.

Magnetic resonance (MR) scans were obtained for each subject prior to self-administration (Aim I). A template was generated using Bradberry lab monkeys and monkeys from a collaborating lab. MRI images (0.5mm voxels size) were acquired using a Siemens 3T Allegra scanner with a custom-designed dual stereotaxic holder/secondary coil designed by Dr Seong Gi Kim and colleagues (Univ of Pittsburgh). Images were warped to a merged and fully segmented macaque monkey atlas, analogous to the Montreal Neurological Institute templates used in human imaging studies. A 5mm smoothing kernel was used in pre-processing. All MRI images from individual subjects were processed and analyzed using statistical parametric mapping software (SPM5;http://www.fil.ion.ucl.ac.uk/spm/software/spm5/) operating within Matlab (version 7.6.0, R2008a;MathWorks, Natick, MA) and the VBM toolbox developed by Christian Gaser (http://dbm.neuro.uni-jena.de/).The reconstructed PET images were co-registered to correspond to structural MR images using automated image registration and then transformed spatially into a standard space with an FDG template for rhesus monkeys. Regions of interest (ROI) were drawn on the MR template and applied to the PET images. Specifically, the cerebellum ROI consisted of cruz 1 and lobule 6, a region shown to be involved in working memory (Stoodley and Schmahmann 2009); (Stoodley et al., 2012); (Strick, Dum et al. 2009). The DLPFC consisted of area BA 46, a region shown to be involved in working memory (Goldman-Rakic 1987).

4.1.8 Data analysis

4.1.8.1 Region of Interest Analysis

All PET imaging data analysis was conducted using SPM8. ROI's were drawn on the lab constructed rhesus macaque monkey template and applied to the PET images of each monkey. Raw scanner numbers (counts/s/px over 60-90minutes) were used to generate standard uptake value (SUV; count*calibration*weight/injected dose), a measure of metabolic activity. The final number used for comparison was the SUVR, which is simply the SUV referenced to the whole brain. Previous neuroimaging studies have identified working memory related brain activity using a subtraction method (Smith et al., 1997); (Posner, 1988). Activity from the control task is subtracted from activity during the WM task. Because the control task is designed to engage all the process except the cognitive processes, this method can be used to isolate cognitive processes involved with working memory. The subtraction method was used for the data analysis. For each subject's scan, an SUVR was generated for all ROI's. For each ROI, the control task SUVR was subtracted from the experimental SUVR in order to generate a difference score, which was included in the group average.

In order to avoid multiple comparisons, SUVR's were compared in the dorsolateral prefrontal cortex (DLPFC), and the Cerebellum. We focused our comparison of the DLPFC and cerebellum because of previous work. Previous work (Porrino, 2005 #5571) using FDG-PET showed that the DLPFC activity in healthy, drug free monkeys was increased during a visual working memory task compared to the control task. Because previous work showed that during early cocaine abstinence, cerebellar activity correlated with working memory performance (Tomasi, Goldstein et al. 2007), we also focused our analysis on the cerebellum. A two-way repeated measures ANOVA with factors: ROI (cerebellum and DLPFC) and group

(cocaine vs. control) was conducted in order to examine metabolic activity differences between groups following 20 months of being drug free. Post-hoc analysis was conducted to determine what factor was contributing to the significant interaction.

4.1.8.2 Whole brain voxel comparison

As a more exploratory analysis, a two-stage parametric approach was conducted for the whole brain statistical analysis. In the first stage, normalized FDG-PET scans for each subject (control and experimental task) were used to create a subtraction image representing change in regional cerebral metabolism between the working memory task and the control task (all scans from the working memory task minus the control task). The results reflect the process of working memory by subtracting motor and visual components involved in the task from the higher cognitive functions of visual working memory. Within session variations in global signal were adjusted using proportional scaling. A between group comparison was conducted using a two sample t-test. The subtraction images from each participant were entered into a random effects two-sample t-test.

4.1.8.3 Behavioral analysis

The behavioral parameters examined were accuracy, mean response time and standard deviation of the response time. A t-test was used to compare performance on each task between the cocaine and the control group.

4.2 RESULTS

4.2.1 Behavioral data

A. B.

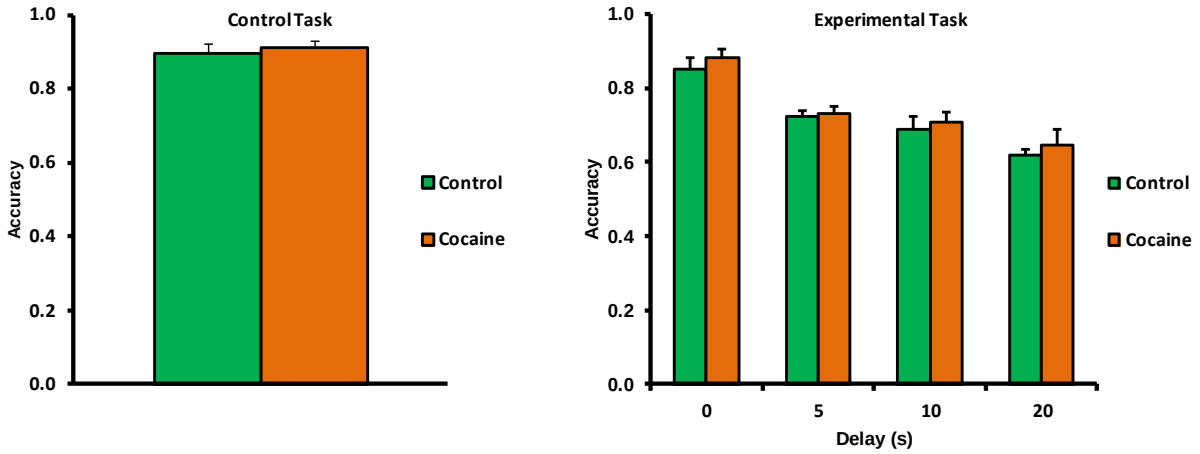

Figure 19 Training period performance between groups comparison on control and experimental task

Training period performance on the (A) control task and the (B) experimental task. There were no between group differences in accuracy on the two tasks. Both groups showed a decrease in accuracy with increase in working memory demand ($p<0.001$).

There were no differences in accuracy on either task during the training period (Figure 19). Both groups were able to perform the task at the same level prior to the initial scan days. There were no between group difference in accuracy (Figure 20) or response time (Figure 21) on the PET scan day. While it may seem like the cocaine group is performing better than the controls on the experimental task, there is no significant difference in performance on the task between the groups. Consistent with working memory performance expectations, there was a decrease in performance with increased delay in both groups ($p<0.001$).

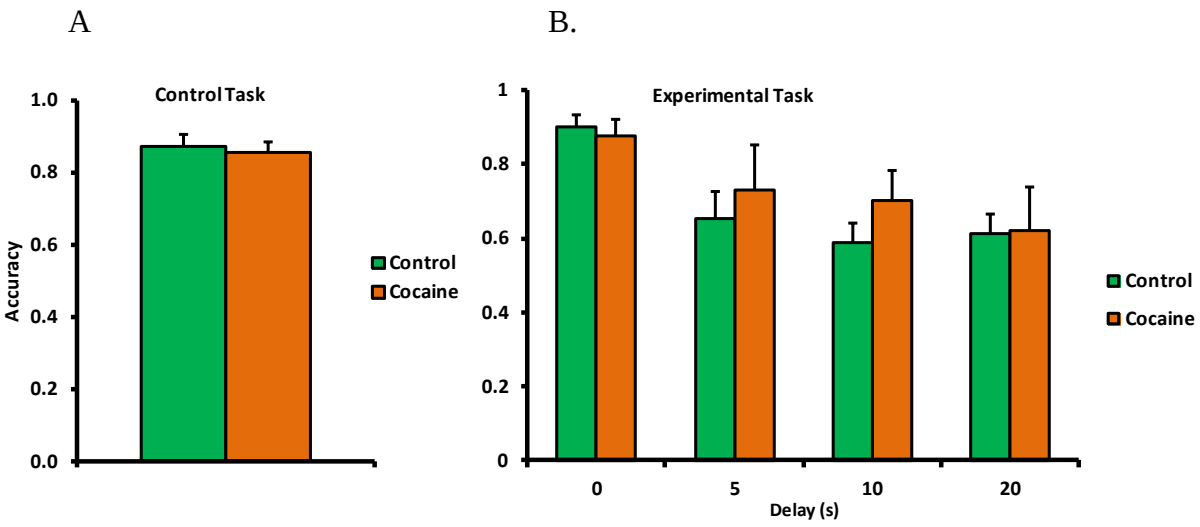

Figure 20 Between-groups comparison of performance on control and experimental task

Behavioral performance on the scan day for the (A) control task and (B) experimental task. There were no between group differences in accuracy. Both groups showed a decrease in accuracy with increase in working memory demand ($p<0.001$)

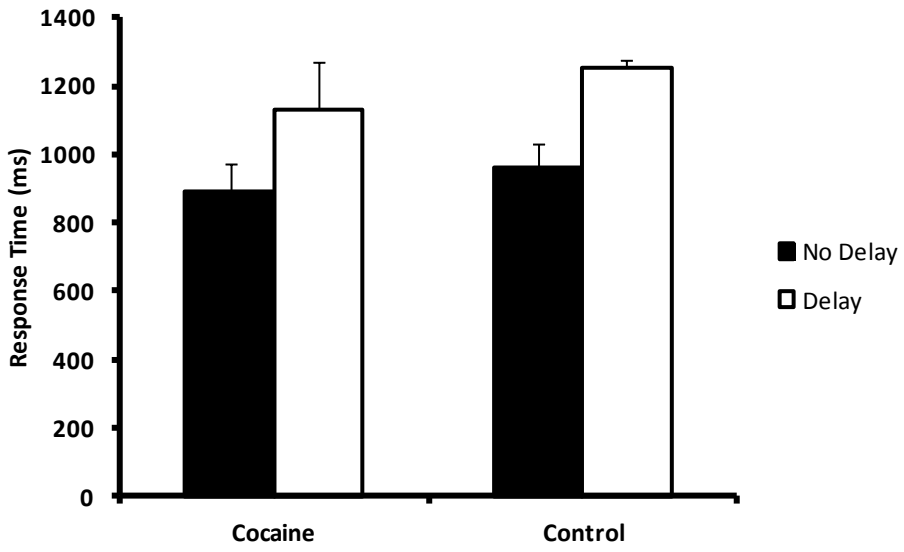

Figure 21 Between-groups comparison of response times during the control and experimental task on scan day

There were no between group differences mean on the control task (no delay) and the experimental task (delay).

4.2.2 Imaging Data

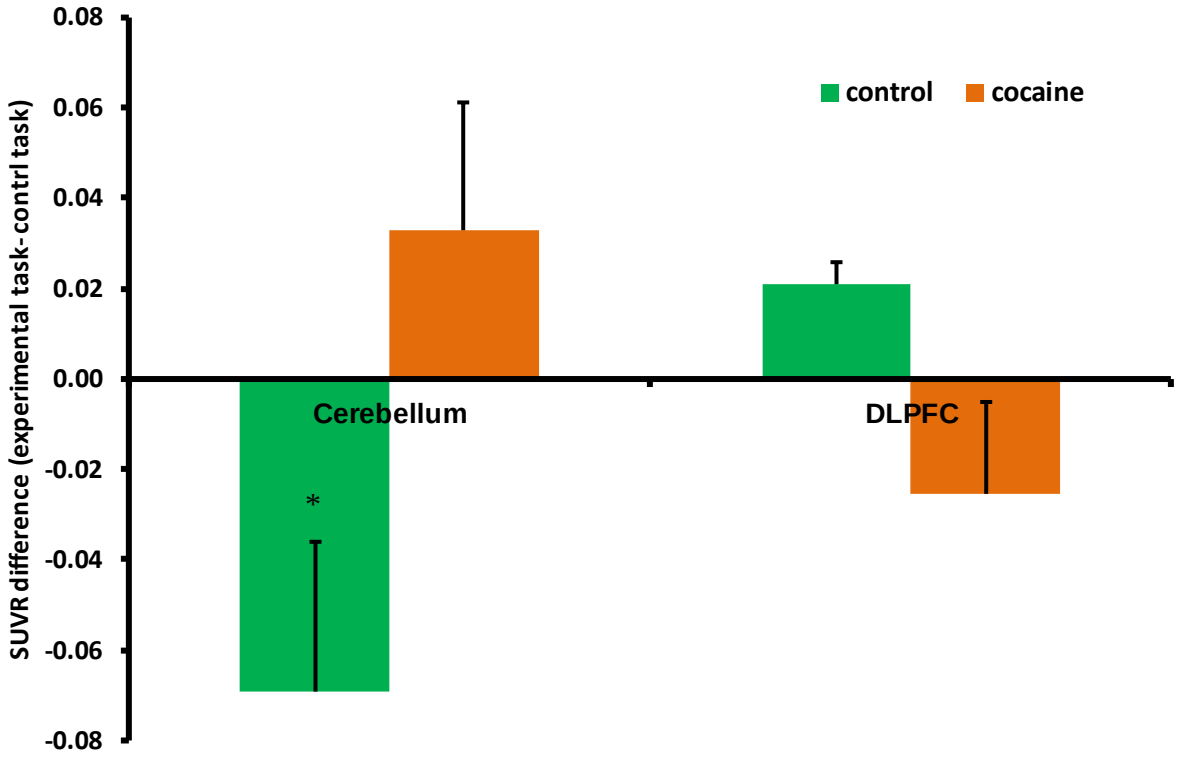

Figure 22 Between-groups comparison of standard reuptake values in the cerebellum and dorsolateral prefrontal cortex

In the cocaine group, the cerebellum had greater activity during the experimental task (relative to control task) compared to the control group. *p<0.05,

When ran t-test for all ROI's, the cerebellum activity was significantly different (p=0.02) between the groups, and there was a trend (p=0.054) in the DLPFC towards a between group difference in activity (Figure 22). A two way-repeated measures ANOVA revealed a group X region interaction ($F_{(1,1)}$=8.09; p=0.019). A multiple comparison analysis revealed that cerebellar activation was significantly different between the groups (unadjusted p=0.005, critical level p=0.050). In the cocaine group, the cerebellum showed an increase in activity during the

experimental task (relative to the control task) compared to the control group. We were unable to observe a significant between group differences in the DLPFC activation (Figure 22). The cocaine group has a negative SUVR, whereas the control group has a positive SUVR. This is not significant possibly due to the increased variability in the cocaine group. A post hoc exploratory analysis of the entire brain did not reveal between group differences in any ROI metabolic activity.

4.3 DISCUSSION

The aim of this study was to examine the long-term effects of chronic cocaine self-administration on cerebral metabolic function in non-human primates. We found metabolic brain differences between control and cocaine monkeys that were drug free for 20 months after 12 months of chronic cocaine self-administration. Specifically, the cocaine group showed increased activation in the cerebellum during a visual working memory task (relative to the control task) compared to the control group. These results suggest long lasting changes in brain metabolic activity after chronic cocaine exposure. This data is consistent with findings in active (Hester and Garavan 2004) and early abstinent (Tomasi, Goldstein et al. 2007) cocaine users who show increased cerebellar activity when performing a task with increased working memory load. These data add to previous findings showing that even after an extended drug-free period, a history of cocaine exposure was associated with long lasting changes in brain metabolic function.

Our data show an increase in cerebellar activity in the cocaine group compared to the control while performing a working memory task. Hester and Garavan (2004) were the first to report increased cerebellum activity using fMRI in active cocaine users performing a Go/No-

Go task with increasing memory load (Hester and Garavan 2004). They reported significant between groups differences in performance, in that controls performed significantly better than cocaine users. Of interest to this paper, they observed an increase in cerebellum responding with increased WM demand in the cocaine users only. In addition, they observed increased ACC activity in controls with increasing working memory load, but ACC activity in the cocaine group remained nonresponsive. Tomasi et al., (2007) conducted a similar study, but in acute abstinent cocaine users acute vs. longer periods of abstinence) performing a verbal working memory task (Tomasi, Goldstein et al. 2007). They hypothesized that abnormalities would be more accentuated during earlier stages (<72hrs) of abstinence compared to later stages (>72hrs). Accuracy on the 2-back task was lower for the early abstinence cocaine abusers compared to controls. In cocaine abusers, larger activation was seen in the cerebellum and larger deactivations in the amygdala were observed with increasing working memory. Group comparisons revealed that cocaine abusers had lower working memory load activation in cortical areas such as the MFG9 (left DLPFC BA 9) and precuneous BA7 and higher working memory load activation in the cerebellum than controls. Interestingly, for cocaine abusers only, high performance accuracy during the 2-back task was associated with increased activation of the cerebellar and lower activation of the post central gyrus 5. These data, taken together suggest that the cerebellum becomes necessary for efficient performance on working memory tasks in cocaine users whether active or abstinent. There is also some indication that increases in cerebellar activity might compensate for cortical hypoactivation. It appears that the cerebellar activity is increased in both active users and in abstinent cocaine abusers, but only during abstinence is the cerebellum able to compensate in a manner that improves working memory performance. With the increase in cerebellar activity, performance on working memory tasks is improved in early abstinent

abusers. Tomasi and colleagues (2007) still observed group differences in behavior, but this may be due to differences in cocaine populations (acute abstinence vs. longer periods of abstinence) (Tomasi, Goldstein et al. 2007). They were still able to show that in abstinent cocaine users, when the cerebellum activity is increased, so is working memory performance.

Our data support the idea that the increase in cerebellar activity might compensate for reduced DLPFC metabolic function, a region shown to be important for working memory (Goldman-Rakic 1987);(Petrides, Alivisatos et al. 1993);(Bechara, Damasio et al. 1998). In the cocaine group, activity in the cerebellum is increased, whereas it is decreased in the control group. While we did not observe a significant between group differences in DLPFC metabolic function, the data trend in the hypothesized direction. The cocaine group showed negative standard uptake values (SUV's), whereas the control group had positive SUVR's in the DLPFC. We did not observe this trend in any other cortical regions. The high variability in SUV's in the cocaine group might have precluded between group differences in DLPFC function. Hester and Garavan (2004) were the first to suggest that the cerebellum may compensate for prefrontal regions in active cocaine users (Hester and Garavan 2004). While cocaine users relied heavily on their cerebellum, their performance did not match that of controls. It can be hypothesized that during active cocaine use the cerebellum is not capable of compensating enough to cause improved working memory or overcome hypoactivation in cortical regions. Consistent with this hypothesis, a compensatory mechanism has also been proposed in an alcoholic population with equivalent working memory performance to controls (Desmond, Chen et al. 2003). This was supported by relative increases in the right cerebellar regions and reduced activity in the left PFC with increasing task demands in alcoholics relative to controls.

The cerebellum has long been considered mainly involved in motor function but, over recent years, neuroimaging studies have shown that the cerebellum may also be involved in executive function (Parkins 1997); (Schmahmann and Sherman 1998); (Owen, McMillan et al. 2005). The most consistent finding in the functional neuroimaging literature is that verbal working memory reliably activates areas of the cerebellar cortex (Fiez 1996); (Desmond and Fiez 1998); (Chen and Desmond 2005). Patients with cerebellar damage are impaired in verbal working memory (Ravizza, McCormick et al. 2006); (Desmond, Chen et al. 2005); Burkk et al., 2003; (Silveri, Di Betta et al. 1998). While lesions of the cerebellum do not produce the same degree of impairment as seen in frontal lesion patients (Ravizza, McCormick et al. 2006), it remains clear that there is a role for the cerebellum in working memory performance. Given the cerebellum's role in working memory, the hypothesis that the cerebellum works in some sort of compensatory mechanism to improve working memory is compelling. The cerebellum increases in activity that were observed in the current study were most likely not related to motor impairments, as we did not see between group differences in response times or see changes in activity in cortical motor regions such as M1 that the cerebellum project to and receive projects from. Working memory has been shown to be impaired in the cocaine abusing population (Hoff, Riordan et al. 1996); (Bechara and Martin 2004); (Kubler, Murphy et al. 2005); (O'Malley, Adamse et al. 1992). We were able to show impaired working memory following chronic cocaine self-administration in non-human primates (Porter, Olsen et al. 2011). We were unable to show a correlation between working memory performance and cerebellar activity in the current study, but this may be due to low subject number and session numbers.

Anatomical and imaging studies suggest an interaction between the cerebellum and DLPFC in cognitive function, working memory specifically. Using rabies virus as a

transynaptic tracer in cebus monkeys, Kelly and Strick (2003) demonstrated that regions of the cerebellar cortex that receive input from area 46 are the same as those that project to area 46 (DLPFC), suggesting a cerebrocerebellar circuit (Kelly and Strick 2003). In healthy human subjects, Desmond et al. (1997) reported increased activation in cerebellar regions that are interconnected with DLPFC, when subjects performed a working memory task (Desmond, Gabrieli et al. 1997). Additional support for a frontal-cerebellar linkage comes from functional connectivity studies conducted in healthy subjects. Krienen and Buckner (2009) identified intrinsic functional connectivity between the DLPFC and the cerebellum in healthy human subjects (Krienen and Buckner 2009). The neuroanatomy supports the hypothesis that there is a strong link between the cerebellum and the DLPFC, specifically. More functional connectivity studies will need to be conducted in cocaine users and abstainers in order to better understand how the relationship between the cerebellum and the DLPFC changes during cocaine use and over periods of abstinence.

Future studies using fMRI and event-related designs in long-term abstainers are needed to determine more detailed roles of the DLPFC and cerebellum in complex cognitive processes and how chronic cocaine self-administration may cause long lasting alterations in these regions. It would be of great interest to look at functional connectivity of the cerebellum and DLPFC in cocaine users and abstainers, to better understand the compensatory mechanism we are hypothesizing in this current study. Our data suggest chronic cocaine self-administration is enough to cause long-term changes in metabolic function in monkeys that were drug free for 20 months. We hypothesize a compensatory mechanism may be activated following cocaine exposure, in order to maintain efficient working memory performance in cocaine abusers. This makes sense given the abundant literature showing that chronic cocaine use results in

hypoactivity in frontal regions (Bolla, Ernst et al. 2004) ;(Bolla, Eldreth et al. 2003); (Volkow, Hitzemann et al. 1992); (Volkow, Fowler et al. 1991) and recent imaging literature suggesting a role for the cerebellum in active and abstinent cocaine abusers (Hester and Garavan 2004);(Tomasi, Goldstein et al. 2007). It is possible that due to the hypoactivity in cortical regions that are necessary for efficient cognitive function, the cerebellum becomes more active. This compensatory mechanism may act as a protective mechanism in some abusers to ensure efficient performance and possibly prevent relapse during longer periods of abstinence. These data are important for future studies and treatment because it suggests that not just cortical areas are altered in chronic cocaine self-administering monkeys, but the cerebellum also.

5.0 SUMMARY AND CONCLUSIONS

5.1.1 Chronic cocaine self-administration is sufficient to cause impairments in reversal performance and visual working memory

Studies designed to assess neurocognitive effects of cocaine abuse are equivocal in respect to the specific types of deficits observed. However, the vast majority of these studies indicate that at least some deficit in a certain broad function such as attention and executive function (decision making, cognitive flexibility, inhibitory control) exists. Jovanovski and colleagues (2005) conducted a meta-analysis to determine the magnitude of effects on specific deficits found in clinical studies (Jovanovski, Erb et al. 2005). They report the largest effect size on tasks that probe attention, moderate effects on working memory and mixed effects on tests of executive function; some had large effect size and others were below the median. This in itself may suggest general cognitive impairments in the cocaine users. Furthermore, tasks that tax attentional processes, might require more cognitive control than those that are not as attentionally demanding. The literature suggests that cognitive control in the form of cognitive flexibility (Ersche, Roiser et al. 2008); (Fillmore and Rush 2006); (Grant, Contoreggi et al. 2000) and inhibitory control (Garavan and Hester 2007); (Hester and Garavan 2004); (Kaufman, Ross et al. 2003); (Fillmore and Rush 2002) are greatly affected in cocaine users. Due to limitations in clinical studies, such as differences in drug history, and lack of information on subjects before

use, it is still unclear whether drug use by itself causes cognitive deficits, and if so, whether there are specific deficits or general impairments across broad cognitive domains. Here, we examined the effects of chronic cocaine self-administration on associative learning, reversal performance and visual working memory in non human primates.

We conducted a longitudinal study in nonhuman primates that chronically self-administered cocaine for 12 months. We assessed their cognitive performance on associative learning, reversal performance and visual working memory prior to self-administration. Animals were placed into evenly matched groups based on their performance on a range of cognitive tasks. After animals began to self-administer, we conducted weekly cognitive assessments after a 72 hour drug free period. This approach allowed us to examine the direct effects of chronic cocaine self-administration on specific cognitive domains. We found that chronic cocaine self-administration causes impairments in reversal performance and visual working memory. Animals self-administered for 12 months and then were drug free for 3 months. During the initial 3 month drug free period, we continued to assess associative learning, reversal performance and visual working memory. We did not observe any between group differences in cognitive performance, which may suggest a recovery of performance or that the effects of chronic cocaine on reversal performance and visual working memory were acute effects. Interestingly, 3 months post cessation, when animals were exposed to environmental auditory and visual attentional distractors, both novel and appetitive, we saw similar patterns of cognitive impairments as that which we observed when animals were chronically self-administering cocaine. Novel and appetitive attentional distracters were able to disrupt reversal performance. Novel distracters were disruptive to visual working memory, but the appetitive attentional distractor had no effect on performance. We saw no effect of either attentional distractor on cognitive performance in the

control group. There are a few ways to explain these findings. This final discussion section, will better lay out the big picture framework for our observations. More specifically, it will touch on how chronic cocaine self-administration may have a more general effect on attention and cognitive control. Taxing attention via the attentional distractors may reveal latent cognitive vulnerabilities during an extended period of abstinence. Our data may be showing are general effects of chronic cocaine self-administration on in these cognitive domains. What may underlie some of the inconsistencies found in the literature outside of inconsistencies in drug history is the inability to tax attention and assess cognitive control in a consistent manner across studies.

Cognitive control can be defined as the cognitive process needed to complete a goal directed behavior. Cognitive control processes are typically executive functions such as decision making, cognitive flexibility, and inhibitory control. They are processes that are non-routine, attentionally demanding and are important for goal directed behavior. In chapter 2, we observed impairments in reversal performance in the cocaine group relative to the control group. The reversal part of the stimulus discrimination/reversal task is more demanding than the stimulus discrimination/associative learning section of the task. What makes the reversal section more demanding is that it is a rapid, unannounced reversal of reward contingencies. The reversal component requires a great deal of attention and cognitive control to first attend to the fact that the reward contingencies have switched, and inhibit choosing previously correct responses. What our data show is that chronic cocaine self-administration is sufficient to cause impairments in cognitive control as measured via decreased accuracy during the initial reversal trials. Animals in the cocaine group are less able to adjust their behavior following the reversal of the reward contingencies. Understanding what aspects of cognitive control are altered is very important in treating addiction given the relationship between treatment outcome and cognitive performance.

It is the lack of cognitive control that may lead to repeated drug use despite the negative consequences. These data suggest that cocaine self-administration is sufficient to impair cognitive control. This impairment might contribute further to cocaine abuse.

Our study adds to previous studies examining the effects of cocaine on cognitive flexibility. Fillmore and Rush (2006) conducted a study in poly-drug users consuming mainly cocaine and some alcohol, and reported significant impairments in reversal learning and elevated perseverative responding (Fillmore and Rush 2006). Similarly, Ersche et al (2008) showed that cocaine users performed poorly on a probabilistic reversal learning task compared to controls and other drug users (Ersche, Roiser et al. 2008). Cocaine users perseverated more on previously rewarded responses. This same reversal impairment has been observed in non-human primates performing a stimulus discrimination/reversal task after chronic investigator-administered cocaine (Jentsch, Olausson et al. 2002). Our study differs from the latter study in that our monkeys self-administered cocaine for a longer period of time. The route of administration (contingent vs. non contingent) has been shown to impact the brain differentially with respect to both dopamine release (Hemby, Co et al. 1997). In chapter 2, we were able to extend both studies in that we collected baseline cognitive performance prior to cocaine exposure to allow us to show a direct relationship between chronic cocaine self-administration and impaired cognitive flexibility and visual working memory.

We also observed marginal impairments in visual working memory during the chronic self-administration period. There are inconsistencies in the literature as to whether working memory is impaired in cocaine users. Some studies show that chronic cocaine users perform poorly on experimental and neuropsychological tasks that probe working memory function (Hoff, Riordan et al. 1996); (Bechara and Martin 2004); (Kubler, Murphy et al. 2005);

(O'Malley, Adamse et al. 1992), whereas other studies do not (Bolla, Rothman et al. 1999); (Colzato, Huizinga et al. 2009). Our data show that in a controlled population, chronic cocaine self-administration is enough to cause marginal impairments in visual working memory. These inconsistencies in the literature and the marginal impairments that we observe in working memory may be partly due to underlying attentional impairments. Some theories suggest a strong relationship between working memory and attention. Although working memory can be distinguished from attention, it has been suggested that working memory places demands on simple immediate space of attention or how much information can be grasped at once (Lesak, 1995). It is important to note that attention is not a unitary phenomenon. Attention can be broken down into 3 constructs: executive attention, orienting and alerting (for review see (Posner and Rothbart 2007)). While we are not assessing attention directly, we are hypothesizing that executive attentional impairments might be underlying the working memory impairments that we report. In our study, the highest delay requires the most attention. Not only must the animal remember the sample stimuli over the delay, but attention must be sustained despite internally or externally generated distractions. We did not observe a significant increase in omissions (a failure to respond within the response window) in the cocaine group, which are indicative of attentional impairments (Dalley, Laane et al. 2005); (Robbins 2002). However, there was a marginal trend towards an increase in omission in the cocaine group relative to the control group.

We were able to probe attentional impairments and how it might interact with cognitive control by presenting attentional distracters during an extended period of abstinence. Novel and appetitive attentional distracters were presented during associative learning, reversal trials and a block of trials during the visual working memory task. Interestingly, we saw similar cognitive impairments during abstinence as during chronic cocaine exposure. What this shows is that

during periods of abstinence it may seem like there is a recovery of cognitive function, but by taxing attention using environmental distractors, more cognitive control is required. Taxing the attentional system during abstinence, revealed this latent cognitive impairment. In clinical populations, the interaction between attention and cognitive control is assessed using a Stroop task. A drug-related version of the Stroop task has been used to measure the degree of attentional bias toward drug-related words compared with neutral words. Stimulant dependent individuals have been reported to have a significant attentional bias in favor of drug related cues (Hester, Dixon et al. 2006); (Carpenter, Schreiber et al. 2006); (Copersino, Serper et al. 2004); (Ersche, Bullmore et al. 2010). This attentional bias interferes with their decision making ability and the ability to maintain sobriety. Greater attentional bias predicts poorer treatment outcomes during drug treatment programs (Streeter, Terhune et al. 2008); (Carpenter, Schreiber et al. 2006).

We were unable to demonstrate an attentional bias toward the cocaine cues. Both the novel and appetitive distracters impaired cognitive performance in the cocaine group. We hypothesized that the drug cue would be more disruptive to cognitive performance than novel attentional distractors, but instead observed an overall increase in distractibility. Due to the conservative design of the experiment, we tried to balance distractor presentations across stimulus discrimination trials, reversal trials and the working memory trials. We first presented novel distractors across all tasks and then the appetitive distractor was presented across tasks. Therefore, animals may have habituated to the distractors or experienced extinction prior to being re-exposed to the appetitive distractors during working memory. We observed a more generalized effect of attentional distracters on cognitive control, in that both distractors were disruptive to cognitive performance. The only time the monkeys were exposed to any sort of environmental stimuli was during daily self-administration sessions. At no other point during

training or testing were visual or auditory tones presented. Thus, they may have generalized the novel attentional distractors to the appetitive distractors given that the stimuli were presented in a similar manner. Even with these confounds, it is clear reversal performance is most vulnerable to disruption by environmental factors. Both the novel distractor and appetitive distractor disrupted reversal performance in the cocaine group, while having no effect on the control group's performance. These data suggest that increasing attentional demands via environmental distractors results in impaired cognitive control during the period of abstinence.

Our data suggest that there are broad impairments in cortical regions as a result of chronic cocaine self-administration. Executive attention has been shown to rely on regions such as the anterior cingulate, lateral ventral cortex, prefrontal cortex and basal ganglia (for review see (Posner and Rothbart 2007). Reversal performance and working memory rely on the OFC and dorsal striatum, and DLPFC, respectively. Frontal areas have been shown to be hypoactive in abstinent cocaine abusers (Volkow, Fowler et al. 1991); (Volkow, Hitzemann et al. 1992). Given the brain regions that underlie these cognitive functions and their inter-connectedness (For review (Duncan and Owen 2000)), it is possible that chronic cocaine abuse alters a network of cortical regions and as a result causes general cognitive impairments. The inter-connectedness of the cortex likely results in the general cognitive deficits observed in cocaine abusers and in the cocaine group in the current study.

Considering that cocaine is associated with impairments in function of dopamine receptors and transporter, there are quite a few reasons to believe cocaine might impact functional aspects of attention and cognitive control. With repeated use, cocaine leads to changes in dopaminergic functions (for review see (Volkow, Fowler et al. 2009); (Volkow, Fowler et al. 2007)). These changes may contribute to the executive cognitive dysfunction reported in cocaine

abusers (Volkow, Wang et al. 1997); (Goldstein, Leskovjan et al. 2004) and in the current study. Animal models of pathologies with abnormal levels of dopamine (for review see (Boulougouris and Tsaltas 2008) suggest that disturbances in attentional processes may be modulated by dopamine. DA facilitates a wide range of cognitive functions (Nieoullon 2002); (Aalto, Bruck et al. 2005); (Goldman-Rakic, Muly et al. 2000). It is hypothesized that the dopamine dysregulation might be modulated by alterations in D2 receptor density in the striatum. Imaging studies conducted in the cocaine abusing population (Volkow, Fowler et al. 1993) and nonhuman primates (Moore, Vinsant et al. 1998); (Nader, Morgan et al. 2006) report reduced DA D2 receptors in striatal regions. Reduction in D2 receptors in the striatum correlates with decreased activity in cortical regions (Volkow, Fowler et al. 1993). Preclinical work has shed light on the relationship between D2 receptor density and cognitive control. Reversal learning is impaired following infusions of a D2 agonist into the rat medial striatum (Goto and Grace 2005). Lee and colleagues, using pharmacological manipulation, reported that D2 receptors are important for reversal learning in monkeys (Lee, Groman et al. 2007). Future studies would need to be conducted in monkeys that chronically self-administered cocaine to assess changes in dopamine D2 receptors and the relationship between hypoactivity and impaired cognitive control.

The association between decreased striatal dopamine D2 receptors and decreased metabolism within the cortex (OFC, ACC and PFC) could reflect either striatal modulation of frontal regions via striatothalamacortical projections (Morecraft, Geula et al. 1992; Haber, Fudge et al. 2000); (Alexander, DeLong et al. 1986)) or frontal modulation of the striatal regions by glutamatergic fronto-mesencephalic and fronto-striatal projections (Graybiel and Ragsdale 1979). A functional connectivity study conducted in humans revealed functional connectivity between the striatum and cortex during resting state (Di Martino, Scheres et al. 2008); (Gu,

Salmeron et al. 2010); (Kalivas and Volkow 2005). Reduced functional connectivity was reported between VTA and thalamus and between amygdala and medial PFC in cocaine users (Gu, Salmeron et al. 2010). In our study, repeated cocaine self-administration may be resulting in an alteration in functional connectivity between cortical and striatal areas and causing the impairments that we observed. It would be of importance to understand whether cocaine is sufficient to cause a decrease in functional connectivity between cortical and striatal regions and how it relates to the cognitive impairments observed in the clinical population.

In summary, we found that chronic cocaine exposure is sufficient to cause impaired cognitive flexibility and visual working memory. These findings provide information that could be helpful in developing better cognitive therapies for treating cocaine abuse. Given that we have an understanding of the impairments caused by addiction, this can be combined with what we know about the circuitry that underlie these cognitive functions thus resulting in better pharmacological treatments that can be paired with cognitive therapy. Furthermore, this work highlights the importance of future studies exploring the potential neurobiological substrates that are altered as a result of chronic cocaine exposure to determine how chronic cocaine exposure directly alters brain regions that underlie the cognitive impairments seen here. We observed that attentional distractors are able to reveal latent vulnerability in cognitive performance during an extended period of abstinence (Chapter 3), which recapitulated the pattern which we observed during chronic cocaine self-administration (Chapter 2). This knowledge is important for addiction research as a whole because during periods of abstinence, one may need more cognitive control to ignore distracters in the environment whether they are drug related or novel.

We were able to show that chronic cocaine self-administration causes long term impairments in cognition. While it may have appeared that cognitive performance was the same

between the groups or may have recovered, exposing the groups to novel distractors and appetitive attentional distractors during cognitive tasks, revealed that the cocaine group still suffered from cognitive impairments in reversal learning and marginal impairments in working memory.

5.1.2 Increased metabolic function in the cerebellum of drug free monkeys that previously self-administered cocaine

One consistent finding in the neuroimaging literature is that cocaine abusers suffer from hypofrontality or decreased function of the prefrontal cortex compared to healthy controls. Initial reports using PET have demonstrated lower rates of glucose metabolism (Volkow, Fowler et al. 1991); (Volkow, Hitzemann et al. 1992). These rates of metabolic activity have been reported to persist 1 week to 4 months post cessation (Volkow, Fowler et al. 1993). Using fMRI, others have also reported dysfunction in lateral PFC and OFC (Bolla, Ernst et al. 2004); (Bolla, Cadet et al. 1998), anterior cingulate, and cerebellum (Hester and Garavan 2004). Structural changes have also been shown in the cocaine abusing population (Franklin, Acton et al. 2002); (Matochik, London et al. 2003); (Hanlon, et al., 2011). Many of these reports have been in active users and during early abstinence. What is still unclear are the long term changes in cerebral metabolic function in cortical and subcortical areas after an extended period of abstinence.

We examined cerebral metabolic function using FDG-PET in monkeys that were drug free for 20 months. We found an increase in cerebellar metabolism in the cocaine group compared to the control group during a visual working memory task. We did not observe a between group difference in DLPFC metabolic activity. The cocaine group had negative activity values in DLPFC, whereas the control group had positive activity values, when subtracting

control task activity values from working memory values, but this did not reach significance. Due to variability within the cocaine group, we were unable to show a significant difference. This variability may be a result of D2 receptor recovery. Three of the five monkeys studied in abstinence after 1 year of cocaine self-administration showed complete recovery of D2 DVR within 3 months of abstinence, whereas two monkeys did not recover D2 receptor availability even after 12 months of abstinence (Nader, Morgan et al. 2006). These monkeys averaged 22% reductions in D2 receptor availability due to cocaine exposure. In light of this study, it is possible that we see such variability in metabolism in the DLPFC within the cocaine group because some of the monkeys are showing recovery in their D2 receptors. As stated earlier, decreased dopamine D2 receptors correlate with hypoactivation in cortical areas (Volkow, Fowler et al. 1993). With an increase in D2 receptors within the striatum, it is possible that some of the monkeys are showing an increase in cortical activity, whereas others still may show reduced cortical activity.

While the cerebellum is mainly known for its involvement in movement, recent evidence has shown an important role for the cerebellum in cognition and more specifically a role in working memory (Stoodley and Schmahmann 2009); (Stoodley, et al., 2012); (Tomasi, Goldstein et al. 2007); (Chen and Desmond 2005). Hester and Garavan (2004) were the first to show increased cerebellar activity in active cocaine users performing a GO/No-Go task with increasing working memory load (Hester and Garavan 2004). A similar study conducted by Tomasi and colleagues (2007) reported a relationship between cerebellar activity and increased working memory performance in cocaine users only (Tomasi, Goldstein et al. 2007). The more cerebellar activity, the better their performance. Our data extends these findings in that the cocaine group,

who where drug free for 20 months showed an increase in cerebellar activity while performing a working memory task. This shows that the changes in the cerebellum are long lasting.

Our data further shines light on a compensatory role for the cerebellum in cocaine users. In addiction disorders, in which frontal lobes are known to be compromised (Volkow, Fowler et al. 2003), cerebellar activity appears to increase to support several tasks involving frontal lobe function including monetary reward response (Martin-Soelch, Leenders et al. 2001), response inhibition/working memory (Hester and Garavan 2004) and working memory (Desmond, Chen et al. 2003). Previous work conducted in humans suggest a compensatory role for cerebellum in active users and abstainers performing a working memory task. Hester and Garavan (Hester and Garavan 2004) actually saw a decrease in performance in active cocaine users compared to controls even though the cerebellum showed increased activity. This suggests the cerebellum was unable to compensate or modulate activity in the hypoactive DLPFC enough to improve working memory performance. Farther down the addiction timeline, Tomasi et al., (2007) reported in early abstinent cocaine abusers an increase in performance with increased cerebellar activity, suggesting that with acute periods of abstinence the cerebellum is more efficient at modulating DLPFC dependent behavior (Tomasi, Goldstein et al. 2007). Our study extends this timeline into long term abstinence. Our monkeys were drug free for 20 months before their PET scans. We observed an increase in cerebellar activity and no difference in DLPFC activity, but there were no differences in behavior between the groups, suggesting that the cerebellum may be modulating cortical activity. Future studies will need to examine whether the cerebellum is modulating cortical activity in cocaine users and if so, what the mechanism might be. It is clear that the cerebellum is altered in cocaine users in a way that allows for it to compensate for hypoactivity in cortical regions. These data do not suggest that the cerebellum is performing

working memory functions, but may be modulating cortical function. Given the closed loop anatomy linking the cerebellum to the DLPFC (Strick, Dum et al. 2009), it is possible the cerebellum, modulates frontal activity. As activity in the DLPFC decreases in cocaine users, the cerebellum may ramp up its activity to efficiently modulate activity in the DLPFC resulting in improved working memory. The cerebellum may be better able to do this during periods of abstinence.

Given the link between working memory and attention (Lezak 1994); (McCabe et al., 2010), it is possible that the cerebellum is improving working memory by increasing attention. A study in epilepsy patients showed that increased stimulation of the cerebellum resulted in increments in working memory as well as subjective reports of increased alertness (Riklan, Kabat et al. 1976). In light of these data, the cerebellum may be improving working memory by increasing attention.

6.0 CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, we observed that chronic cocaine self-administration is enough to cause general cognitive impairments. Specifically, impairments in reversal performance and marginal impairments in visual working memory in cocaine self-administering monkeys compared to drug free controls. These impairments were long lasting. Even after 3 months of abstinence, exposure to novel distractors and appetitive distractors revealed latent vulnerabilities in cognition. These findings suggest that cocaine is sufficient to cause long lasting impairments in cognitive flexibility and visual working memory. While there may be some recovery of cognitive function, it remains vulnerable in interaction with the environment.

We also discovered increased cerebellar activity in the cocaine group compared to the control group while performing a visual working memory task after a 20 month drug free period. While this increase in metabolic activity in the cerebellum has been observed previously (Hester and Garavan 2004); (Tomasi, Goldstein et al. 2007), our data extend these findings to show that the increases in cerebellar activity are long lasting. My data support an increased modulatory role for the cerebellum in working memory following chronic cocaine self-administration. What these data were unable to show is whether this change in metabolic activity is directly a result of cocaine exposure. We did not collect pre-cocaine baseline in our monkeys. Future studies will need to look at baseline metabolic activity as it relates to baseline cognition. I hypothesize that the increase in cerebellar activity is a result of cocaine exposure given that none of the control

monkeys showed increased cerebellar activity. Our data also hint at the possibility of recovery in the DLPFC in a subset of the monkeys. The majority of the monkeys in the cocaine group showed negative DLPFC values, suggesting that the DLPFC was still impacted after a 20-month drug free period. This study warrants further studies to better characterize cerebellar roles in other cocaine related cognitive behavior and identify substrates mediating these effects. Most importantly, it would be important to understand how exactly the cerebellum is modulating the DLPFC, if at all, and if this is in fact improving working memory or merely increasing alertness.

Future work is necessary to better understand the role of the cerebellum in cocaine related behavioral impairment. Most importantly, it would be advantageous to find out whether the cerebellum might be modulating cortical activity during working memory task using fMRI and pharmacological manipulations of the cerebellum. Also examine whether the modulation is via direct projections from the cerebellum or through indirect pathways like connections including the basal ganglia loop, which are mediated by mesencephalic dopaminergic pathways. Furthermore this information could elucidate a mechanism by which subcortical areas might interact with cortical areas to improve executive functions that are impaired as a result of cocaine exposure. A better understanding of recovery and compensatory mechanisms can be helpful for developing better pharmaco-cognitive therapeutic treatments.

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