COCAINIE SELF-ADMINISTRATION IN RHESUS MONKEYS: EFFECTS ON NEUROBIOLOGY AND COGNITION AND EVALUATION OF COGNITIVE ENHANCEMENT FOR ADDICTION TREATMENT

BY

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I could not have reached this milestone in my scientific career without the support and guidance from a large group of people.

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I appreciate the unwavering love and support from my family. I hope I have maintained most of the positive qualities they instilled in me. My father once told me that keys to success included surrounding oneself with smart people, working ~60 hours/week, and becoming irreplaceable by learning multiple skill sets.

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ABSTRACT

Gould, Robert W.

COCAINE SELF-ADMINISTRATION IN Rhesus Monkeys: EFFECTS ON NEUROBIOLOGY AND COGNITION AND EVALUATION OF COGNITIVE ENHANCEMENT FOR ADDICTION TREATMENT

Dissertation under the direction of Michael A. Nader, Ph.D., Professor of Physiology and Pharmacology

No effective drug treatments for cocaine dependence exist, although combined behavioral and pharmacological interventions, reviewed in Chapter I, may lead to better treatment outcomes. The goal of the current research is to characterize neurobiological and cognitive deficits associated with cocaine use in monkeys, and examine cognitive enhancement as a pharmacotherapeutic approach to compliment behavioral methods.

Cocaine users show cognitive deficits in working memory (WM) and behavioral flexibility that persist into abstinence, although the extent and duration are not well established. In Chapter II, WM was assessed in rhesus monkeys with an ~6 year cocaine self-administration (SA) history using a delay match-to-sample (DMS) task. High-dose cocaine SA in afternoon sessions resulted in impairments on WM in subsequent morning sessions; across 30 days of abstinence, DMS performance improved. In Chapter III, cocaine-experienced monkeys performed significantly worse and FDG-PET imaging showed lower neuronal activity in the anterior cingulate cortex, an area associated with error-detection, compared to cocaine-naive monkeys during set shifting, a measure of behavioral flexibility. In addition to effectively modeling cognitive deficits in monkeys, these data suggest pharmacological interventions may ameliorate deficits in neural function and cognition.
Nicotinic acetylcholine receptor (nAChR) agonists can stimulate dopamine function and enhance cognition. In Chapter IV, nAChR availability, assessed via \[^{11}C\]-nicotine and PET was greater in the hippocampus in cocaine-experienced monkeys compared to controls, suggesting a target for further study. Acutely nicotine, a nonselective nAChR high-efficacy agonist, varenicline, a low-efficacy \(\alpha_4\beta_2\) and PNU-282987, an \(\alpha_7\) selective high-efficacy nAChR agonist improved WM in cocaine SA and control monkeys.

In Chapter V, chronic administration of varenicline increased the reinforcing strength of cocaine, while nicotine, varenicline and the nonselective antagonist mecamylamine potentiated the discriminative stimulus effects of cocaine. Thus, compounds that enhanced cognitive performance in monkeys with a cocaine SA history also increased the abuse liability of cocaine.

Integrating cognitive and behavioral pharmacology with PET imaging extends our knowledge of cocaine-related cognitive deficits, their neurobiological manifestations, and the utility of potential pharmacological adjuncts to behavioral treatments, providing a stronger assessment regarding therapeutic potential than any single method alone.
INTRODUCTION: PRECLINICAL MODELS AND CLINICAL STUDIES OF COCAINE ABUSE: INSIGHTS FOR FUTURE PHARMACOTHERAPY DEVELOPMENT

Portions of this Chapter were published: Gould RW, Porrino LJ, Nader MA. Nonhuman primate models of addiction and PET imaging: Dopamine system dysregulation. In JW Dalley, CS Carter (eds), “Brain Imaging in Behavioral Neuroscience”, Springer, Heidelberg, Germany, in press.
COCAINE ABUSE

Drug dependence remains a consistent societal problem resulting in deleterious consequences on an individual’s health, work, and family that resonates throughout communities worldwide and bears with it an overwhelming financial burden (WHO, 2004). In the United States alone, over 20 million people over the age of 12 met the DSM-IV criteria for drug abuse or dependence in 2008 (translating to nearly 1 in every 15 people; NSDUH, 2009) including 1.6 million cocaine users (SAMHSA, 2010). In 2010 nearly 3% of 12th graders surveyed admitted using cocaine once in the last year (Johnston et al., 2011). These statistics suggest not only a current problem for our society, but also a perpetuating one given the likelihood of future drug dependence.

Cocaine binds with near equal affinity to dopamine (DA), serotonin (5-HT), and norepinephrine (NE) transporters (DAT,SERT, NET, respectively; Ritz and Kuhar, 1989; Bennett et al., 1995) acutely elevating synaptic concentrations of all three monoamines (e.g. Di Chiara, and Imperato, 1988; Bradberry et al., 1993; Florin et al., 1994). Autoradiography studies in humans or monkeys with a cocaine self-administration history showed higher DAT (human, Little et al., 1993; monkey, Letchworth et al., 2001), NET (monkey, Macey et al., 2003) and SERT (human, Mash et al., 2000) binding compared to control groups, demonstrating potential neuroadaptations across each monoaminergic system as a result of cocaine exposure. Although multiple neurotransmitter systems contribute to aspects of the addiction cycle (i.e. craving, stress, reinstatement, cognitive disruptions) and are pursued as treatment targets for cocaine
dependence, the reinforcing effects of cocaine are attributed to elevated synaptic DA levels due to DAT blockade (Di Chiara and Imperato, 1988).

The DA system is comprised of four neuronal pathways originating from the midbrain with projections to various brain structures (see Beaulieu and Gainetdinov, 2011 for review). The nigrostriatal pathway originates in the substantia nigra pars compacta, innervates the dorsal striatum (caudate-putamen) and is involved in motor control. The mesolimbic pathway projects to the ventral striatum (nucleus accumbens), and other limbic structures including the amygdala, hippocampus, and cingulate gyrus and mediates actions related to reward, reinforcement, emotion, and motivation. The mesocortical pathway innervates cortical regions and is implicated in learning and memory. Lastly, the tuberoinfundibular pathway projects to the hypothalamus and influences anterior pituitary gland function. Dysregulation of the DA system through neurodegeneration or pharmacological insult can contribute to a number of disease states including Parkinson’s Disease (PD), depression, attention-deficit/hyperactivity disorder (ADHD), schizophrenia, and addiction (for reviews see Vallone et al., 2000; Beaulieu and Gainetdinov, 2011). Therefore, drug development strategies for these conditions, including addiction, focus on direct and indirect mechanisms that influence dopaminergic tone.

There are two superfamilies of DA receptors, the D1- and D2-like G-protein coupled-receptors, originally distinguished by their ability to stimulate and inhibit adenyl cyclase activity, respectively. D1-like receptors are primarily located postsynaptically whereas D2-like receptors are located pre- and post-synaptically functioning as autoreceptors as well as post-synaptic effectors. These superfamilies are
subdivided into D₁ and D₅ (D1-like) and D₂, D₃, and D₄ receptors (D2-like; subscripted numbers represent subtype). Both D₁ and D₂ receptors are predominately expressed in areas associated with the aforementioned DA pathways including the dorsal and ventral striatum, nucleus accumbens, substantia nigra, amygdala and frontal cortex with lesser expression in the hypothalamus, thalamus, cerebellum, and hippocampus. In contrast, D₄ and D₅ receptors are expressed in relatively low levels in various cortical and limbic regions with minimal expression in the striatum. D₃ receptor expression is limited to limbic regions. Also located on presynaptic DA nerve terminals in the striatum are DA uptake transporters (DAT) that function to transport synaptic DA intracellularly where it can be repackaged in vesicles by vesicular monoamine transporters (VMAT) or degraded by catechol-O-methyltransferase or monoamine oxidase (COMT and MOA; for reviews of the DA system see Vallone et al., 2000; Beaulieu and Gainetdinov, 2011).

Although numerous advances have been made to improve our understanding of drug addiction including effectively demonstrating addiction is a brain disease, pharmacological treatments for cocaine abuse have remained elusive. However, behavioral treatment strategies, with a primary goal of modifying deleterious, drug-oriented behaviors to more positive, goal-directed behaviors, are moderately successful. The most efficacious treatment strategies will most likely involve both pharmacological and behavioral treatments. Thus, one strategy is to develop drug treatments that will improve success of current behavioral modification strategies. One avenue for drug development that will be evaluated in the current research is cognitive enhancement.
BEHAVIORAL TREATMENT APPROACH

The overarching goal of behavioral treatment is sustained abstinence through relapse prevention, measured directly via drug-free urinalysis and indirectly via treatment retention rates (regarding the latter, attrition is assumed to be a result of relapse). Three common and sometimes overlapping strategies are the community-reinforcement approach (CRA), contingency management (CM) and cognitive behavioral therapy (CBT). CRA and CM are based on principles of operant conditioning whereby presentation of alternative reinforcers to drug use are the primary focus. CBT is based on social learning principles. Together psychosocial approaches aim to modify maladaptive, drug-related behaviors and introduce positive, goal-oriented behaviors. A recent meta-analysis of psychosocial interventions through 2005 using the aforementioned approaches found an average rate of abstinence of 32% compared to 13% across respective control groups not employing behavioral strategies (Vocci and Montoya, 2009). These results suggest that although drug treatments are largely ineffective in treating cocaine addiction, behavioral modification strategies are at least resulting in modest success. Behavioral modification approaches will be introduced and when appropriate, relevant preclinical models will be discussed.

The “community-reinforcement approach” was coined in the early 1970’s by Hunt and Azrin who stated that when intoxicated to a point that one’s social, family and employment obligations are impaired, one enters a state of “postponement or omission of positive reinforcement as a result of alcohol intake” (Hunt and Azrin, 1974). Therefore, treatment involves rearranging the temporal distribution of positive reinforcers in the environment (e.g. vocational, social, recreational, and familial) so that one reduces drug
use to maintain such positive, enriching interactions. This concept relies on both positive alternative reinforcement to drug use and negative reinforcement (preventing the absence of loss of job, family, friends) to increase abstinence. This initial study paired patients with a counselor that facilitated marital, family, social, and vocational counseling and included assistance in preparing, interviewing and procuring a job. By increasing the frequency of positive reinforcement, the relative reinforcing strength of alcohol presumably diminished. In this initial study, alcoholics in the CRA group spent less time away from home, unemployed, institutionalized or drinking and more time sober-an effect extending months after the end of treatment (Hunt and Azrin, 1974). Diminution of the reinforcing strength of the abused substance by increasing the positive reinforcing aspects of other environmental influences on behavior remains the core component of many behavioral treatment strategies.

Preclinical studies have rigorously examined the effects of environmental influences on self-administration behavior by exposing animals to “enriched” or “stressful” environments (for reviews, see Nader et al., 2008; Stairs and Bardo, 2009; Solinas et al., 2010). Enrichment can be defined as greater access to resources within one’s environment such as food, novel stimuli, etc., either through frequent experimenter introduction, or through social housing or dominance attainment within a social hierarchy. While more difficult to quantify, in most models “stress” is often introduced through an event that is unfamiliar, startling, and unpredictable (cage shaking/tilt, flashing lights, loud noises) or in a social setting whereby one is exposed to repeated aggression by others and resources are not as easily obtained (e.g. Rodriguez-Echandia et al., 1988; Morgan et al., 2002). Across several different species and models of
environmental stress and enrichment, enrichment results in neurobiological changes that reduce the vulnerability to self-administer psychostimulants including cocaine (for reviews, see Nader et al., 2008; Stairs and Bardo, 2009; Solinas et al., 2010). One human study (Martinez et al., 2009) complementing animal data (Morgan et al., 2002) showed that higher socioeconomic status (or social rank in animals) is associated with greater DA D2-like receptor availability, a state variable associated with decreased vulnerability to drug self-administration in animals (Morgan et al., 2002; Nader et al., 2006; Dalley et al., 2007). Further, studies suggest that strong social relationships (e.g. between spouses) can facilitate recovery from addiction (Kosten et al., 1987) whereas weak social attachments may increase the vulnerability to substance abuse (Brennan and Shaver, 1995; Caspers et al., 2005).

Similarly, increasing activity level within one’s environment via aerobic exercise may decrease the vulnerability for initial illicit drug abuse during adolescence (Terry-McElrath and O’Malley, 2011) as well as potential for relapse during abstinence (Van Rensburg et al., 2009; Roessler, 2010). Preclinical evidence also supports this theory. Aerobic exercise via wheel or treadmill running reduced the reinforcing potency of cocaine (Smith et al., 2008) and resulted in fewer injections of morphine self-administered by rats (Hosseini et al., 2009). Cue- and stress-induced reinstatement of cocaine seeking was significantly lower in rats exposed to wheel running than in sedentary rats (Lynch et al., 2010). Conversely, exposure to acute stressors increased cocaine seeking in rodents (e.g. Brown and Erb, 2007; Conrad et al., 2010) and increased the relative reinforcing strength of cocaine in subordinate monkeys following exposure to an unknown group of monkeys (Czoty et al., in preparation). Collectively, these data
support the primary goal of behavioral treatment strategies such that increasing exposure to positive, enriching environments (e.g. socialization with family, employment) and minimizing isolation and stress may reduce vulnerability to relapse and aid in sustaining abstinence.

CM approaches are based on operant conditioning techniques such that emitting a predetermined desired behavior (e.g. drug free urine) results in delivery of a positive reinforcer with the primary aim of reduced drug use. Unlike CRA, CM focuses on smaller, tangible reinforcers. A number of variations have been tested such that the reinforcer is either money, a voucher to be exchanged for household goods (e.g. food, diapers, etc) or chances to win prizes (e.g. Higgins et al., 2002; Stitzer and Vandrey, 2008). Higgins and colleagues (Higgins et al., 1991, 1993, 1994, 2000) have implemented a point system that progressively increases following each subsequent negative urine sample and is reset following a positive sample or skipped appointment. This series of studies demonstrated that CM nearly doubled (75% vs 40%) the percentage of participants that completed treatment compared to CRA alone, doubled (50% vs 20%) the percentage of participants that reached 12 weeks of sustained abstinence from cocaine and improved abstinence rates 4-6 months after treatment ceased (for review see Higgins et al., 2002). Further, the effects of CM on sustained abstinence were distinguishable from the effects on treatment retention, as determined by comparing voucher presentation contingent on drug-free urine samples versus noncontingent voucher presentation regardless of urine results (for review see Higgins et al., 2002). The dissociation between treatment retention and sustained abstinence is important because, although treatment retention correlated with overall treatment success (e.g. Simpson et al., 1999),
maintaining abstinence during treatment should translate to greater rates of abstinence following treatment completion.

Employment-based reinforcement has been implemented such that patients are employed in a low-stress work setting and continued employment with full financial compensation is contingent on monthly negative urine screens (e.g. Donlin et al., 2008; DeFulio and Silverman, 2011). A positive urine for drug metabolites results in denied access to the workplace until drug abstinence is achieved, and a reduction in compensation. In one recent study, greater than 80% of abstinent-contingent employees returned negative urine samples compared to ~50% of employees without employment contingencies (DeFulio and Silverman, 2011). Employment-based abstinence reinforcement maximizes two factors integral in the success of CM, reinforcer magnitude and length of abstinence reinforcement. This approach uses a necessary reinforcer (money) for a therapeutic goal and the duration of CM can exceed several years. CM approaches have shown greater success in reducing relapse compared to other behavioral strategies.

Schedules of reinforcement utilized by CM approaches are similar to those used extensively in preclinical models, including simple ratio schedules and variable or intermittent schedules. CM schedules of reinforcement are concurrently available choices between two reinforcers such that allocation of behavior results in either drug use or non-drug reinforcement, similar to concurrent choice studies in animals and humans (e.g. Macenski and Meisch, 1998; Johnson and Bickel, 2003; Negus et al., 2003; Czoty et al., 2005b). Increasing the magnitude of the alternative reinforcer to drug use increases success, often in individuals unresponsive to less efficacious alternative reinforcers
(Silverman et al, 1999) similar to animal models whereby altering the magnitude or
temporal distribution of a non-drug reinforcer affects the relative reinforcing efficacy of a
drug (e.g. Meisch et al., 1988; Nader and Woolverton, 1991, 1992; Campbell and Carroll,
2000; Negus, 2003; Hamilton et al., 2011). It is therefore important to consider
reinforcement frequency as well as magnitude when applying CM approaches to treat
addiction and the awareness that successful approaches may vary based on population
demographics (e.g. cocaine vs heroin users, high vs low impulsive, male vs female, age,
drug history; cf. Vocci and Montoya, 2009) similar to animal studies (Czoty et al., 2005b;
Hamilton et al., 2011).

Unlike concurrent choice studies in animals, drug use in human CM applications
is often associated with delayed reinforcement for sustained abstinence. For example, a
once-weekly negative urine sample might result in reinforcement, but 7 days of drug-free
behavior must precede reinforcement delivery. On the opposite spectrum, drug use one
day would not immediately forfeit continued access to work or voucher delivery if the
subsequent urine sample was not required for several days or weeks. Several animal
models have incorporated negative (aversive) aspects in combination with drug self-
administration (e.g. concurrent delivery of drug and shock; Cooper et al., 2007) but the
temporal dynamics are simultaneous, not delayed. Another difference between concurrent
schedules of reinforcement and CM approaches in clinical trials is that to produce a drug-
free urine one must inhibit a response (drug taking) whereas preclinically animals
actively respond between two concurrently available reinforcers. Few preclinical studies
schedule the availability of one non-drug reinforcer contingent upon inhibiting drug-
maintained responding. LeSage (2009) attempted to design an animal model of CM to
reinforce abstinence from nicotine self-administration. In this paradigm, delivery of a sucrose pellet was contingent on an emitted response on one manipulandum following a set duration of time during which a lack of responding occurred on a separate, drug-associated manipulandum (LeSage, 2009). However, these schedules of differential reinforcement of “alternative” or “other” behaviors (DRA or DRO schedules, respectively) are infrequent in current preclinical addiction literature.

CBT is based upon social learning principles and involves individual and group counseling sessions. Numerous variations of CBT integrate didactic lecture and role playing scenarios to identify and adjust maladaptive behaviors to positive, goal-oriented behaviors based on 7 goals: “1) reducing exposure to drugs and drug cues; 2) fostering resolution to stop drug use through exploring positive and negative consequences of continued use; 3) self-monitoring to identify high-risk situations for relapse; 4) recognition of conditioned drug craving and development of strategies for coping with craving; 5) identification of seemingly irrelevant decisions that could culminate in high-risk situations; 6) preparation for emergencies and coping with relapse; and, 7) developing alternate activities to drug use” (Carroll et al., 1994a). Often, additional tasks are assigned to implement these strategies in the outpatient environment. Several modifications have been integrated from depression or anxiety-disorder treatment approaches including dialectical behavioral treatment (DBT) and motivational enhancement therapy (MET). The former strongly emphasises the importance of acceptance and change and weekly sessions follow a hierarchical progression to focus on the most relevant hurdle faced by the patient at that present time (including dealing with and minimizing harm of relapse and associated worries, e.g. overdose, infection, etc;
Dimeff and Linehan, 2008). MET has been applied in conjunction with classic CBT such that the first few sessions focus on the motivational aspects of abstinence from drug use by increasing personal awareness of future consequences related to relapse or abstinence (e.g. McKee et al., 2007). CBT and numerous variations have shown promise in preventing relapse to substance abuse during treatment (Linehan et al., 1999, 2002; Carroll et al., 1994a; Rawson et al., 2002, 2006). A secondary goal of CBT is treatment retention, a factor that positively correlated with success rates of sustained abstinence post treatment (Carroll et al., 1994b; Vocci and Montoya, 2009). In theory, treatments that improve cognitive performance should increase abstinence rates for CBT-related strategies.

Often, CM is used in conjunction with CRA or CBT. Despite equivocal rates of sustained abstinence post treatment, CM +CRA, CM+CBT or MET+CBT increased length of abstinence during treatment (Higgins et al., 2000; Rawson et al., 2002, 2006; McKee et al., 2007). One hypothesis is that smaller goals such as producing drug-free urine by not using drugs for one week may seem more attainable than the long-term goals of keeping a job or repairing relationships with family or friends (for review see Dutra et al., 2008; Vocci and Montoya, 2009). Due to the strong societal influences associated with deterrence from or adherence to goals for behavioral modification strategies, preclinical models are difficult to establish with substantial validity to human CBT conditions. For example, weekly therapy sessions may discuss strategies to avoid environments associated with drug-related cues or effective techniques to decline drug-related activities with other drug users.
However, the underlying cognitive processes integral for successful behavioral modification can be examined in animal models and include behavioral flexibility, the ability to adapt to changing environmental stimuli and behavioral inhibition, the ability to inhibit impulses or actions, as well as measures of attention, motivation, and memory. Further, neuropsychological assessments at treatment initiation have correlated with overall treatment success (Teichner et al., 2001; Aharonovich et al., 2006; Turner et al., 2009; Schmitz et al., 2009; Moeller et al., 2010). Therefore, a pharmacological approach aimed at improving cognitive performance may facilitate success of behavioral modification strategies. In chapters II and III cognitive performance, specifically measures of behavioral flexibility and working memory were assessed in monkeys with a chronic cocaine self-administration history and cocaine-naïve monkeys. In chapter IV, nAChR agonists were examined for their effects on working memory performance in the same monkeys during periods of abstinence from cocaine, effectively modeling the period of abstinence during which treatment-seeking cocaine users undergo behavioral modification strategies. In Chapter V, the effects of nAChR agonists were assessed on their ability to influence the abuse liability of cocaine. Ideal drugs to improve behavioral modification strategies must enhance cognitive performance without increasing likelihood of relapse.

LONG-TERM CONSEQUENCES OF COCAINE ABUSE

Specific consequences of cocaine use on an individual’s health include increased risks of cardiovascular, respiratory or cerebrovascular anomalies. Cardiovascular effects include increased perivascular fibrosis, myocardial infarctions, and small vessel diseases
such as coronary artery dissection (see Milroy and Parai, 2011 for review). Since cocaine (crack) is often inhaled in addition to intravenous injection, alveolar hemorrhage, respiratory congestion pulmonary edema, and emphysema are all symptoms associated with ‘crack lung’ (Kissner et al., 1987). While less predominant, gastrointestinal, kidney, and liver complications have also been associated with cocaine use (Milroy and Parai, 2011). Within the CNS, cerebral infarctions or intracerebral hemorrhages are also reported (Milroy and Parai, 2011). Changes in vascular function are a result of enhanced catecholamine activity and chronic cocaine use disrupts homeostatic regulation within these neurotransmitter systems and leads to neurobiological alterations (see below). Corresponding disruptions in behavior such as increased irritability, depression, anorexia, fatigue and disrupted sleep cycles are often side effects exhibited during abstinence following cocaine use (Gawin and Kleber, 1986).

One of the hallmark consequences of cocaine addiction is a disruption of executive function. Broadly defined, executive function includes all processes involved in learning, monitoring and adapting to stimuli to produce complex, goal-oriented behaviors. Delineated cognitive domains include 1) updating, monitoring and adapting to cues relevant to a current goal and discarding/suppressing irrelevant information, 2) shifting, the ability to redirect focus between multiple modalities or tasks, and 3) inhibition, the ability to suppress or withhold a preplanned or impulsive response (see Miyake et al., 2000; Beveridge et al., 2008 for review). Compared to control groups, chronic cocaine users show impaired cognitive performance across each of these cognitive domains, effects that extend into abstinence, influencing treatment and vulnerability to relapse.
For example, working memory tasks are classically used to assess the updating component of executive function. Using a simple n-back task, a test requiring an individual to recall and identify a letter or number from a series that occurred n images prior to the current one, cocaine users showed poorer working memory performance (Verdejo-Garcia et al., 2006; Tomasi et al. 2007). Similar to other tasks assessing verbal or visual working memory (Hester and Garavan, 2004; Woicik et al., 2009; Hanlon et al., 2011), increasing cognitive demand either by the amount of information to recall, the length of time, or the amount of distractions embedded between sample and recall phases, magnifies cognitive deficits between cocaine users and controls. In Chapter II, the effects of cocaine and abstinence on working memory were assessed in monkeys with a chronic cocaine self-administration history and compared to performance in cocaine-naive monkeys.

Cocaine users also show impairments in shifting or behavioral flexibility as measured via reversal learning and set-shifting tasks (Kubler et al., 2005; Fillmore and Rush, 2006; Beveridge et al., 2008; Ersche et al., 2008; Hanlon et al., 2011). Both tasks probe different aspects of the shifting component of cognition. In a rule reversal task, following a learned discrimination between two distinct stimuli the rules are reversed such that a previously non-reinforced stimulus is now reinforced and the previously reinforced stimulus now has no consequences. Set-shifting tasks require multiple overlapping stimuli (i.e. shapes and lines). As before, a discrimination is established with one set of stimuli (i.e. responding on one shape is reinforced and the other shape is incorrect, lines serve as distracters). Once established, contingencies are shifted such that an individual must attend to the alternative stimulus (lines) and discriminate within this
new attentional set, disregarding the previous set (shapes). Set-shifting tasks require a redirection of attention whereas reversal-learning tasks directly require the inhibition and dissociation of a previously established rule. In Chapter III, set-shifting behavior was examined in monkeys with a chronic cocaine self-administration history and compared to performance of cocaine-naive monkeys.

Tasks measuring response inhibition include Go/No-Go tasks and stop-signal reaction time (SSRT) tasks. Both tasks involve training to associate responding to one stimulus and a lack of response upon presentation of a different stimulus. However, each task measures a slightly different aspect of motoric inhibition (Eagle and Robbins, 2008). The Go/No-Go task examines “action restraint”. Trials involve either the presentation of stimuli signaling responding (Go trial) or stimuli signaling withholding a response (No-Go trial). Go/No-Go tasks may rely on an attentional component, since an individual must focus on multiple stimuli to signal and withhold a response. In contrast, SSRTs measure “action cancellation”, the ability to inhibit a response during its execution. Similar stimuli signal emission of or withholding a response, yet the latter stimuli is presented at varying millisecond durations following the stimuli signaling responding. Eagle and Robbins (2008) provide an indepth differentiation of the two inhibitory tasks. Importantly, cocaine users show impaired inhibitory performance across both forms of inhibitory activity (Kaufman et al., 2003; Fillmore and Rush, 2002).

Decision-making tasks, such as the Iowa Gambling Task (IGT) require concurrent use of updating, shifting, and inhibitory domains. In this task, participants must choose between 4 decks of cards that are associated with various advantageous and disadvantageous scenarios (monetary gains and losses) each occurring with different
frequencies. Participants develop a choice selection strategy for perseverating on or choosing between decks based on updating information from previous trials. As one would expect, cocaine users show impaired performance on this task compared to controls (e.g. Bechara et al., 2000; Verdejo-Garcia et al., 2007).

Of importance for treatment development, cognitive deficits persist through weeks and months of abstinence (Verdejo-Garcia et al., 2006; Tomasi et al., 2007). However, most human studies compare current or recently abstinent cocaine dependent groups to cocaine-naïve control groups at only one timepoint. While informative, these studies do not provide information as to whether acute abstinence from cocaine (e.g. several days) affects cognition differently than longer durations of abstinence (e.g. weeks to months). For example, Woicik and colleagues (2009) showed that measures of attention and executive function were impaired to a greater extent in humans abstinent from cocaine greater than 72 hours compared to humans that had used cocaine within 72 hours suggesting that continuous cocaine use may mask deficits that become magnified during abstinence. In contrast, a recent study by Hanlon et al. (2011) showed greater impairments on tasks measuring working memory, planning and reaction time in current cocaine users compared to a group that maintained abstinence for 30 days. Both groups showed structural and cognitive deficits compared to cocaine-naïve controls (Hanlon et al., 2011). Therefore, more empirical research is needed to elucidate the effects of cocaine, and abstinence from cocaine on cognitive performance controlling for variables such as polydrug use, environment, stress and duration of cocaine use and/or abstinence. In Chapter II, the effects of cocaine and subsequent abstinence were assessed in monkeys with an ~6 yr chronic cocaine self-administration history and compared to cocaine-naïve
monkeys, providing an assessment of working memory performance during a critical window of abstinence during which treatment-seekers initiate and attempt to remain in treatment.

NONHUMAN PRIMATES (NHPs) AS RESEARCH SUBJECTS

As it relates to translational research, NHPs are more phylogenetically related to humans and, along with baboons, Old World macaques (rhesus, *Macaca mulatta*, and cynomolgus, *M. fascicularis*) are the closest relatives of humans approved for biomedical research in the United States. Macaques have close homology to humans in terms of developmental and aging processes, neurotransmitter distribution, and complex social and cognitive behavioral repertoires (see Weerts et al., 2007 for review). For example, humans and NHPs share greater than 95% overall gene homology and greater than 98% homology in monoaminergic transporters (Hacia et al., 1998; Miller et al., 2001). Further, documented differences in DA neuron innervation (Berger et al., 1991; Joel and Weiner, 2000) and affinity of DA for receptors between monkeys and rodents (Weed et al., 1998) may be indicative of other differences in drug biodistribution, pharmacokinetic or pharmacodynamic interactions within the DA system (e.g., Lyons et al., 1996; Roberts et al., 1999; Lile et al., 2003). Related to the current research, monkeys share similar nAChR localization, distribution and affinity states to humans (Cimini et al., 1992; Han, 2000; Quik et al. 2000; Papke et al., 2005). An additional advantage of NHP research is the ability for long-term studies and within-subject designs in a controlled laboratory setting. Baseline behavioral, neurochemical, and hormonal measures can be correlated
with changes following an experimental manipulation (e.g. chronic drug administration) while controlling for such factors as stress and nutrition over many years.

Additionally, NHPs can learn complex cognitive tasks analogous or homologous to those administered to humans. Following a stable cognitive baseline, the effects of environmental or pharmacological manipulations can be evaluated. Such studies can be conducted to gain a better understanding of the acute and chronic drug effects on specific cognitive domains, or elucidate the neural substrates underlying cognitive performance through lesioning or imaging techniques (e.g. Dias et al., 1996 a,b; Porrino et al., 2005). Neurocircuitry of the primate brain, specifically the prefrontal cortex is similar to the human brain (see Roberts et al., 1996 for discussion) and therefore provides relatively accurate extrapolation from animals to humans.

**COGNITIVE ASSESSMENT IN NHPS**

NHPs can be trained to perform tasks probing specific cognitive domains known to be impaired in human cocaine users. Importantly, tasks taken from human neuropsychological batteries have been administered unaltered to monkeys using touch sensitive computer screens. Additional tasks have been modified for use in monkeys to examine similar cognitive domains as tasks administered in humans and these have been extensively characterized. For example, the Cambridge Neuropsychological Test Automated Battery (CANTAB), the apparatus utilized in the current research, is comprised of a series of visual and spatial tasks designed to probe regional brain function by challenging specific cognitive components (Weed et al., 1999). Initially employed in humans, recent applications include extension to NHPs to examine such variables as age,
disease progression, pharmacological manipulation, and lesions within the CNS on task performance (Cirillo et al., 1989; Dias et al., 1996a,b, 1997; Voytko 1999; Weed et al., 1999, 2008; Porrino et al., 2005; Hampson et al., 2009). Two specific domains associated with cognitive impairments in human cocaine users that were assessed in the current research using CANTAB were working memory (Chapter II, IV) and behavioral flexibility (Chapter III).

Working memory can be examined in animals using visual or spatial cues. Classic working memory tasks include delay match- and non-match-to-sample tasks (DMS and DNMS, respectively). In both tasks a stimulus is presented to the animal that must be retained across a variable delay interval. Following this delay, the animal must select from an array of stimuli either the same stimulus (match) or the opposite stimulus (non-match) presented prior to the delay. Tasks assessing spatial memory are similar except that instead of recalling a specific stimulus, the location of the stimulus on a screen must be recalled. Similarly, in a delay-alternation task two stimuli are presented and each subsequent correct response must be made on the stimulus not touched in the preceding trial. In all measures of working memory, increasing the delay value or the number of distracter images increases the cognitive demand.

Two measures of behavioral flexibility include reversal of a previously acquired stimulus discrimination and attentional set shifting. In the former, two stimuli are simultaneously presented. In the simplest form, one stimulus is associated with a reward (S+) and the other stimulus has no reward (S-). In other versions, both stimuli are associated with a reward but of differing magnitudes. In both scenarios, the animal must learn to discriminate between the two stimuli and choose the stimulus associated with the
reward (or the reward of greater magnitude). Stimuli can be presented using a touch-sensitive computer apparatus as in the current studies to deliver food pellets, or manually using the Wisconsin General Testing Apparatus (WGTA; e.g. Jentsch et al., 2002). For the WGTA, two boxes are presented per trial, each with a distinct visual cue on top. A palatable treat is hidden within one of the two boxes and the monkey must discriminate between the two based on the visual cues. For all behavioral flexibility tasks, initial discrimination is said to have been acquired when performance meets an experimenter-determined criteria such as 6 correct responses in a row, or a serial acquisition criteria such as 18 correct responses out of 20 consecutive trials. Following acquisition, the contingencies are reversed such that the previously reinforced (or higher magnitude of reinforcer) is no longer associated with reinforcement (or lower magnitude reinforcer); in both scenarios the S+ now becomes the S- and vice versa. Acquisition of a simple discrimination can serve as a measure of associative learning while reversal learning provides an assessment of response inhibition, one aspect of behavioral flexibility.

Another measure of behavioral flexibility involves discrimination within and between multiple attentional sets. As before, a simple discrimination (SD) is acquired (e.g. two shapes, an S+ and S-). Second, a compound discrimination (CD) is introduced such that shapes are still discriminated but another attentional set (lines) overlay the shapes, and are not associated with any reward-related contingencies (serving only as distracter images); all four stimuli are randomly distributed independent of each other. Following this stage, two new shapes and two lines are introduced but shapes continue to be the attentional set associated with reinforcement. This stage employs an intra-dimensional (ID) shift, such that the previously established set remains in focus. The last
stage is an extra-dimensional (ED) shift. One stimulus from the previously ignored attentional set (lines) now becomes the S+ and the previously reinforced set (shapes) serves as a distraction. While similar, a reversal task requires termination of a previously established stimulus-reinforcement association before the formation of a new association whereas set shifting requires a re-direction of attention, to a new, relevant attentional set and inhibition of an irrelevant stimulus set.

Despite similarities between human and NHPs and the numerous clinical studies demonstrating cognitive dysregulations in cocaine-experienced humans, few studies have examined the effects of cocaine on cognition in NHPs. Of these studies, the cognitive domains frequently assessed are associative learning, measured via a simple discrimination task, behavioral flexibility, measured via a reversal learning task, and measures of working memory, assessed by either a delayed alternation task or DMS task. One additional study validated a model of impulsivity, the SSRT in a rhesus monkey model. Employing similar conditions as the human task and using 2 monkeys with a moderate cocaine SA history (~360 mg/kg cumulative intake) this study reported increased impulsivity, as measured by long SSRT measures following 18 months of abstinence from cocaine SA (Liu et al., 2009).

Only two previous studies have examined working memory performance in NHPs following a cocaine SA history. In one study, 2 rhesus monkeys with a cocaine (average intake 360 mg/kg) and cocaethylene history (a metabolite produced by the interaction of cocaine and alcohol; average intake 90 mg/kg), self-administered 0.5 mg/kg/injection cocaine (max 3.0 mg/kg cumulative daily intake) once weekly (Liu et al., 2008). These monkeys were trained on a delay alternation task. Monkeys with a cocaine SA history
required a greater number of trials to acquire this task (80% accuracy with a zero sec
delay). There were no initial differences over 10 sessions when delays were introduced
(0-10 sec) but over 17 weeks of assessment the cocaine-naïve monkeys improved at a
significantly greater rate than the two monkeys continuing to self-administer cocaine (Liu
et al., 2008). In another study by the same group, 8 monkeys self-administered the same
dose as above 4 days/week and visual working memory was assessed weekly using a
DMS task, following 72 hours of abstinence (Porter et al., 2011). Compared to a control
group (n=6), percent accuracy at the longest delay was lower during the first 4
assessments (1 month) but tolerance developed to the disruptive effects of cocaine. In
both of these studies, cocaine was intermittently self-administered and 72 hours elapsed
before each cognitive assessment. In Chapter II, monkeys with a chronic cocaine self-
administration history more extensive than subjects from Liu et al. (2008) and cocaine-
aïve monkeys were trained to perform a DMS task, 5-7 days/week. Monkeys continued
to self-administer cocaine (or food pellets, control) in afternoon sessions and DMS
sessions took place on subsequent mornings. Using this model, we extend the previously
described studies by examining the effects of high doses of cocaine and subsequent
periods of abstinence on daily working memory performance. This study design modeled
chronic and current cocaine users and the residual effects on working memory the day
after a cocaine self-administration session. Secondly, this assessment characterized a
critical window during which treatment-seeking cocaine users would initiate treatment.

Jentsch and colleagues (2002) used the WGTA to administer an object
discrimination and reversal test to vervet monkeys prior to and following 14 days of once
daily, experimenter-administered injections of 2 or 4 mg/kg cocaine, (im). Monkeys
exhibited impaired abilities to learn the reversal portion of this task, but not to acquire the initial discrimination 9 and 30 days following cocaine administration (Jentsch et al., 2002). Two additional studies used a touch-sensitive computer screen to administer a discrimination and reversal task prior to and following various cocaine self-administration paradigms (Liu et al., 2008; Porter et al., 2011). In both studies, the discrimination contingencies were between high and low liquid reinforcers (1.0 mls and 0.3 mls H2O) and a correct response required choosing the high magnitude stimulus. SD/SDR performance occurred 72 hours following the last self-administration session and showed that initial SD acquisition (18 correct out of 20 consecutive trials) was hindered such that the SDR component could not be reliably examined (Liu et al., 2008). Porter and colleagues (2011) showed that compared to a control group (n=6), initiation of cocaine SA did not impair initial percent accuracy (defined as the % of correct trials in the first 15 trials) on the SD but sustained accuracy (defined as 27 correct out of 30 consecutive trials) was impaired. Accuracy on the first 15 trials following reversal was also lower in the monkeys with a cocaine SA history compared to controls. In Chapter III, we extended this work by examining another measure of behavioral flexibility, set shifting.

In addition to neuropsychological test batteries administered to current or recently abstinent cocaine users, functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) have provided new opportunities to examine specific brain function underlying specific cognitive domains. Recent fMRI studies have shown functional deficiencies associated with impaired executive function compared to cocaine-naïve control groups. Various regions of the prefrontal cortex are typically activated (e.g.
dorsolateral, ventromedial, or orbital prefrontal cortex) depending on the cognitive domain being assessed. Consistently, cocaine users showed lower activity in the anterior cingulate cortex (ACC) and relevant regions of prefrontal cortex (PFC), notably the dorsolateral PFC (dlPFC) and orbital PFC (orbPFC) during tasks measuring updating, shifting, and inhibitory domains compared to cocaine-naïve control groups (Fillmore and Rush 2002; Bolla et al., 2004; Hester and Garavan, 2004; Kubler et al., 2005; Goldstein et al., 2007, 2010; Woicik et al., 2009).

Broadly, the PFC controls executive function. The orbPFC receives projections from and reciprocates projections to limbic reward-related areas (striatum, ACC) and sensory posterior cortical and PFC (dlPFC, mPFC) regions (Carmichael and Price, 1995a,b; see Wallis 2007 for review). Functionally, the orbPFC is implicated in maintaining stimulus-reinforcement associations based on feedback from these inputs and relaying pertinent information to other PFC regions such as the dlPFC and ACC (for reviews see Volkow and Fowler, 2000; Wallis, 2007; Liu et al., 2011). The ACC is involved in error-detection and conflict-monitoring and signals other PFC and limbic brain regions such as the striatum to re-direct goal-oriented behaviors in response to task-specific errors, or following deviations from expected reward values (e.g. Aron and Paulus, 2007; Liu et al., 2011). The striatum, a DA-rich limbic region is thought to play a filtering role between the PFC (with projections to and from the ACC) by updating and establishing stimulus-reward associations (Bar-Gad et al., 2000; Volkow and Fowler, 2000; Leblois et al., 2006; Nagano-Saito et al., 2008) and the posterior sensory cortical regions by activating relevant and inhibiting irrelevant circuits specific to sensory modalities (e.g. shapes vs lines, objects vs faces; see van Schouwenburg et al., 2010).
Additional PFC subregions, such as the dlPFC and ventromedial PFC (vmPFC) receive projections from temporal and parietal sensory regions that encode information regarding task specific physical properties (e.g. colors and shape vs rank order) that then project to the orbPFC and ACC to execute behavior (cf. Romanski, 2004). Disruption to the PFC regions or the limbic structures filtering information between PFC and posterior cortical regions will impair aspects of executive function, including behavioral flexibility and impulsivity, largely due to the inability to accurately evaluate and adapt to changing contingencies. As previously stated, cocaine use is associated with disruptions in cognitive performance and underlying function across the PFC and limbic system depending on the cognitive domain (e.g. reversal learning or set shifting) and sensory domain (e.g. visual or spatial) being assessed (e.g. Hester and Garavan, 2004; Kubler et al., 2005; Tomasi et al., 2007; Moeller et al., 2010).

Cognitive deficits associated with cocaine use may perpetuate the cycle of drug use and increase relapse through 1) dysregulation in pathways controlling behavioral inhibition and/or impulsive behavior, and 2) predisposing abstaining drug users to failure in behavioral treatments in which cognitive-based approaches are designed to educate and modify maladaptive behaviors (cf. Rogers and Robbins 2001). In fact, neurobiological and behavioral measures obtained from cocaine-dependent treatment seekers just prior to treatment initiation have been directly linked to rates of attrition and treatment success rates (e.g. Teichner et al., 2001; Turner et al., 2009; Martinez et al., 2011). Therefore, understanding the neurobiological alterations underlying impaired executive function as a result of cocaine self-administration and the persistence of these effects is integral in developing effective treatment strategies. In Chapter III, glucose
metabolism was assessed during a set-shifting task using PET imaging in monkeys with an extensive cocaine SA history and cocaine-naïve monkeys to identify potential CNS targets for drug development.

**POSITRON EMISSION TOMOGRAPHY (PET) IMAGING**

One application for using animals and cognitive testing is to determine the underlying neural substrates mediating cognitive performance. For example, Dias and colleagues (1996a,b, 1997) systematically lesioned either the dlPFC or orbPFC to determine the respective involvement of each subregion in successful acquisition of set-shifting and rule reversal tasks. Their findings suggested that chemically induced lesions to the orbPFC selectively inhibited reversal learning while lesions to the dlPFC selectively inhibited set-shifting behavior; these results were similar to cognitive deficits seen in human patients with natural lesions to similar brain regions. These studies highlight similar neurobiological substrates underlying specific cognitive function between NHPs and humans (Dias et al., 1996a,b, 1997). Similarly, transection of the temporal stem, the white matter tract connecting the temporal lobe to the thalamus and PFC impaired working memory in monkeys performing a DMS task implicating the temporal cortex in mediating working memory performance (Cirillo et al 1989).

However, lesion studies only implicate the involvement of one component of a neural pathway in mediating a specific behavior. PET, SPECT, and fMRI techniques can provide a broader assessment of CNS function, including multiple regions within the brain that are active during cognitive performance. PET is a neuroimaging technique used to visualize and quantify the interaction of a radiolabelled molecule within an organism
in a noninvasive manner. Briefly, a radioactive isotope, typically $^{18}$-Flourine, $^{11}$-Carbon or $^{15}$-Oxygen, is attached to a molecule of interest. Once injected into an organism, the radioactive decay of the isotope is recorded over minutes to hours by an array of detectors. The exact location and amount of radiation is then visualized and quantified using kinetic modeling algorithms and computer analysis to reconstruct 3-dimensional data into 2-dimensional images (see Kegeles and Mann, 1997 for review). PET imaging techniques allow an examination of neuronal function and regulation through utilization of radiolabelled molecules with affinity and selectivity for specific neuronal structures (mitochondria) or proteins (e.g., neurotransmitter receptors or transporters). PET imaging techniques in NHP models of addiction have substantially increased our understanding of the effects of cocaine on the brain and the malleability of brain function through pharmacological and environmental factors (see Nader et al., 2008; Howell and Murnane, 2011, Murnane and Howell, 2011 for reviews).

One utility of PET imaging is to examine protein expression, notably neurotransmitter receptor availability or changes in availability following an environmental or pharmacologic manipulation, *in vivo*. The reinforcing effects of cocaine are attributed to elevated synaptic DA levels due to DAT blockade (Di Chiara and Imperato, 1988). Therefore, the DA system has been extensively characterized regarding neurobiological alterations associated with cocaine exposure. PET imaging showed that recent human cocaine users had lower dopamine D2-like receptor availability in the DA-rich striatum compared to cocaine-naïve controls (Volkow et al., 1993). In NHP studies, repeated PET imaging demonstrated cocaine-induced reductions in D2-like receptor availability (Nader et al., 2006) corresponding to lower DA D2-like receptor densities in
monkeys with a cocaine self-administration history confirmed by in vitro receptor autoradiography (Moore et al., 1998; Nader et al., 2002).

Another use for PET imaging is the in vivo investigation of blood flow or energy use as a means of measuring neural activity. \(^{15}\text{O}\)H\(_2\)O for example, is a marker of blood flow and can be used to characterize the acute effects of a pharmacological stimulus. Another tracer, \(^{18}\text{F}\)-fluorodeoxyglucose (FDG), is an analog of glucose that can be used to examine metabolic rates of cerebral glucose utilization (MRglu). The primary energy source in the brain under normal circumstances, glucose is transported into cells to restore and maintain chemical gradients. Increased rates of FDG uptake reflect increases in local energy use, representing a measure of functional activity. These methods are intended for the evaluation of manipulations that occur over relatively short time frames (e.g. drug administration or brief behavioral task) with changes in MRglu calculated by comparison to scans obtained during baseline conditions. However, differential metabolic activity in response to a brief challenge reflects changes within the CNS as a result of accumulating environmental and behavioral histories (e.g. chronic cocaine self-administration).

Primarily, FDG-PET has been used in NHPs to understand the neural substrates associated with natural behaviors such as the stress response, including response to mate competition, maternal separation, or freezing behavior (Rilling et al., 2001, 2004; Kalin et al., 2005; Fox et al., 2008; Kern et al., 2008; Jahn et al., 2009). A series of studies has recently examined environmental and pharmacological influences on working memory performance. For example, DMS performance was associated with increased glucose metabolism in the temporal cortex, PFC and dorsal striatum (Porrino et al., 2005). Sleep
deprivation impaired percent accuracy and was accompanied by reductions in glucose metabolism in working memory-related cortical areas (Porrino et al. 2005). Administration of the ampakine, CX717 or orexin-A attenuated the deleterious effects of sleep deprivation on both percent accuracy and glucose metabolism (Porrino et al. 2005; Deadwyler et al. 2007; Hampson et al. 2009). Lastly, when cocaine was substituted for juice as the reward for correct DMS performance, accuracy declined but glucose utilization in the dlPFC increased, which was interpreted as a disruption in neuronal firing patterns within the dlPFC (Hampson et al., 2011).

In recent cocaine users, glucose metabolism was lower in the ACC and orbPFC compared to cocaine-naive control groups, and metabolic activity positively correlated with D2-like receptor availability in the striatum (Volkow et al., 1993). A similar relationship between D2-like receptor availability using PET and glucose metabolism in the PFC has been documented in NHP studies using a 2-[14C]deoxyglucose (2DG) method and autoradiographic analysis (for reviews see Porrino et al., 2007; Beveridge et al., 2008). Using this ex vivo assessment of glucose metabolism the effects of cocaine on glucose metabolism expand over time. For example, following five days of cocaine SA (~1 mg/kg/day), lower glucose metabolism was apparent in the ventral striatum (nucleus accumbens), VTA, and the medial-caudal aspect of the ACC, compared to controls (Porrino et al., 2002). Following 100 days of high dose (9 mg/kg/day) cocaine SA, the pattern of lower glucose metabolism expanded both rostro-caudally and dorso-laterally, including both dorsal (caudate-putamen) and ventral striatum, and extended across the ACC and portions of the dlPFC and orbPFC (Porrino et al., 2004). Together these studies showed the progressive expansion of cocaine’s effects on glucose utilization from limbic
areas predominately associated with reward, to encompass areas associated with executive function (for review see Beveridge et al., 2008). A similar study using FDG-PET also showed a progressive involvement of limbic and cortical brain regions following acute and extended cocaine SA (Henry et al., 2010) although the direction of change was opposite from the 2DG methods. Reasons for similar effects in opposite directions may include cocaine dose or schedule of reinforcement, or analytical methodology. It is also noteworthy that autoradiography studies following the same cocaine SA paradigm as the 2-DG studies described above revealed changes in the metabotropic glutamatergic system (Beveridge et al., 2011), dopamine system (including increased D1-receptor and reduced D2-receptor binding: Nader et al., 2002), increased DAT (Letchworth et al., 2001) and NET binding (Macey et al., 2003) illustrating that cocaine-induced alterations extend beyond the DA system. To our knowledge, with the exclusion of these studies, FDG-PET has not been readily used in animals to assess the long-term effects of cocaine on cognition.

In Chapter IV, FDG-PET was used to assess MRglu during a set-shifting task in cocaine SA monkeys and cocaine-naive monkeys to extend our understanding of a) the neural substrates responsible for accurate set-shifting behavior and b) cocaine-related alterations throughout the brain that underly cognitive deficits, without the confounds associated with human drug use. The results of these studies can be used to focus drug treatment strategies on neurotransmitter systems and receptor subtypes that are distributed heavily within areas of abnormal metabolic activity.
COGNITIVE ENHANCEMENT AS A THERAPEUTIC STRATEGY

In rodents, D2-like receptor binding in the striatum and hippocampus was directly correlated with accuracy in the radial-arm maze, an assessment of working memory (Levin et al., 1997). In monkeys, D2-like receptor availability was directly correlated with performance on a reversal-learning task measuring behavioral flexibility (Groman et al., 2011). Further, DAT binding in the striatum of humans correlated with functional changes in response to increasing cognitive demand in the frontal and posterior cortical areas (Tomasi et al., 2009). These examples support the notion that dysregulation within the DA system may be an underlying cause of cognitive impairments associated with cocaine use. Thus, one avenue for therapeutic development is to explore mechanisms to increase DA function. Specifically, improving CNS function and cognitive performance during addiction treatment may improve overall treatment success (for review, see Sofuoglu, 2010). For example, methylphenidate, an indirect DA agonist, increased activity in hypoactive brain regions, including the ACC, and improved cognitive performance in cocaine-dependent individuals compared to control groups (Goldstein et al., 2010). Validation of cocaine-associated cognitive and neurobiological deficits in monkeys lacking confounds associated with human subjects (e.g. stress, polydrug use, diet, etc.) will allow a systematic assessment of novel pharmacotherapies to aid behavioral modification strategies.

Both D1- and D2-like DA receptors have been implicated in mediating executive function. However, the effects of DA modulation appear to be dependent on a number of variables including task, region, DA receptor subtype, and most notably basal DA function. For example, D1-like receptors in the PFC appear to be implicated in mediating
tasks focused on goal-relevant stimuli such as working memory performance (for review see van Schouwenburg et al., 2010). In general, D1-like agonists improved spatial working memory in monkeys, but only within a limited dose range; high doses induced disruptions in performance (see Mehta and Riedel, 2006 for review). In contrast, D2-like receptors may be more influential in mediating cognition relevant to updating and shifting tasks when there is competition between task-relevant and irrelevant stimuli (for review see van Schouwenburg et al., 2010). The striatum is thought to play a filtering role in communicating with both PFC areas responsible for updating and adapting for future responses while simultaneously activating relevant, and suppressing irrelevant posterior cortical circuits specific to various sensory perceptions (e.g. lines, shapes, faces, colors; see Cools, 2011 for review).

Effects of pharmacological agents on cognition are dependent on both baseline cognitive function and neurotransmitter tone. Often, equivocal results of DA modulation in human (and animal) studies are attributed to individual differences or examination of healthy individuals. Cools and D’Esposito (2011) described the balance between DA function and working memory performance as an inverted-U-shaped curve such that at an optimal dopaminergic tone performance is most accurate, least likely to be enhanced, and most likely to be disrupted. In healthy individuals at the peak of this curve, either agonism or antagonism of DA function can inhibit cognitive performance through over-stimulation or DA depletion, respectively. The majority of clinical and preclinical data showing cognitive enhancing effects of DA modulation involve patients with Parkinson’s Disease (PD), schizophrenia or ADHD or animal models of these conditions associated with hypodopaminergic function (see van Schouwenburg et al., 2010). Cocaine use is
also associated with a hypodopaminergic state. Therefore, when evaluating potential cognitive enhancers it is crucial to utilize a model with disruptions in DA function similar to the clinical condition one hopes to improve.

In Chapters II and III, data will be shown indicating that cognitive performance is significantly disrupted in rhesus monkeys with a history of cocaine self-administration. Drugs directly targeting the DA system have largely been unsuccessful in maintaining abstinence in treatment-seeking cocaine users (see below). Therefore, an indirect mechanism through which to modulate DA function is proposed for further study. In humans, nicotine and nicotinic acetylcholine receptor (nAChR) compounds can increase neural function and improve cognition (e.g. Lawrence et al., 2002; Rezvani and Levin, 2001). In Chapter IV, nicotinic acetylcholine receptor agonists were examined as putative cognitive enhancers during periods of abstinence from cocaine. Of clinical relevance, nicotinic agonists can indirectly stimulate DA function which may improve executive function in treatment-seeking cocaine users and improve the success of behavioral treatment strategies. In Chapter V, the effects of nAChR compounds were assessed for their ability to influence the abuse liability of cocaine, using cocaine self-administration and cocaine discrimination models.

NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR) SYSTEM

Within the CNS, the acetylcholine (ACh) neurotransmitter system serves a neuromodulatory role to influence signaling of other neurotransmitters such as dopamine. There are two primary ACh projection pathways within the CNS. Cell bodies in the basal forebrain project to the thalamus and cortex and are implicated in mediating aspects of
cognition including attention and memory (for review see Perry et al., 1999). Of relevance to the DA system, ACh neurons project from the pedunculopontine nuclei in the midbrain and synapse on DA neurons within the ventral tegmental area (VTA); additional ACh interneurons in the striatum synapse on striatal DA dendrites (see Exley and Cragg, 2008 for review). Nicotinic ACh receptors are ligand-gated cation channels that influence Ca^{2+} levels both intra- and extracellularly to affect ion gradients and neurotransmitter function (see Dajas-Bailador and Wonnacott, 2004, Dani and Bertrand, 2007 for reviews). Nicotinic AChRs located on DA, as well as GABA and glutamate nerve terminals can thereby directly influence neuronal excitability and subsequent neurotransmitter release (Jones et al., 2001; Zhou et al., 2001; Rice and Cragg, 2004). For example, nAChR agonists (e.g. nicotine, cytosine, varenicline) have been shown to increase DA release in the striatum and in the PFC in both in vitro and in vivo studies (Zhou et al., 2001; Zoli et al., 2002; Rice and Cragg, 2004; Rollema et al., 2007; Abin-Carriquiry et al., 2008; Chan et al., 2007; Livingstone et al., 2009).

Nicotinic AChRs are comprised of 5 subunits of homogenous (primarily containing α7 subunits) or heterogenous (α1-10; β1-4) makeup with subtype composition affecting ligand binding affinity and efficacy (Corringer et al., 2000; Le et al., 2002). Although prevalent across a number of brain regions including the striatum and PFC, homomeric α7 receptors are more prevalent within the hippocampus and the most prevalent heteromeric receptor makeup within the CNS, the α4β2* receptor, is more densely located in striatal and PFC regions (Quik et al., 2000). β2-containing nAChRs have been identified directly on DA neurons, α7-subtype nAChRs on glutamatergic inputs to DA neurons in the VTA, and non-α7 subtype receptors (primarily α4β2*-
subtype receptors) are located on GABA neurons projecting to DA neurons (for review see Dani and Bertrand, 2007). Thus, differential localization and distribution with the CNS support different influences on DA function, including tonic versus phasic activity (see Picciotto et al., 2008 for review). Therefore, stimulation of various nAChR subtypes may differentially affect DA function, including cognition and the reinforcing effects of cocaine.

However, differences in nAChR availability and distribution have been shown in several neuropathological diseases. For example, postmortem studies in PD patients showed reduced α3 and α4 receptor subunit binding in the caudate nucleus and increased α7 subunit binding in the temporal cortex compared to non-PD postmortem tissue (Guan et al., 2002). Using 2[18F]F-A-85380 (2FA), a PET ligand specific for α4β2* nAChRs and [18F]-L-DOPA a DA D2-like receptor ligand, Kas and colleagues (2009) reported lower α4β2* nAChR and DA D2-like receptor availability, respectively in the striatum in PD patients compared to healthy controls. Studies in Alzheimer’s Disease (AD) patients have shown lower [11C]-nicotine binding in the frontal and temporal cortex, including the hippocampus compared to healthy patients (Nordberg et al, 1990, 1995) suggesting a role of nAChR subtypes either in the etiology of disease progression, or as a compensatory mechanism to other neurobiological alterations. Further, 12 weeks of treatment with rivastigmine, a cholinesterase inhibitor, increased [11C]-nicotine binding and these increases directly correlated with improvements on measures of attention (Kadir et al., 2006, 2007).

Regarding addiction studies, nicotine exposure in animals and smoking in humans is consistently associated with nAChR up-regulation. For example, in rats, chronic
nicotine exposure resulted in greater $\alpha 4\beta 2*$-subtype nAChR binding measured via autoradiography studies (Nguyen et al., 2003; Metaxas et al., 2010). Higher $\alpha 4\beta 2*$-subunit binding was reported in smokers compared to non-smokers in frontal, striatal and midbrain sites (Muhkin et al., 2008). Post-mortem tissue from long-term smokers showed higher nAChR binding sites in the thalamus and hippocampus using $[^3]H$-nicotine compared to controls (Breese et al., 1997). There is a paucity of PET studies examining specific effects on $\alpha 7$-selective receptor subtypes, in part due to few commercially available subtype-specific compounds and radiotracers. While nicotine produces upregulation of nAChRs in humans and animals, chronic smoking is associated with lower DA D2-like receptor availability measured via PET (Fehr et al., 2008), similar to cocaine and other drugs that elevate extracellular DA (see Goldstein and Volkow, 2002 for review), a finding that has yet to be examined in animal models.

To our knowledge, no animal study and only one human study has indirectly investigated nAChR distribution associated with cocaine exposure using PET imaging. Adinoff and colleagues (2010) examined regional cerebral blood flow (rCBF) in cocaine users. Alterations in rCBF were present between cocaine users and non-drug abusing controls in regions underlying learning and memory including the hippocampus following pharmacological challenges, although similar differences were seen with the muscarinic AChR antagonist scopolamine and the nonselective muscarinic and nicotinic AChR agonist physostigmine. While this study implicated changes in the ACh system, per se, effects could not be directly attributed to nAChRs exclusively. Further, cocaine users in this study were also nicotine-dependent, suggesting differences were not specific to the effects of cocaine (Adinoff et al., 2010). Direct examination of nAChR availability

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following cocaine self-administration in humans or animals has not yet been performed. Characterizing nAChR availability is important when attempting to evaluate drugs targeting this receptor system. Therefore, in Chapter IV nAChR availability was evaluated in monkeys with a cocaine self-administration history and cocaine-naive monkeys using $[^{11}C]$-nicotine and positron emission tomography (PET).

Nicotinic AChR agonists have shown cognitive-enhancing effects across a number of species and cognitive domains. For example, various nAChR agonists including nicotine have improved measures of attention and working memory in healthy cohorts and individuals with neuropathologies associated with hypodopaminergic function such as Parkinson’s Disease, depression and schizophrenia; in many cases such results were replicated in various animal models (for reviews see Rezvani and Levin 2001; Forgacs and Bodis-Wollner, 2004; Wilens and Decker 2007; Cincotta et al., 2008; Radek et al., 2010). Nicotinic AChR agonism may enhance cognition in subjects with a cocaine self-administration history by indirectly stimulating DA function. Drugs that enhance cognitive performance across domains associated with cocaine-related deficits may be applied in clinical settings as an adjunct treatment to facilitate the goals set forth by behavioral treatment strategies, namely behavioral modification, impulse control, treatment retention and ultimately, abstinence from drug use (Sofuoglu, 2010).

In addition to stimulating DA release and improving cognition, nAChR agonists increase measures of CNS function in regions associated with hypoactivity in cocaine users. In healthy volunteers, nicotine improved performance during a sustained attention task, the Rapid Visual Information Processing task (RVIP), and increased neuronal activity quantified as an increased BOLD response using fMRI, in the caudate nucleus.
and parietal cortex (Lawrence et al., 2002). Varenicline, an FDA-approved aid for smoking cessation with low abuse liability, is an α4β2* subtype-selective nAChR partial agonist and an α7 subtype-selective low-affinity, high efficacy agonist (Mihalak et al., 2006; Rollema et al., 2007) with promise as a cognitive enhancer (Patterson et al., 2009; for review see Rollema et al., 2009). In rats, varenicline acutely increased activity in a number of brain regions including the hippocampus, nucleus accumbens, VTA, ACC, PFC, temporal and occipital cortex and improved spatial learning performance (King et al., 2011). Similarly, in chronic smokers, varenicline increased activity, specifically in the ACC and dlPFC and improved working memory performance (Loughead et al., 2010); each of the above studies used fMRI. In addition, chronic treatment with varenicline increased D2-like receptor density measured via autoradiography (Crunelle et al., 2009, 2011), similar to increased D2-like receptor availability following chronic treatment in monkeys with the DA antagonist raclopride, measured via PET (Czoty et al., 2005a).

Alpha 7 subtype-selective nAChR agonists also increase DA release (Livingstone et al., 2009) and produce cognitive enhancing effects in various animal and human studies including tasks measuring working memory (see Thomsen et al., 2010c). One novel, potent α7 subtype-selective compound, PNU-282987 (Bodnar et al., 2005) has shown cognitive enhancing effects specifically on measures of attention and working memory, but to our knowledge has yet to be examined in monkey models of cognition. In Chapter IV, the effects of nicotine, the nonselective high-efficacy nAChR agonist, varenicline, and PNU-282987 were examined on working memory performance in both cocaine-naïve monkeys and monkeys with a cocaine SA history. Despite each drug demonstrating cognitive-enhancing effects in healthy animal or human studies, efficacy
and nAChR subtype-selectivity of the chosen compounds may differentially influence working memory performance in monkeys with cocaine-associated cognitive deficits.

Chapters II and III examined cognitive function in rhesus monkeys with a cocaine self-administration history and cocaine-naïve monkeys. These assessments established a monkey model of cocaine-associated cognitive and neurobiological deficits. In Chapter IV, nAChR agonists were examined on working memory performance in these same monkeys. NAChR agonists have reliably shown cognitive-enhancing effects in humans and animal models (see Rezvani and Levin, 2001 for review), acutely increased neural function (Lawrence et al., 2002; Loughead et al., 2010; King et al., 2011) and increased DA D2-like receptor availability (Crunelle et al., 2009, 2011). The effects of nAChR agonists on neurobiology and cognition suggest that they may provide beneficial effects towards reversing cocaine-induced hypofunction of the CNS and have not previously been examined as a therapeutic strategy. However, cognitive enhancement alone may not be sufficient to maintain abstinence from cocaine. It is important to assess drugs across multiple behavioral assays. For example, the ability to enhance cognitive performance, while a promising therapeutic strategy, might be overshadowed if the target drug also influences the direct effects of cocaine such that it alone might increase the likelihood of relapse. Therefore in Chapter V, we examined the effects of varenicline on cocaine self-administration and discrimination to assess its ability to influence the abuse liability of cocaine.
CLASSIC PHARMACOLOGICAL TREATMENT APPROACH

Two opposing strategies in developing pharmacological treatments for addiction are agonist replacement therapy and receptor antagonism (e.g. reviews see Dutta et al., 2003; Grabowski et al., 2004; Xi and Gardner, 2008; Herin et al., 2010). A third, novel approach that will only be mentioned is immunization such that the drug is sequestered before it can enter the CNS (for review see Hall and Gartner, 2011). Briefly, agonist replacement strategies attempt to decrease drug use by partially producing the positive effects of the abused drug. Optimal agonist compounds bind to receptors with higher affinity but lower efficacy as the drug in question (e.g. cocaine), to induce less robust neurochemical effects, have a slower rate of onset and a longer duration of action. In this manner, patient compliance is typically not a concern, although abuse liability may be problematic. Agonist approaches may reduce psychological withdrawal symptoms or craving. Successful agonist treatments include buprenorphine and methadone for opioid dependence and varenicline or nicotine patch or gum for smoking cessation, but drugs targeting cocaine dependence have not garnered similar clinical success. Antagonist approaches, in theory, decrease repeated drug use by blocking drug effects during relapse. Antagonists block a receptor so that other substrates either cannot bind to or must compete for receptor occupancy and therefore produce less or no net effect. Antagonism may potentiate withdrawal symptoms or induce negative side effects and is often associated with poor compliance.

A more recent approach utilizes partial agonist compounds. Partial agonists, or agonists with less than full efficacy, bind to receptors but induce a lesser degree of activity (Hoyer and Boddeke, 1993). Partial agonists compete with endogenous ligands or
exogenous compounds and, based on receptor affinity, may produce antagonist-like
effects through receptor blockade or competition for binding sites. For example, low-
efficacy DA agonists can induce small elevations of synaptic DA levels when
administered alone, but effectively antagonize the effects of increased DA in the synapse
through competition at receptors (e.g. following blockade of DAT by cocaine). A partial
agonist approach attempts to apply both agonist replacement and antagonism principles.

At doses known to be reinforcing in rodents, cocaine elevated extracellular DA
levels by ~250 percent and ~150 percent whereas nicotine only elevated extracellular DA
by ~100 and 50 percent in the nucleus accumbens and caudate, respectively, in rats (Di
Chiara et al., 1988). Varenicline only elevated extracellular DA levels by ~ 1/3 the extent
of nicotine (Rollema et al., 2007, 2009). Although nicotine is considered a high-efficacy
nAChR agonist and varenicline a low-efficacy agonist, when referring to their potential
to increase extracellular DA levels, both must be considered partial efficacy agonists.

DRUG SELF-ADMINISTRATION

There are two primary animal models utilized to examine potential
pharmacotherapies for addiction, drug self-administration (SA) and drug discrimination.
Self-administration studies involve operant training such that a discriminative stimulus
(e.g. light, tone) signals the occasion during which a drug will be delivered following a
behavioral response (e.g. pressing a lever or poking a nose into a hole). A drug is
considered to have reinforcing effects if the behavior maintained by drug delivery occurs
at levels greater than that same behavior engendered by a neutral stimulus (e.g. a vehicle
administration, typically physiological saline). Drug SA studies have been incorporated
for decades to examine the reinforcing effects of compounds and to examine effects of putative pharmacotherapies on established SA (e.g. Johanson and Fischman, 1989; Woolverton and Nader, 1990; Mello and Negus, 1996). Drugs that are abused by humans including stimulants, opiates, and barbiturates/benzodiazepines are self-administered by rodents and monkeys and can be delivered via routes identical to those associated with human drug use (e.g. oral, intravenous, inhaled). Further, chronic drug SA in monkeys produces neurobiological effects that parallel those reported in human drug users including metabolic, structural and functional CNS alterations (e.g. cocaine; Strickland et al., 1993; Volkow et al., 1993; Lyons et al., 1996; Beveridge et al., 2006). In these respects, drug SA studies have strong predictive and construct validity to human drug addiction.

A number of variations on this paradigm have increased the generalizability of findings across species and drug classes, as well as the specific aspect of the addiction cycle being modeled. For example, a simple fixed-ratio schedule of reinforcement (FR; a constant number of responses results in delivery of a stimulus) allows assessment of the reinforcing effects of a drug. However, due to the influence of direct drug effects on behavior, as well as parametric limitations and variability between laboratories, FR schedules preclude direct comparisons of reinforcing “strength”. Progressive-ratio (PR) schedules of reinforcement can be employed to examine the reinforcing strength of a compound. By increasing the response ratio required to deliver each subsequent stimulus, a breakpoint (BP; highest ratio completed before responding ceases for a set duration of time) can be determined and interpreted as a threshold that represents the highest
behavioral allocation an animal will emit to produce subsequent drug delivery. Increasing the timeout duration between drug availability also minimizes drug accumulation.

Regardless of the contingencies set forth by the experimenter, a major strength of the SA paradigm is that following minimal training, animals respond reliably and consistently. Deviations from this response profile can subsequently be characterized based on pharmacological or parametric manipulations. One goal of Chapter V was to examine the effects of nAChR compounds on the reinforcing strength of cocaine, as measured via a PR schedule of reinforcement.

Related to addiction studies and cocaine, nicotine has been shown to have a synergistic effect with cocaine by stimulating DA release measured via microdialysis in rats (e.g. Sziraki et al., 1998; Gerasimov et al., 2000). In behavioral studies, nicotine and cocaine combinations resulted in higher break points than cocaine alone in monkeys responding under a progressive ratio schedule of reinforcement (e.g. Freeman and Woolverton, 2009), supporting the high prevalence of co-abuse and/or potentiating effects of these two drugs in humans (e.g. Schorling et al., 1994; Roll et al., 1996). These data suggest that nonselective nAChR agonist-based treatment would not be successful.

In contrast, mecamylamine, a nonselective nAChR antagonist attenuated cocaine SA during maintenance (e.g. Levin et al., 2000) and escalation in rodents (Hansen and Mark, 2007). One clinical trial examined mecamylamine in treatment-seeking cocaine-dependent patients and reported no differences in cocaine intake, rates of abstinence, or self-reports of cocaine craving compared to a placebo-controlled group (Reid et al., 2005). Although only one dose was tested, this study suggests that nonselective antagonist-based treatment may also not be successful. However, similar to studies in
smokers (Rose et al., 1994; Gonzalez et al., 2006), a low-efficacy agonist may prove to be a better treatment approach for treatment-seeking cocaine-dependent patients than a high efficacy agonist or antagonist alone. Therefore varenicline, which has provided moderate success as a smoking cessation agent via a partial agonist approach at nAChRs (Rollema et al., 2007; Gonzalez et al., 2007; Jorenby et al., 2007), was evaluated in cocaine-related behavioral assays in Chapter V.

**DRUG DISCRIMINATION**

Drug discrimination (DD) studies provide a behavioral assay to examine the central mechanisms of action of drugs (see Seiden and Dykstra, 1977 for review). Whereas an external stimulus (e.g. light) sets the occasion for response-contingent delivery of cocaine in self-administration sessions, in drug discrimination studies administration of a drug provides an interoceptive stimulus to the animal regarding reinforcement contingencies (for clarity and because it is directly related to the studies described in Chapter V, positive reinforcement will be the example). For example, an animal is trained through repetitive pairing that when a drug is administered (e.g. cocaine), responding on one manipulandum (e.g. right lever) will result in delivery of a food pellet. Alternately, on days where the internal cue produced by cocaine is absent (e.g. saline administration) delivery of a food pellet is contingent upon responding on a different manipulandum (e.g. left lever). Following successful acquisition of the discrimination, test sessions can be implemented by varying doses of the training drug, substituting different drugs, or by administering another drug prior to the training drug. During discrimination sessions the dependent variables are the percent of total responses
allocated on the drug-associated and non-drug associated manipulandi and response rates. Dose-response functions are generated such that the training dose engenders near 100% responding on the drug-appropriate manipulandum and as the dose of the training drug decreases, response allocation shifts to the non-drug associated manipulandum. Substitution of other drugs during test sessions can provide information regarding whether the mechanism of action is similar to or different from the training drug. Pretreatment of various drugs prior to the training drug can assess the ability of a novel drug to potentiate or attenuate the discriminative stimulus effects through direct or indirect modulation of similar receptor systems. The discriminative stimulus effects of a drug in animals can provide a model of the subjective effects of a drug in humans.

Consistent with the observation that nAChR agonists can increase DA release (e.g. Rollema et al., 2007, 2010), nicotine fully substituted for cocaine in monkeys and rodents in discrimination trials (de La Garza and Johanson, 1983; Desai et al., 2003). Similarly, nicotine, along with indirect DA agonists, fully substituted for methamphetamine in rats while partial nAChR agonists such as varenicline engendered partial substitution for methamphetamine (Desai and Bergman, 2010). In human studies, nicotine enhanced and mecamylamine diminished subjective measures of craving for cocaine (Reid et al., 1998; Reid et al., 1999). To date, no other studies have examined the effects of varenicline on the discriminative stimulus effects of cocaine, or whether varenicline generalizes to the discriminative stimulus effects of cocaine. A second goal of Chapter V was to examine varenicline as well as nicotine and mecamylamine, a nonselective antagonist, in monkeys trained to discriminate cocaine from saline.
It is important to examine the effects of novel pharmacotherapies on both SA and DD as each assay may provide different information about the therapeutic potential of a drug. While SA provides information about the abuse liability (drug substitution) of a compound and the ability of a novel compound to influence the reinforcing effects (FR) or strength (PR) of cocaine, DD studies provide information regarding the discriminative stimulus effects, modeling the subjective effects experienced in humans (e.g. ratings of “liking” or “high”). While both are important variables associated with drug use, and an early presumption assumed that similar drug actions mediated both the reinforcing and discriminating effects, a dissociation between the two exists. For example, in humans, d-amphetamine was rated more pleasurable than placebo (based on the positive subjective effects) but only participants with high anxiety ratings chose to repeatedly self-administer d-amphetamine (Uhlenhuth, 1981). In several NHP studies, drug doses that were reliably self-administered did not engender discriminative stimulus effects (e.g. Hoffmeister, 1988; Ator, 2002) including cocaine (Martelle and Nader, 2009). It is therefore important to examine potential therapeutic agents in both assays.

The goal in assessing potential pharmacotherapies in drug SA is the reduction or complete elimination of drug-maintained responding indicative of the attenuation of reinforcing effects. However, more theoretical debate revolves around the optimal therapeutic goal for preclinical DD assessments. For example, if no overlap exists in the mechanisms underlying the discriminative stimulus versus reinforcing effects of a drug, a drug that engenders similar discriminative stimulus effects but is not self-administered might be beneficial in alleviating withdrawal symptoms. However, if at minimum, some overlap exists in the underlying mechanisms, a drug that engenders discriminative
stimulus effects might be hypothesized to also increase “craving” and likelihood to relapse. These assumptions must also account for the mechanism of action under investigation. For example, alcohol did not substitute for cocaine in cocaine DD studies in rats (Gatch et al., 2003), although alcohol is reliably self-administered. These finding suggest that the discriminative stimulus effects of alcohol do not overlap with those of cocaine, not that alcohol has low abuse liability or wouldn’t influence the reinforcing effects of cocaine, and reiterates the need to examine novel compounds across multiple behavioral assays.

DISCONNECT BETWEEN PRECLINICAL AND CLINICAL PHARMACOTHERAPEUTIC EFFICACY

Over the past few decades a plethora of studies have examined the modulation of various neurotransmitter systems on cocaine-related behaviors in animal models. Since the primary reinforcing effects of cocaine, are mediated by the DA system, preclinical assessments have focused on direct DA modulation. However, neurotransmitter systems that indirectly influence the DA system have also been examined. Within each neurotransmitter system, preclinical assessments have produced positive signals (e.g., reduced cocaine SA or DD) and yet not a single compound has successfully translated to an FDA-approved, clinically efficacious drug to treat cocaine dependence (see Vocci and Ling, 2005; Karila et al., 2008). The nAChR system has been less rigorously examined regarding its influence on cocaine-related behaviors, notably because the influence of various receptor subtypes and subtype-specific compounds have only more recently
become available. Hence, the focus of the current research will be to examine the influence of nAChR function on cocaine-related behaviors, as previously described. Briefly, an overview of significant preclinical assessments on cocaine-related behaviors across the major neurotransmitter systems will be highlighted.

Both D1- and D2-like dopamine receptor families have been studied extensively to elucidate their involvement in cocaine-related behaviors. Although D1 receptors have been associated with the reinforcing effects of cocaine as evidenced by reduced rates of cocaine self-administration by some but not all D1 antagonists, D1 receptors have been implicated in mediating the locomotor-increasing effects of cocaine, as well as partial involvement in mediating the discriminative stimulus effects of cocaine (Katz et al., 1999; for review see Spealman et al., 1992). Dopamine D2-like receptor agonists substitute for cocaine in drug discrimination and self-administration studies, suggesting their involvement in mediating the discriminative stimulus and reinforcing effects of cocaine (for review see Spealman et al., 1992). DA D2-like low efficacy agonists can attenuate cocaine-maintained responding (Platt et al., 2003) and relative reinforcing efficacy of cocaine (Thomsen et al., 2010b) similar to full antagonists (e.g. Platt et al., 2003). However, full dose-effect evaluations often show a right-ward shift in the cocaine dose-response curve suggesting competition between the partial agonist and DA at receptors that is overcome by higher doses of cocaine (for review see Spealman et al., 1992). Often, antagonism at either D1-like or D2-like receptors produces nonspecific effects such that response rates maintained by cocaine and non-drug reinforcers (e.g. sucrose pellets, milk) are both diminished (e.g. Platt et al., 2003) and are preclinical
signals of unwanted side effect profiles that may predict non-compliance in a clinical setting.

More recent mechanistic approaches for pharmacotherapies to treat cocaine dependence include DA D₃ receptor specific compounds. D₃ receptors are localized predominately in limbic regions (Sokoloff et al., 1990). Therefore, modulation spares motoric side effects often associated with the nigrostriatal dopamine system. Ample evidence supports the evaluation of D₃-selective compounds. Cocaine overdose victims demonstrated greater receptor densities than controls (Staley and Mash, 1996) and monkeys with an extensive cocaine-self-administration history displayed greater sensitivity to low efficacy D₃ agonists (Blaylock et al., 2011) compared to cocaine-naïve monkeys. D₃-selective partial agonists reduced cocaine seeking in rodents (Pilla et al., 1999) and decreased rates of cocaine self-administration in monkeys (Martelle et al., 2007).

DAT blockers or DAT substrates acting as indirect DA agonists have also shown preclinical success in reducing cocaine SA. DAT blockers including GBR-12909 and methylphenidate have various affinities for DAT, show similar onsets for DA uptake inhibition, yet vary in peak DA inhibitory effects (Yorgason et al., 2011). DAT blockers attenuated cocaine SA (e.g. Rothman et al., 2008; Batman et al., 2010). DAT substrates block reuptake and induce DA release via the DAT. D-amphetamine, the classic DAT substrate, has shown widespread preclinical success across different schedules of reinforcement (FR, PR, food-drug choice procedures), species (rodents and non-human primates) and durations of administration (Negus 2003; Negus and Mello, 2003a, b; Chiodo et al., 2008, 2009; Czoty et al., 2010, 2011). Further d-amphetamine treatment
showed positive results in a preliminary clinical trial (Grabowski et al., 2001). Unfortunately, both DAT blockers and substrates are typically self-administered in preclinical studies when substituted for cocaine (e.g. Rothman et al., 2008, Batman et al., 2010). Similarly, amphetamines are readily abused in human populations thereby proving a hurdle based on abuse liability and social stigma associated with prescribing one stimulant to reduce use of another (for reviews, see Vocci and Ling, 2005, Karila et al., 2008). Therefore, other lines of research have focused on indirect modulation of the DAergic system in an attempt to mimic the positive aspects of DAergic agents while minimizing their negative aspects.

A growing literature describes the interactions between the ACh and DA neurotransmitter systems (e.g. reviews, Lester et al., 2010; Maskos et al., 2010). ACh receptors are divided into muscarinic and nicotinic subtypes. Briefly, the non-selective muscarinic receptor agonist oxotremorine, and M1/4-preferring agonists have attenuated cocaine SA and discriminative stimulus effects of cocaine (Thomsen et al., 2010a). The antagonist scopolamine attenuated the cocaine-primed reinstatement of previously extinguished cocaine-maintained responding (Yee et al., 2011). However, at high doses scopolamine, similar to other muscarinic antagonists, induced cognitive deficits (e.g. Voss et al., 2010).

As previously described, nAChR agonists increase DA release, and potentiate the reinforcing strength and discriminative stimulus effects of cocaine (Desai et al., 2003; Rollema et al., 2007, 2009; Freeman and Woolverton, 2009) while the antagonist mecamylamine attenuated cocaine SA (Levin et al., 2000) and cocaine-primed reinstatement of previously extinguished cocaine-maintained responding (Schmidt et al.,
In humans, mecamylamine decreased cocaine craving in non-treatment seeking cocaine users (Reid et al., 1999) but did not reduce the number of cocaine-free urines or duration of abstinence in one clinical trial, although only one dose was assessed (Reid et al., 2005). This literature suggests that antagonism of nAChRs may be a mechanism to reduce cocaine-related behaviors while agonism of nAChRs is necessary for cognitive enhancement. For example, nicotine is a cognitive enhancer and could prove beneficial in aiding behavioral modification strategies but nicotine increases craving for cocaine that may increase likelihood of relapse (e.g. Reid et al., 1998, Katner et al., 2004). In cognitive studies in humans, extremely low doses of mecamylamine have been shown to improve cognition (e.g. Potter et al., 2009), but have no effect or may impair cognition at doses that reduced craving (Reid et al., 1999). These issues highlight the importance of examining multiple behavioral paradigms and dose ranges when examining putative pharmacotherapies. Two avenues to reconcile these apparently divergent effects include evaluating intermediate-efficacy agonists and subtype-selective nAChR compounds. One focus of this dissertation was to examine the α4β2* subtype-selective nAChR partial agonist, varenicline on working memory performance (Chapter IV) and on cocaine self-administration and discrimination (Chapter V) in monkeys.

Other monoaminergic neurotransmitter systems have been examined as potential treatment mechanisms for cocaine addiction. 5-HT neurons innervate DA neurons of both nigrostriatal and mesolimbic DA pathways and are thought to play an inhibitory role on the DA-mediated reinforcing effects of cocaine (e.g. Herve et al., 1987). Currently, there are more than 14 different 5-HT receptors identified. Interactions at a number of receptor subtypes have yielded positive results in preclinical models of cocaine-related behaviors,
including attenuation of cocaine SA, cocaine or cue-induced reinstatement of previous cocaine-maintained behavior, and the discriminative stimulus effects of cocaine (for review see Filip et al., 2010). Alternatively, 5-HT subtype-selective compounds attenuated behavioral disinhibition following cocaine use, an aspect of impulsive choice associated with chronic cocaine SA (Anastasio et al., 2011; Fletcher et al., 2011) and can produce anxiolytic effects, a potentially positive effect during acute cocaine abstinence (see Filip et al., 2010). Further, the SERT inhibitors alaproclate, citalopram, and fluoxetine (e.g. Kleven and Woolverton, 1993; Howell and Byrd, 1995; Czoty et al., 2002), as well as the 5-HT releasing agent fenfluramine (acting via SERT uptake and intracellular-evoked 5-HT release) reduced cocaine-maintained responding in monkeys (Negus et al., 2007). However, positive results from preclinical studies have not translated to clinical success (Vocci and Ling, 2005). For example, fluoxetine (Grabowski et al., 1995) did not significantly reduce the percent of drug-free urine samples in methadone-maintained cocaine users compared to control groups.

The NE system may not be directly implicated in mediating the reinforcing effects of cocaine, but may be involved in the discriminative stimulus effects or in pathways mediating stress or anxiety that may trigger relapse (for review, see Weinshenker and Schroeder, 2007). Clinical studies support the notion that modulation of the NE system may be a viable treatment option to prevent relapse. The NET inhibitors desipramine, reboxetine, (FDA-approved antidepressants), and atomoxetine, (approved to treat ADHD) have shown positive indications in limited human studies either through the reduction of physiological or subjective effects of acute cocaine or reduced stress (for review, see Sofuoglu and Sewell 2008). The role of NE in mediating mood, attention, and
stress may suggest positive outcomes of medications targeting the NE system in regards
to relapse prevention as opposed to blocking the effects of cocaine during lapses, through
minimizing stress or psychological withdrawal symptoms that may influence relapse (cf.
Sofuoglu and Sewell, 2008). However, in one clinical trial atomoxetine reduced ADHD-
like symptoms in treatment-seeking cocaine users but did not reduce cocaine intake and
still resulted in a high drop-out rate, suggesting that alone, NE manipulation may not
produce large enough net effects to reduce cocaine use (Levin et al., 2009).

The primary excitatory and inhibitory neurotransmitter systems glutamate and
GABA, respectively, are also targets for drug development due to the relative abundance
of neurons in mesocorticolimbic structures and evidence of neurofunctional alterations
following chronic cocaine exposure (Beveridge et al., 2011; see Dackis and O’Brien,
2003). GABA agonists increase net inhibitory tone. Baclofen, a GABA\textsubscript{B} receptor agonist
attenuated DA and glutamate release and attenuated the reinforcing effects and strength
of cocaine under various schedules of reinforcement in rats (for review, see Cousins et
al., 2002). Group II metabotropic glutamate receptor (mGluR) agonists, acting at
autoreceptors to reduce glutamatergic transmission, have attenuated cocaine SA in
monkeys as well as cue- and cocaine-induced reinstatement of cocaine-seeking in
monkeys and rats (Baptista et al., 2004; Adewale et al., 2006). Topiramate, an FDA-
approved anticonvulsant medication has shown promise in maintaining abstinence from
cocaine in humans (Kampman et al., 2004). Topiramate also has a dual mechanism of
action, raising GABA levels in the CNS and facilitating GABA release, and inhibiting
 glutamatergic activity (cf. Kampman et al., 2009). However, in a single-dose assessment
of baclofen in severe cocaine-dependent individuals, the percent of cocaine-positive urines were not different from a group receiving placebo (Kahn et al., 2009).

As highlighted by this brief review, a variety of cocaine-related behaviors in animal models are attenuated by compounds across neurotransmitter systems, and yet none have successfully translated to an FDA-approved, clinically efficacious compound to treat cocaine dependence (see Vocci and Ling, 2005; Karila et al., 2008). Interestingly, several drugs with positive signals in animal studies (e.g. reduced cocaine SA, blocked reinstatement) have produced similar effects in a human laboratory setting (e.g. reduced cocaine SA, reduced craving; see Haney and Spealman, 2008 for review). For example, one FDA-approved medication to treat sleep-wakefulness disorders (modafinil, Provigil®; Keating and Raffin, 2005; Schwartz, 2009) has provided positive signals in a variety of preclinical and clinical studies. While the full extent of its interactions with monoaminergic systems are unclear, studies showed that this substrate binds with weak affinity to the DAT, as evidenced via PET imaging studies in both nonhuman primate and human studies (Volkow et al., 2009; Andersen et al., 2010), to inhibit DA uptake and increase extracellular DA (e.g. Andersen et al., 2010). In addition, modafinil enhanced glutamate synthesis and release and reduced GABA release (cf. Dackis and O’Brien, 2003). Modafinil engendered at least partial substitution for cocaine in rodents (Gold and Balster, 1996; Paterson et al., 2010), monkeys (Newman et al., 2010) and humans (Rush et al., 2002), suggesting a shared discriminative stimulus with cocaine. Importantly, the onset of action is longer than that of cocaine (Dopheide et al., 2007; Newman et al., 2010) and despite reinforcing efficacy at doses outside of the therapeutic window in monkeys (Gold and Balster, 1996), clinical studies testing therapeutic doses have shown
no preference over placebo (Vosburg et al., 2010), a reduction in cocaine SA (Hart et al., 2008), and decreased subjective and physiological effects of cocaine (Dackis et al., 2003; Malcolm et al., 2006). While clinical trials are ongoing, one preliminary study showed that the percentage of cocaine-free urines in a double-blind, placebo controlled study nearly doubled in abstaining cocaine users compared to the group receiving placebo (Dackis et al., 2005). The current data regarding modafinil as a treatment for cocaine dependence appear promising. However, the potential for abuse, albeit at high doses, may be problematic. Additionally, no single drug treatment works for the entire affected population. Therefore, additional medications need to be developed.

Numerous other drugs showed positive signals both preclinically and in a human laboratory setting yet were unsuccessful in reducing cocaine-related behaviors in clinical trials. For example ecopipam, a D1-like antagonist attenuated cocaine-primed reinstatement of previously extinguished cocaine-maintained responding in squirrel monkeys (Khroyan et al., 2003) and dose-dependently decreased both the subjective ratings of “high” and “good drug effects” and the “desire” for cocaine in a human laboratory setting in non-treatment seeking cocaine-dependent volunteers (Romach et al., 1999). As stated above, fluoxetine pretreatment reduced cocaine SA in squirrel monkeys (Spealman, 1993) and also reduced the “high” and “good” subjective effects in healthy adult volunteers when administered prior to cocaine (Walsh et al., 1994). Together, these studies suggest that animal SA and drug discrimination procedures have strong predictive validity when assessing the abuse liability of compounds and the ability of a compound to reduce the reinforcing efficacy or discriminative stimulus effects of self-administered drugs in non-treatment seeking humans in a laboratory setting. Unfortunately, both
fluoxetine (Grabowski et al. 1995) and ecopipam (see Grabowski et al., 2000) accompany greater than 65 other drugs across neurotransmitter systems that failed to show positive results in clinical trials when administered alone (Vocci and Ling, 2005).

Several factors may account for this striking disparity. For example, in the two human laboratory studies described above, non-treatment-seeking cocaine-dependent individuals or healthy volunteers served as subjects rather than treatment-seeking cocaine-dependent populations. Another overwhelming contrast between preclinical/human laboratory studies and clinical trials in treatment-seeking drug users is an individual’s control over the environment and alternative reinforcement relative to drug use. For example, animals and humans in laboratory studies are placed in, or directed by the experimenter to, the environment where the occasion is set via external stimuli for the drug SA session. Following an established baseline pattern of drug SA, the effects of a treatment are evaluated by a change in subsequent SA compared to baseline performance. In animals, drug pretreatments are always administered by the experimenter. Alternately, humans can and probably often do choose not to take a medication before actively pursuing drug-related behaviors. Compliance is always a concern in the clinic and is not modeled in animal studies. Moreover, the drug-seeking component, while purportedly modeled in reinstatement studies, still hinges on animals being placed in the environment associated with drug SA. Animal studies lack a component of actively engaging behavior to gain access to the environment where drug SA occurs. The monkeys in the current study live within the environment that they self-administer cocaine, and thus we are not improving on this limitation. However, when assessing varenicline or mecamylamine treatment on SA, drugs were administered orally.
Delivered in a small palatable treat, one benefit of this administration route is that each monkey can choose not to ingest the drug. If a previously palatable treat is no longer ingested, one may infer a negative effect of drug treatment that might predict poor compliance clinically.

Secondly, numerous animal studies examine the acute effects of a drug pretreatment on SA. Tolerance to a drug effect may develop over chronic dosing such that initial effects on drug-maintained behavior may diminish over time. In fact, D1-like antagonists such as ecopipam reduced the subjective ratings of cocaine when administered acutely (Romach et al., 1999) but increased similar subjective ratings and increased low-dose cocaine self-administration in humans following a week of dosing (Haney et al., 2001). Chronic dosing regimens are becoming more prevalent in preclinical evaluations to model the long-term treatments hypothesized to be necessary for clinical success (e.g. Negus and Mello 2003a,b; Czoty et al., 2010, 2011; Gould et al., 2011). However, chronic dosing studies in animals diverge from the human condition regarding exposure to relevant drug-related cues within the environment. For example, Newman and colleagues (2010) examined the effects of chronic modafinil treatment on cocaine SA. Following determination of a baseline cocaine dose-effect curve, modafinil treatment was initiated and the cocaine dose-effect curve was redetermined with each dose of cocaine being available for a minimum of 5 consecutive sessions. Under these repeated sessions, modafinil significantly reduced cocaine-maintained responding. However, the interactions of modafinil with cocaine may have, over repeated sessions, altered the effects of the conditioned stimuli signaling cocaine availability. An interaction between a pharmacological treatment and repeated exposure to drug-related conditioned stimuli is
an interesting concept not often incorporated into clinical studies. Functional MRI studies showed that drug-related stimuli produced substantial increases in brain activity in reward-related pathways (e.g. Wilcox et al., 2011), highlighting the importance of drug-associated cues and their potential facilitation to relapse, and suggest that extinction-based learning may aid in relapse prevention (e.g. Price et al., 2011). Systematic assessment of the effects of chronic drug treatments in the presence of conditioned stimuli through repeated daily SA sessions compared to chronic drug treatment in the absence of these repeated stimuli have not been empirically evaluated, and may provide valuable information for future treatment strategies. Studies described in this dissertation involve chronic drug treatments.

Another model more relevant to the clinical condition used in our laboratory involves 7-8 days of drug treatment without access to cocaine followed by a single cocaine SA session (e.g. Czoty et al., 2010, 2011; Gould et al., 2011). This procedure is repeated weekly and models relapse, such that the chronic effects of a drug treatment occur predominately without exposure to drug-associated stimuli. For example, in rhesus monkeys a chronic i.v. infusion of d-amphetamine showed significant reductions in the reinforcing strength of cocaine when assessed weekly (Czoty et al., 2010, 2011). *In Chapter V, the effects of chronic treatment with varenicline and mecamylamine were evaluated on once-weekly cocaine SA.*

Additionally, the human addict must weigh the benefits of drug use (euphoria, relieving withdrawal, etc) with negative consequences (potential loss of job, family, etc). Notably, these choices are not mutually exclusive - drug use one weekend may not immediately result in job loss. The temporal distribution of the cost-benefit analysis is not
always immediate or equal and such aspects are extremely difficult to model. Further, preclinical evaluations of abstinence are artificially induced by the experimenter - animals rarely abstain from self-administering reinforcing doses of a drug on their own volition once initial SA behavior has been acquired.

Lastly, there are different expectations for characterizing a successful drug treatment in clinical versus preclinical studies. Often the goal of medication evaluation in clinical trials is drug-free behavior and relapse is constituted as a treatment failure. In preclinical studies the effects of a drug treatment on SA behavior are compared to baseline behavior. A significant reduction in drug-related behavior is considered a success - not the absolute elimination of drug-maintained responding.

These examples highlight several disconnects between preclinical and clinical examinations of putative pharmacotherapies. Numerous preclinical studies examine only one aspect of the addiction process (e.g. vulnerability, maintenance, abstinence or relapse) using only one assay (e.g. self-administration, reinstatement, drug discrimination or cognition). Addiction is complex and drug treatment strategies need to be multi-faceted. Current lead compounds (e.g. modafinil) affect multiple neurotransmitter systems that have shown positive signals in multiple drug-related behaviors or changes in behavior resulting from chronic drug use or withdrawal (e.g. clinically: sleep disturbances, depression, cognitive impairments). Successful treatment strategies need to incorporate multiple therapies (behavioral and pharmacological) and possibly multiple drugs to treat different stages of the addiction cycle.

Currently, behavioral treatment approaches garner greater success in maintaining abstinence in treatment-seeking cocaine users than pharmacological interventions.
Therefore, both preclinical and clinical researchers need to strive to incorporate successful aspects of behavioral and pharmacological approaches when designing studies to develop better treatment strategies for drug addiction. For example, numerous preclinical studies are improving validity through integration of environmental influences (e.g. Morgan et al., 2002) or introducing alternative reinforcers or delays associated with drug SA (see above; Negus, 2003; Czoty et al., 2005b; Woolverton and Anderson, 2006; Hamilton et al., 2011). Conversely, while preclinical models are specifically designed to reflect clinical conditions, clinical studies may need to incorporate aspects of preclinical assessments to improve success. For example, new drug formulations are being tested, such as a depot naltrexone for opiate users that will last for several weeks (e.g. Everly et al., 2011). This dosing formulation would increase the likelihood that a treatment would be in an addict’s system during periods of relapse, effectively employing an extinction-based approach and accepting the mantra that isolated relapse is a part of the healing process, not an immediate failure. Alternately, behavioral strategies are attempting to devalue reinforcing efficacy of drugs through alternative reinforcers and through extinction of conditioned effects of drug-related environmental stimuli (e.g. pipes, lighters, crack houses) such as exposure to visual imagery in a laboratory setting that does not result in drug use.

PHARMACOLOGICAL + BEHAVIORAL TREATMENT APPROACH

From the limited reviews above of preclinical and clinical literature assessing pharmacological or behavioral treatment approaches for cocaine dependence, one overarching theme emerges. The etiology of addiction is complex and successful
treatments will be neither simple nor singular. Targeting multiple aspects of addiction is more efficacious than any one facet. Similarly, combination behavioral strategies are more likely to improve treatment retention or sustained abstinence to a greater extent than single methodologies. Although pharmacotherapies are largely unsuccessful administered alone, evidence suggests that drug treatments can complement existing behavioral strategies to increase overall treatment retention and sustained abstinence. Briefly, combined drug and behavioral therapies will be discussed relating to existing studies and potential future applications. Unless otherwise noted, all clinical studies described in this section utilized some variation of CBT, at minimum once weekly individualized or group counseling sessions.

The first studies examining combination pharmacotherapy plus behavioral strategies explored the use of buprenorphine or methadone agonist replacement therapy for opiate addicts. Originally implemented to minimize the exaggerated physiological effects of withdrawal from opiate use, buprenorphine- or methadone-maintenance was extended to opiate/cocaine users in an attempt to reduce polydrug use in treatment seekers. Following success of this strategy in opiate addicts, buprenorphine- or methadone-maintenance has been utilized in cocaine users, often as a control for other drug combination therapies. For example, \(d\)-amphetamine (Grabowski et al., 2004) and tiagabine, a GABA agonist (Gonzalez et al., 2007) reduced the percentage of cocaine-free urines in methadone-maintained cocaine users compared to placebo-controlled methadone-maintained groups.

In preclinical models, antidepressants have shown success in reducing cocaine related-behaviors (see above). However, in methadone-maintained cocaine users the
antidepressants fluoxetine (Grabowski et al., 1995) and desipramine (Kosten et al., 1992) did not significantly reduce the percent of drug-free urine samples compared to control groups when treatment included CBT sessions alone. In combination with CM techniques, both fluoxetine (Schmitz et al., 1999) and desipramine (Kosten et al., 2003) resulted in fewer drug-positive urine samples over the treatment period. It should be noted that in the Kosten et al. (2003) study, patients were also maintained on buprenorphine treatment. These results are similar to other studies examining antidepressants in combination with CM techniques including citalopram (Moeller et al., 2007) and bupropion (Poling et al., 2006). It is noteworthy that the efficacy of antidepressants to improve treatment success is not specific to one mechanism and is similar with the tricyclic antidepressant (desipramine), SSRIs (fluoxetine, citalopram) and the mixed DAT/SERT/NET inhibitor (bupropion) when combined with CM strategies.

Similarly, CM strategies and indirect DA agonists are more efficacious in reducing drug-free urine samples compared to DA agonists alone, or DA agonist + CBT. In a recent meta-analysis examining 26 clinical studies that assessed indirect DA agonists in cocaine or cocaine/opiate dependent patients in combination with various behavioral treatments, \(d\)-amphetamine and bupropion were the only two drug treatments that trended toward an overall significant effect on the primary outcome measure of percent drug-free urines (Perez-Mana et al., 2011). Of these 26 studies, only 3 implemented CM strategies. Similar to the previously described results with antidepressants, these three studies assessing disulfiram (Petrakis et al., 2000), bupropion (Poling et al., 2006) and levodopa (Schmitz et al., 2008) resulted in a greater percentage of drug-free urines than the
placebo-control group in combination with CM techniques. Clinical trials with these same drugs and CBT alone did not produce significant reductions in drug-free urines (e.g. Shoptaw et al., 2005, 2008; Pettinati et al., 2008).

These results highlight the importance of implementing combined behavioral and pharmacological strategies to treat drug dependence. It has been hypothesized that CM strategies provide more immediate positive reinforcement compared to CBT in which the emphasis is long-term abstinence through behavioral modification, and thus may reduce the relative reinforcing effects of the alternative drug reinforcer, resulting in longer rates of abstinence. While research is ongoing to design the most effective behavioral treatment platform to pair with pharmacological agents, and more successful drug treatments, presently CM appears to be most efficacious when implemented with CBT and pharmacological adjuncts (e.g. Carroll et al., 2004; Schmitz et al., 2008). The overarching goal of this dissertation research is to utilize NHP models of cocaine abuse to examine a novel pharmacological approach that alters the effects of cocaine related to abuse liability and reverses the cognitive effects caused by chronic cocaine exposure. It is hypothesized that cognitive enhancement may be used to improve behavioral treatment strategies to aid in treatment of cocaine dependence.
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CHAPTER II

EFFECTS OF COCAINE SELF-ADMINISTRATION AND ABSTINENCE ON WORKING MEMORY IN Rhesus Monkeys

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The following manuscript is in preparation to be submitted to *Psychopharmacology* in October, 2011. Stylistic variations are due to the requirements of the journal. Robert W. Gould performed the experiments, analyzed the data and prepared the manuscript. Michael A. Nader acted in an advisory and editorial capacity.
ABSTRACT

Rationale: Cocaine use is associated with neurobiological and cognitive deficits that persist into abstinence, hindering success of behavioral treatment strategies, and increasing likelihood of relapse. The effects of current cocaine use on cognition and the long-term consequences during abstinence are not well characterized. Objective: To examine the effects of cocaine self-administration and abstinence on working memory in a monkey model of cocaine abuse. Methods: Adult male rhesus monkeys with an extensive cocaine self-administration history (n=3; ~6 years, mean 1588.7 mg/kg cumulative cocaine intake) and age-matched controls (n=4) performed a delayed match-to-sample (DMS) task in morning and self-administered cocaine (0.1 mg/kg/injection; max 1.5 mg/kg/session) or food (1-g pellets) in afternoon sessions. Following stable DMS performance, the dose of cocaine was increased for 10 days (0.3 mg/kg/injection; max 4.5 mg/kg/session) followed by 30 days of abstinence from cocaine. Results: There was no difference in baseline working memory performance between groups, with equal delay-dependent declines in performance. Self-administration of high cocaine doses disrupted DMS performance at the longest delay during the first 5 days, but tolerance developed to the disruptive effects within 10 days. Acute abstinence did not affect performance. However by day 30 of abstinence, accuracy increased significantly while performance of cocaine-naive monkeys was unchanged. Conclusions: These data suggest that tolerance can develop to cocaine-induced cognitive impairments and that improvements in working memory can occur within the first month of abstinence, a critical period for treatment seekers.

Key Words: abstinence, CANTAB, cocaine, cognition, delay match-to-sample, nonhuman primates, self-administration, working memory
INTRODUCTION

Chronic cocaine use is associated with structural and functional alterations within the central nervous system that underlie cognitive deficits in attention, memory, impulsivity, and behavioral flexibility (Fillmore and Rush 2002; Bolla et al. 2004; Hester and Garavan 2004; Tomasi et al. 2007; Goldstein et al. 2007, 2010; Moeller et al. 2010). These deficits extend into periods of abstinence (e.g. Volkow et al. 1992; Matochik et al. 2003; Tomasi et al. 2007; Hanlon et al. 2011) and are correlated with treatment retention and success (Aharonovich et al. 2006; Turner et al. 2009; Moeller et al. 2010). However, the effects of duration, such as acute versus long-term (e.g. week 1 versus week 4) abstinence on executive function are not well characterized. Understanding the effects of abstinence from cocaine during the first month, a critical period of treatment (e.g. Gawin and Kleber 1985, 1986), may lead to more successful treatment strategies.

Deficits in attention, memory, strategic planning and decision-making have all been shown following approximately 30 days of abstinence from cocaine (Ardila et al. 1991; O’Malley et al. 1992; Beatty et al. 1995; Hoff et al. 1996; Gillen et al. 1998). However, these cognitive deficiencies were qualified as poorer performance compared to cocaine-naive control groups, not compared to within-subject measures obtained prior to abstinence from cocaine. More recent studies are beginning to examine the influence of abstinence on cognition by comparing recently abstinent cocaine users to current users or through repeated, within-subject assessment. For example, cocaine users showed deficits in verbal recognition memory, attention, and executive function compared to cocaine-naive controls, but such deficits were magnified in users that were abstinent for less than 72 hours compared to those abstaining for greater than 72 hours (determined via
Urinalysis; Tomasi et al. 2007; Woicik et al. 2009). Further, an inpatient study showed that attention was impaired during a three day cocaine self-administration “binge” compared to controls, and that measures of attention were further impaired throughout two weeks of abstinence compared to performance during the three day binge (Pace-Schott et al. 2008).

However, recent studies suggest that cognitive deficiencies, specifically memory-related, may dissipate over extended periods of abstinence (van Gorp et al. 1999; Di Sclafani et al. 2002; Pace-Schott et al. 2008; De Oliveira et al. 2009; Hanlon et al. 2011). For example, in the Pace-Schott et al. (2008) study, spatial memory performance improved such that abstinent cocaine users showed significantly greater accuracy than the control group by week two. In another study, cocaine users that remained abstinent for 30 days performed worse on measures of attention and visual working memory compared to a drug-naive control group, but performed significantly better than current cocaine users (Hanlon et al. 2011). Similarly, abstinent cocaine users showed improved cognitive function, including verbal memory at 6 months compared to earlier assessments at 6 weeks of abstinence (Di Sclafani et al. 2002). Together, these studies suggest the effects of abstinence from cocaine on cognition may vary based on duration of abstinence. However, human studies cannot control for factors such as cognitive predisposition, environmental stressors or polydrug use. Further, the criteria used to define abstinence are limited to urinalysis results and self-reports, hindering temporal refinement regarding recent cocaine use. Therefore, the current study sought to examine the effects of cocaine self-administration and subsequent abstinence on visual working memory in a rhesus monkey model of cocaine abuse.
Cocaine self-administration in animals results in similar neurobiological and cognitive deficits as seen in human cocaine users (e.g. Volkow et al. 1993; Porrino et al. 2004; Nader et al. 2006; see Beveridge et al. 2008 and Nader et al. 2008 for reviews). Cocaine self-administration has also been shown to result in impairments across tasks measuring attention, memory, impulsivity and behavioral flexibility in rodents (Schoenbaum et al. 2004; Dalley et al. 2005; Briand et al. 2008; George et al. 2008; Winstanley et al. 2007, 2009) and monkeys (Liu et al. 2008, 2009; Porter et al. 2011) and such impairment has been documented from 1 day to 3 months of abstinence in rodents (e.g. Dalley et al. 2005; Calu et al. 2007) and up to 18 months in monkeys (Liu et al. 2009). In the current study, adult rhesus monkeys were trained to perform a delayed match-to-sample (DMS) task to assess working memory performance in morning sessions. Three monkeys with a chronic cocaine self-administration history continued to self-administer cocaine in afternoon sessions throughout DMS training, while cocaine-naive monkeys performed similar operant behavioral sessions, except that responding was maintained by food reinforcement. Following determination of a stable cognitive baseline, the effects of high-dose cocaine self-administration on DMS performance were examined for 10 days, followed by 30 days of experimenter-induced abstinence. We hypothesized that monkeys self-administering cocaine would perform worse on the DMS task across increasing delays compared to control monkeys and that increasing the cocaine dose would further disrupt DMS performance. Abstinence from cocaine was hypothesized to produce transient disruptions in DMS performance, with improvements over the 30-day abstinence period.
MATERIALS AND METHODS

Subjects. Seven singly housed adult male rhesus monkeys (Macaca mulatta; age 11-14) of Indian origin served as subjects. Four monkeys (R-1681, R-1682, R-1696, R-1756) were cocaine naive and three monkeys had an extensive cocaine self-administration history at the initiation of this study (~6 yrs; mean 1588.7 mg/kg cumulative cocaine intake; see Table 1). Each monkey was fitted with an aluminum collar (Primate Products, Redwood City, CA) and trained to sit in a primate restraint chair (Primate Products). Monkeys were weighed weekly and fed enough food daily (Purina Monkey Chow and fresh fruit) to maintain ~95% free-feeding body weight; water was available ad libitum in their homecage, which measured 0.71 x 0.84 x 0.84 m (Allentown Caging Inc., Allentown, NJ). Environmental enrichment was provided as outlined in the Institutional Animal Care and Use Committee’s Nonhuman Primate Environmental Enrichment Plan.

All monkeys (except R-1756) were surgically implanted, under ketamine anesthesia and aseptic conditions, with indwelling vascular access ports and intravenous catheters (Access Technologies, Skokie, Il) into a major vein (see Czoty et al. 2007). Heparinized saline (100 U/ml) was infused into the catheter following surgery and self-administration sessions to maintain catheter patency.

General Procedure. All animals underwent two daily operant behavioral sessions (5-7 days/week) consisting of cognitive testing (5-7 days/week) using the Cambridge Neuropsychological Test Automated Battery apparatus (CANTAB; Lafayette Instruments, Lafayette, IN) between 7:00-10:00 AM and either food (controls) or cocaine self-administration during afternoon sessions between 1:00-4:00 PM (see below). A
minimum of 2 hours elapsed between morning and afternoon sessions during which monkeys were returned to their home cage. A minimum of 14 hours elapsed between afternoon self-administration sessions and the following day’s cognition session.

Afternoon sessions. Operant panels were located in sound-attenuating chambers (1.5 x 0.74 x 0.76 m; Med Associates, East Fairfield, VT) and contained two photo-optic switches (Model 117-1007; Stewart Ergonomics, Inc., Furlong, PA) with white stimulus lights above each, spaced equidistant from a central food receptacle. Illumination of a white light over the right or left photo-optic switch signaled the initiation of each trial. A response was registered by breaking the infrared beam within a circular recess of the switch. Responding was maintained by delivery of either 1-g banana-flavored sucrose pellets (cocaine-naive control monkeys) via a pellet dispenser (Gerbrands Corp., Arlington MA), or an intravenous cocaine infusion (cocaine-experienced group; ~ 1.5 ml over 10 sec) using a peristaltic infusion pump (Cole-Parmer, Inc., Chicago, IL).

Prior to cocaine self-administration sessions, the area surrounding the vascular access port was shaved and cleaned using betadine followed by a 95% ethanol scrub. The port was connected to an infusion pump (outside the chamber) via a 20-guage Huber Point Needle (Access Technologies) and tubing. A 3-second infusion filled the internal catheter with cocaine hydrochloride. Cocaine (0.1 mg/kg/injection) or food (1.0-g banana-flavored food pellets) was available under a fixed-ratio (FR) 30 schedule of reinforcement followed by a 30-sec timeout. Self-administration sessions lasted for 120 minutes or until 15 total reinforcers were received, whichever occurred first. This dose of
cocaine (0.1 mg/kg) was chosen because it is either at the peak or on the descending limb of the previously determined dose-effect curves (e.g. Blaylock et al. 2011).

*Cognition.* Each CANTAB cognition station (0.38 x 0.56 x 0.31 m) was located in a sound-attenuating, ventilated chamber (0.8 x 0.8 x 1.32 m) and included a touch-sensitive computer screen (0.3 x 0.23 m) with a stimulus light, non-retractable response lever, and a pellet receptacle located to the right side of the front panel. Following a brief training period and several stimulus discrimination and set-shifting experiments (Gould et al. submitted), monkeys began training on a delay match-to-sample task. For the current study, a predefined set consisting of 67 distinct stimuli were used which consisted of differing shapes and colors (Cambridge Cognition PAL stimuli set 0). At the start of each trial the house light was illuminated for 5 seconds followed by the appearance of a “target” stimulus in the center of the screen (sample phase). A response on this stimulus (i.e. touching the screen) was followed by a brief delay (0 or 1 sec) followed by the presentation of 2 shapes situated on the left and right sides of the screen (match phase). One stimulus was the previously shown image and the other was a distracter image. Responding on the matching image during this phase was considered a correct response and resulted in delivery of 2 190-mg sucrose pellets, whereas a response on the distracter image was considered an incorrect response and resulted in trial termination. Following a correct response, a light within the pellet receptacle was illuminated until the pellet was retrieved. The house light remained illuminated during each delay and remained lit throughout post-trial timeouts (5 sec) following correct trials but was extinguished following incorrect responses during the matching phase. If no response was emitted
within 10 seconds during the sample or match phase, the house light was extinguished and the trial was terminated. For each training session, 100 trials were presented daily. Once percent accuracy increased above 70% for three consecutive days a second distracter image was introduced and stimuli during the matching phase could appear in any of the 4 corners of the screen. Increasing the number of distracters decreased the random probability of a correct choice or “chance” performance to 33% which allowed for a greater range of assessment across various cognitive demands. When percent accuracy increased above 80%, defined as a high percent accuracy associated with a low cognitive demand on working memory, monkeys were considered to have acquired the match-to-sample task.

**Experiment 1: Effects of cocaine history on working memory.** When percent accuracy remained above 80% for three consecutive days with 2 distracter images, delays were slowly introduced such that 3 delays were randomly distributed throughout each session (~33 trials/delay). Each new delay was presented for a minimum of 5 sessions and until a stable 3-day mean was acquired such that the percent accuracy within each day was not greater than 1.5 standard deviations from the mean. The three delays were increased in the following order: 0, 1, 5 sec; 0, 5, 10 sec; 0, 10, 30 sec; 0, 30, 60 sec; 0, 60, 90 sec; 0, 60, 120 sec, until percent accuracy at the lowest delay approached chance (33%). R-1756 was not tested with the 90 or 120 sec delays, R-1374 and R-1375 were not tested at the 120 sec delay (see Table 2).
Experiment 2: Increased cocaine self-administration and abstinence conditions. Based on the delay-effect curves in Experiment 1, three delays were chosen for each monkey to produce delay-dependent reductions in percent accuracy (short delay, >75% accuracy; middle delay, 50-75%; long delay, <50%; see Table 2). For monkey R-1682 the number of distractor images was increased to 3 to make baseline performance similar across all monkeys. In addition, the total number of trials was reduced to 60 (20 trials/delay). Following determination of a 5-day stable baseline during which performance did not differ by more than 1.5 standard deviations from the 5-day mean at any delay, the dose of cocaine available was increased from 0.1 mg/kg/inj to 0.3 mg/kg/inj for 10 consecutive days. The increase in dose resulted in increases in total cocaine intake in 2 of the 3 monkeys (Table 1). For R-1377, total cocaine intake steadily decreased from days 1-4 (4.5 to 2.7 mg/kg), so the available dose of cocaine was reduced to 0.2 mg/kg/injection for day 5-10. As a result, all monkeys self-administered at least twice their baseline dose and cocaine intake doubled during this 10-day exposure. Following this period, afternoon sessions were discontinued for 30 consecutive days.

Thirty days of DMS performance in the cocaine-naive monkeys was used for comparison with the 30 days of abstinence in the cocaine-experienced monkeys. Cocaine-naive monkeys were concurrently used to assess the acute effects of nicotine on DMS performance (data not shown). Days in which a drug pretreatment was administered were excluded (average 5.75 days excluded, range 5-7 days). Therefore, 30 sessions from an average of 40.75 (range 40-42) sessions are shown for each monkey compared to a 5 consecutive day stable baseline (as determined above) during which monkeys were consistently performing the DMS task on average ~6 days/week. No lingering effects of
nicotine were apparent on days following acute administration that would have inhibited improvement over time.

**Data Analysis.** The primary dependent variables were percent accuracy, number of omitted trials, latencies to respond during both target and match phases, and pellet retrieval latency. In Experiment 1, the 3-day means used for analysis were taken from the first exposure to each delay. For example, the first set of delays introduced were 0, 1, 5 sec followed by 0, 5, 10 sec. Data for the 0, 1, and 5 sec delays were taken from the first exposure and the 3-day stable mean for all subsequent delays were added to generate the delay effect curve. A two-way repeated measures ANOVA was conducted using group (cocaine-experienced or cocaine-naive) and delay (0-90 sec) to compare percent accuracy during the initial determination of the delay-effect curve. One-way repeated measures ANOVAs were conducted to compare number of omitted trials and response latencies between groups.

In Experiment 2, data were expressed as a percent change from the 5-day baseline averages and then collapsed into 5-day bins. Changes in percent accuracy were examined by conducting two-way repeated measures ANOVAs using delay (short, mid, long) and condition (5-day bins including baseline, high-dose cocaine availability days 1-5 and 6-10, and six bins of 5 days each from the 30 days of abstinence) as factors. Significant main effects were followed by Bonferroni post-hoc tests. Separate one-way repeated measure ANOVAs were conducted to examine the number of omitted trials and response latencies within each group. In Experiment 2, significant main effects were followed by Bonferroni post-hoc tests comparing each 5-day bin to the baseline condition; in all cases,
p<0.05 was considered significant. R-1377 showed robust disruptions in responding during DMS sessions on days 6 and 7 following increased cocaine dose availability, responding in 0 trials on the 6th day and only 3 trials following the 7th day. Therefore, data for these two days were excluded from statistical analyses of percent accuracy and response latencies.

RESULTS

Afternoon sessions. During training and baseline sessions, control monkeys consistently received all 15 food pellets and cocaine-experienced monkeys received all 15 injections of 0.1 mg/kg cocaine for a cumulative session intake of 1.5 mg/kg. Mean response rates (± SEM) were 1.63 (± 0.54) and 0.21 (± 0.05) responses/sec for food and cocaine self-administration groups, respectively, during the 5-day baseline condition (Table 1). Upon increasing the dose of cocaine from 0.1 mg/kg to 0.3 mg/kg/inj, response rates decreased substantially and rarely did monkeys self-administer the maximum 15 injections (4.5 mg/kg cumulative intake; see Table 1). The average cumulative intake over the 10 days for all three monkeys was 3.5 mg/kg cocaine (range 3.19-4.26 mg/kg; see Table 1 for 5-day averages).
Table 1. Daily intake and response rates (RR; responses/sec) during cocaine or food self-administration

<table>
<thead>
<tr>
<th></th>
<th>Cocaine self-administration</th>
<th></th>
<th>Increased Access</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>Intake (RR)</td>
<td>Day 1-5</td>
<td>Day 6-10</td>
</tr>
<tr>
<td></td>
<td>Day 1-5</td>
<td>Intake (RR)</td>
<td>Intake (RR)</td>
<td></td>
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<tr>
<td>Cocaine History</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>R-1374 1.5 (0.18)</td>
<td>4.38 (0.08)</td>
<td>4.14 (0.06)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-1375 1.5 (0.16)</td>
<td>3.06 (0.04)</td>
<td>3.12 (0.04)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-1377 1.5 (0.29)</td>
<td>3.30 (0.05)^</td>
<td>3.00 (0.12)^</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AVE 1.5 (0.21)</td>
<td>3.58 (0.06)</td>
<td>3.42 (0.07)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-1681 15 (2.14)</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R-1682 15 (1.57)</td>
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<tr>
<td></td>
<td>R-1696 15 (2.47)</td>
<td>--</td>
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<tr>
<td></td>
<td>R-1756 15 (0.35)</td>
<td>--</td>
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</tr>
</tbody>
</table>

^ Dose was decreased from 0.3 to 0.2 mg/kg on day 5; averages are for days 1-4 and 5-10.
DMS performance

Experiment 1: Effects of cocaine history on working memory.

When the baseline cocaine dose (0.1 mg/kg/injection) was available in the afternoon sessions, DMS performance between cocaine-experienced and cocaine-naive monkeys was not different. There was a significant effect of delay ($F_{6,29}=24.89$, $p<0.001$) but not group on percent accuracy for the initial delay-effect curve (Figure 1, Table 2). There were no differences between groups in response (target or match phase) or pellet retrieval latencies. There were no differences between cocaine-naive and cocaine-experienced monkeys in the total number of sessions that each delay was available before reaching stable criteria (average ± SEM; 6.62 ± 0.35 versus 6.63 ± 0.37 sessions, respectively) or overall time to complete the entire curve (average ± SD; 39.3 ± 9.3 versus 39.8 ± 4.9 sessions, respectively).
Figure 1. Delayed match-to-sample performance in monkeys. Group delay-effect curves (mean ± SEM) for cocaine-naive monkeys (n=4, except t=90, 120, n=3) and monkeys with a cocaine self-administration history (n=3, except t=120, n=1).
Table 2. Individual percent accuracy across increasing delay values and individualized delays for baseline delay-effect curves

<table>
<thead>
<tr>
<th>Cum Coc Intake (mg/kg)*</th>
<th>Exp 1: Delays (sec)</th>
<th>Exp 2: Individualized Delays (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0  1  5  10  30  60  90  120</td>
<td>Short  mid  long</td>
</tr>
<tr>
<td><strong>Cocaine-Experienced</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-1374</td>
<td>13  2038.7</td>
<td>77.4  76.4  73.3  71.5  42.8  44.1  37.5  --</td>
</tr>
<tr>
<td>R-1375</td>
<td>14  1018.7</td>
<td>80.3  83.2  85.4  89.7  68.3  42.4  18.1  --</td>
</tr>
<tr>
<td>R-1377</td>
<td>14  1708.6</td>
<td>83.6  90.5  96.5  90.4  89.3  75.2  56.1  39.9</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>13.7  1588.7</td>
<td>80.4  83.4  85.1  83.9  66.8  53.9  37.3  --</td>
</tr>
<tr>
<td><strong>SEM</strong></td>
<td>1.8  4.1  6.7  6.2  13.4  10.7  11.0  --</td>
<td></td>
</tr>
<tr>
<td><strong>Cocaine-Naive</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-1681</td>
<td>12  --</td>
<td>68.1  63.4  71.3  74.8  62.5  48.7  50.4  46.7</td>
</tr>
<tr>
<td>R-1682</td>
<td>11  --</td>
<td>80.9  87.3  81.7  77.0  65.4  47.4  50.6  32.2</td>
</tr>
<tr>
<td>R-1696</td>
<td>12  --</td>
<td>75.4  78.2  75.7  64.9  44.7  41.7  39.3  37.9</td>
</tr>
<tr>
<td>R-1756</td>
<td>14  --</td>
<td>83.6  82.2  68.5  59.6  48.4  38.2  --   --</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>12.3</td>
<td>77.0  77.8  74.3  69.1  55.3  44.0  46.8  38.9</td>
</tr>
<tr>
<td><strong>SEM</strong></td>
<td>4.0  5.9  3.3  4.7  5.9  2.9  4.6  5.2</td>
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</tr>
</tbody>
</table>

*Cumulative cocaine intake at the end of Experiment 1
Experiment 2: Increased cocaine self-administration and abstinence.

There was a significant effect of condition on percent change in accuracy from baseline in the cocaine history group ($F_{8,32}=6.92, p<0.001$) and an interaction between condition and delay ($F_{16,32}=2.29, p<0.022$). Following increases in cocaine dose, percent change in accuracy during days 1-5 was significantly different from baseline ($t=2.9$, $p<0.05$; Figure 2, top). There were no significant changes in the number of omitted trials, response or pellet retrieval latencies compared to baseline. However, two monkeys showed disruptions in responding. Percent change in accuracy during days 6-10 was not significantly different from baseline. Percent accuracy increased across days 6-10 such that by day 10, percent accuracy was nearly identical to baseline in each monkey. Other than the aforementioned disruptions seen in R-1377 on days 6 and 7, R-1374 omitted 35 and 21 trials following days 5 and 7 of the high dose cocaine self-administration condition.

Following discontinuation of daily cocaine self-administration sessions, DMS performance was unchanged during abstinence days 1-20 (Figure 2). Days 21-25 and 26-30 of abstinence were significantly different from baseline at the longest delay ($t=3.19$, $p<0.05$; $t=3.10$, $p<0.05$, respectively). Additionally at the middle delay, percent change in accuracy during day 26-30 of abstinence was significantly different from baseline ($t=2.87$, $p<0.05$). There were no significant changes in the number of omitted trials, response or pellet retrieval latencies compared to baseline across either group or any condition. During abstinence, R-1374 omitted 35, 23 and 22 trials on days 18, 27 and 30, respectively (data not shown). On no other occasion did any monkey omit more than 20 trials. DMS performance for control monkeys was also studied for 30 days without access
to afternoon food reinforcement sessions. There were no significant differences in the percent change from baseline over 30 sessions in the cocaine naive group (Figure 2, bottom).

**Figure 2.**

Figure 2. Effects of high-dose cocaine and abstinence on delayed match-to-sample performance. Group data (mean ± SEM) expressed as a percent of baseline for each 5-day period from a 5-day average when 0.1 mg/kg cocaine was self-administered. Ten days of high-dose cocaine self-administration were followed by 30 days of abstinence in monkeys with a cocaine self-administration history (top) and 30 days of DMS performance in cocaine-naive control monkeys (bottom); * denotes significant difference from 5 day baseline average (p<0.05).
There were also individual differences following high-dose cocaine self-administration and abstinence from cocaine. The largest reductions in percent accuracy at the longest delay following high-dose cocaine self-administration occurred at days 4, 5, and 3 for monkeys R-1374, R-1375 and R-1377, respectively (see Figure 3, top, middle, bottom). Throughout the 30 days of abstinence R-1374 did not show signs of improvement at the middle or long delays until day 20. R-1375 showed increased accuracy at the middle delay throughout the 30 days of abstinence such that by day 20 percent accuracy at the middle and short delays was similar. In R-1377, percent accuracy at the long and middle delays was similar from days 16-30.
Figure 3. Effects of high-dose cocaine and abstinence on delayed match-to-sample performance. Individual percent accuracy on the delay match-to-sample task at each delay in monkeys with a cocaine self-administration history, including a 5-day baseline average (BL), 10 days of high-dose cocaine self-administration, and 30 days of abstinence from cocaine. Data were omitted for days 6 and 7 of high-dose cocaine availability for R-1377 due to low responding (see results).
DISCUSSION

The present study examined working memory performance in cocaine-naive monkeys and monkeys with an extensive cocaine self-administration history using a delay match-to-sample task. There were no initial group differences in working memory performance. Increasing the dose of cocaine available in afternoon self-administration sessions resulted in significant disruptions in accuracy on subsequent morning DMS sessions. Tolerance developed to the cognitive-disrupting effects of cocaine by day 10 such that performance was not different from baseline. Acute abstinence did not adversely affect working memory, while continued abstinence resulted in significant increases in accuracy. Similar improvements did not occur in cocaine-naive monkeys over a similar duration of time. The current data adds to rodent and monkey studies demonstrating cocaine-induced deficits in cognition (Jentsch et al. 2002; Schoenbaum et al. 2004; Briand et al. 2008; George et al. 2008; Liu et al. 2008, 2009; Porter et al. 2011) and extends this literature to repeated assessment of working memory performance throughout one month of abstinence, a period when the likelihood of relapse is high (Gawin and Kleber 1985, 1986).

When monkeys self-administered an intermediate cocaine dose (0.1 mg/kg/injection) and delays were gradually increased over time, working memory was not different between groups across delays that engendered near chance-level responding. These results are similar to a study conducted by Liu and colleagues (2008) in which working memory was assessed using a delayed alternation task. In the current study, cumulative cocaine intake was higher at the start of the study, and self-administration sessions occurred 5 days/week as opposed to once weekly in Liu et al. (2008). This
cocaine dose is at the peak or on the descending limb of the dose-effect curve for all monkeys (e.g. Blaylock et al. 2011) and is within a range known to induce changes in glucose utilization and DA D2-like receptor availability in rhesus monkeys (e.g., Porrino et al. 2002; Nader et al. 2002). Therefore, either the maintenance dose of cocaine does not significantly alter cognitive performance or tolerance developed to the initial effects, such that monkeys were performing at levels comparable to cocaine-naive monkeys. We did not assess DMS performance in monkeys prior to and following cocaine self-administration. However, in a recent study, rhesus monkeys were trained to perform a visual DMS task, similar to the present study and then self-administered cocaine 4 days/week. Once-weekly cognitive assessments, each following 72 hours of abstinence, revealed modest disruptions in accuracy at long delays, but these deficits dissipated within the first 5 test sessions following repeated exposure (Porter et al. 2011). The development of tolerance to the disruptive effects of cocaine on next-day cognitive assessment has also been reported in rodents in a 5-Choice Serial Reaction Time Task (5-CSRTT) assessing attention (Winstanley et al. 2009). The fact that we did not see initial differences between monkeys with an extensive cocaine history and cocaine-naive controls supports the hypothesis that tolerance can develop to the disruptive effects of cocaine on cognitive performance.

Human cocaine intake is interspersed between high-intensity binges and subsequent lesser use or abstinence (e.g. Gawin and Kleber et al. 1985, 1986) that may be more disruptive to cognitive performance than stable daily intake, whereby animals may adjust to these conditions over time (e.g. see Koob and Le Moal, 2001 for review), and this was modeled in Experiment 2. Based on delay-effect curves from Experiment 1,
delays were individualized to produce similar dose-dependent reductions in percent accuracy, representing low, middle, and high cognitive load; the middle and long delays were more susceptible to disruption or improvements in cognitive function. As a group, increasing the dose of cocaine disrupted percent accuracy at the longest delay within the first 5 days. Importantly, these disruptions in cognitive performance were not accompanied by effects on response latencies or number of trials omitted, suggesting that the impairments were specific to working memory and not attention or reaction time.

There were individual-subject differences in sensitivity to high-dose cocaine self-administration on cognitive performance. In particular, monkey R-1377 was most sensitive to the disruptive effects of the high dose of cocaine. When the dose of cocaine available for self-administration was lowered to 0.2 mg/kg/inj and total daily intake increased in R-1377, disruptions in responding on subsequent DMS sessions occurred for the first 2 days. Despite these modest differences in sensitivity, tolerance to the disruptive effect of cocaine on working memory developed in all three monkeys within 10 days. In other studies, we have not noted tolerance to disruptive effects, notably rate-decreasing effects of cocaine when studied over 1 year (Nader et al. 2006), suggesting that cocaine-induced disruptions in cognition are more sensitive to continued drug use than other measures of behavior.

To our knowledge, this is the first study in monkeys to utilize a within-subject design to assess both cocaine-induced deficits and changes across abstinence periods during repeated, daily cognitive assessment in monkeys. In contrast to human studies measuring verbal (Kelley et al. 2005) or visual (Tomasi et al. 2007) working memory, acute abstinence from cocaine did not result in further cognitive deficits in visual
working memory. One important difference from those studies is that in the current study, working memory assessment occurred daily and was compared to individual baselines. Further, working memory performance at the middle and long delays significantly improved compared to baseline, for days 21-30 of abstinence. Improvements following repeated task performance are common in both animals and humans (e.g., Weed et al. 1999; Backman et al. 2011), however similar improvements did not occur in the cocaine-naive group. These findings are opposite the results of Liu et al. (2008) who found that cocaine-naive monkeys improved performance on a delayed alternation task to a greater extent than cocaine-experienced monkeys, albeit over 17 weeks, compared to the current 30 days. One difference is that in the current study monkeys were trained on the DMS task while currently self-administering cocaine while in the latter study monkeys were already in extended abstinence upon learning the task (Liu et al. 2008).

Human studies have also shown improvements in memory following extended durations of abstinence from cocaine (e.g. van Gorp et al. 1999; Di Sclafani et al. 2002; Pace-Schott et al. 2008), so the present findings documenting improvement in cognitive performance during abstinence are similar to those seen in humans. With the exception of the Pace-Schott et al. (2008) study where daily testing showed improved performance within 2 weeks, cognitive improvements occurred following 3-6 months of abstinence (van Gorp et al. 1999; Di Sclafani et al. 2002). Due to the high rate of relapse within the first month of abstinence (e.g. Gawin and Kleber 1985, 1986) the likelihood for most treatment-seeking cocaine users to remain abstinent long enough for cognitive improvements to occur naturally is low. The current monkey model, demonstrating cocaine-induced impairments, can be further utilized to examine pharmacological
approaches to improve cognition more rapidly. Lastly, working memory is only one
cognitive domain affected by cocaine self-administration, and is controlled by different
neuronal substrates than other cognitive domains (Dias et al. 1996a,b; Rogers and
Robbins 2001; Porrino et al. 2005). Therefore, a better understanding of the effects of
high-dose cocaine exposure and subsequent durations of abstinence on multiple cognitive
domains assessed through repeated examination is necessary for developing successful
research strategies to treat cocaine dependence.

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253.
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ABSTRACT

Chronic cocaine use is associated with neurobiological dysregulations in the mesocorticolimbic system leading to cognitive disruptions, such as impaired behavioral flexibility, hypothesized to increase compulsive drug use or relapse. The present study examined set shifting, a measure of behavioral flexibility, in adult male rhesus macaques with an extensive history of cocaine self-administration (SA) (n=4; ~ 5 years SA, mean intake, 1395 mg/kg) and age-matched, cocaine-naive monkeys (n=4) using the Cambridge Neuropsychological Automated Test Battery (CANTAB) intra-/extra-dimensional set shifting task (ID/ED). Cocaine SA monkeys required significantly more trials and committed more errors during acquisition of an extradimensional shift (EDS) compared to controls. Using [18F]-fluorodeoxyglucose (FDG) and positron emission tomography (PET) to measure regional cerebral glucose utilization, cocaine-naive and cocaine SA monkeys showed significantly greater glucose utilization during an EDS compared to baseline in the prefrontal cortex and posterior cingulate gyrus. However, only cocaine-naive monkeys showed increases in the anterior cingulate, precentral gyrus, hippocampus, caudate nucleus and precuneus, areas within neural circuits mediating behavioral flexibility. These data document neurobiological sequelae underlying cognitive deficits associated with chronic cocaine SA. Pharmacotherapies designed to improve neuronal function and behavioral flexibility may improve success of current behavioral modification strategies in treatment-seeking cocaine users.

KEYWORDS: [18F]fluorodeoxyglucose (FDG); CANTAB; cocaine self-administration; PET imaging; set shifting
In humans, long-term cocaine use is associated with structural and functional differences that correlate with cognitive deficits across domains measuring attention, memory, inhibition, and decision making compared to control groups (Hester and Garavan, 2004; Kubler et al., 2005; Goldstein et al. 2007; Tomasi et al., 2007a,b; Lane et al., 2010). Specifically, chronic cocaine users showed numerous alterations in the mesocorticolimbic dopamine (DA) system such as lower DA D2-like receptor availability in the striatum that correlated with lower glucose metabolism in the prefrontal cortex (PFC; Volkow et al., 1993). It has been hypothesized that a hypodopaminergic state contributes to cognitive impairments that perpetuate a cycle of compulsive drug use and increased likelihood of relapse (Goldstein and Volkow, 2002; Koob and Volkow, 2010). Given the moderate success of cognitive behavioral therapy and associations between cognitive impairments at treatment initiation with attrition rates (e.g. Aharonovich et al., 2006; Turner et al., 2009), cognitive enhancement may prove beneficial in treating various substance use disorders, including cocaine abuse (Sofuoglu, 2010; Perry et al., 2011). Identification of deficient CNS pathways or regions are necessary prior to development of putative cognitive enhancers. However, the influence of polydrug use, and environmental or social stressors on brain function and cognition are difficult to determine in human studies. Thus, the present study sought to examine brain function and cognitive performance in an animal model of cocaine abuse.

Compared to humans, nonhuman primates (NHPs) possess similar brain cytoarchitecture, structure and function (Weerts et al., 2007). NHPs readily self-administer drugs abused by humans resulting in similar neurobiological alterations such as reduced D2-like receptor availability in the striatum and reduced glucose metabolism
in the PFC (for reviews see Beveridge et al., 2008; Nader et al., 2008). Further, NHPs can be trained to perform complex tasks homologous or analogous to those used in human studies to examine specific cognitive domains (e.g. Dias et al., 1996a,b; Weed et al., 1999). The current study examined set shifting, a measure of behavioral flexibility, between rhesus monkeys with an extensive and current cocaine self-administration history (modeling chronic cocaine users) and age-matched, cocaine-naive monkeys using an intra/extra-dimensional (ID/ED) set shifting task analogous to the Wisconsin Card Sorting Task (WCST) used extensively in human testing (Dias et al., 1996b).

Additionally, we used $[^{18}\text{F}]$fluorodeoxyglucose (FDG) and PET imaging during an extra-dimensional shift to examine glucose metabolism, an indirect measure of neuronal activity. We hypothesized that cocaine-naive subjects would acquire components of the ID/ED task more rapidly, committing fewer errors, and would show greater glucose utilization in brain regions previously associated with set shifting and error detection, primarily the PFC and anterior cingulate cortex (ACC) compared to monkeys currently self-administering cocaine (Dias et al., 1996a,b, 1997; Hester and Garavan, 2004; Goldstein et al., 2007; Ng et al., 2007). Identifying neural substrates associated with cognitive deficits may aid in pharmacotherapeutic development for treatment-seeking cocaine users, perhaps leading to greater success of behavioral treatment strategies promoting development of advantageous goal-directed behaviors.
MATERIALS AND METHODS

Subjects

Eight singly housed adult male rhesus macaques (*Macaca mulatta*) of Indian origin served as subjects. Four monkeys (ages 12-13 years old, mean weight 9.5 kg) had an extensive cocaine self-administration history (~5 yrs; mean 1395 mg/kg cumulative cocaine intake; see Table 1) at the initiation of this study (Czoty et al., 2007). Four age-matched, experimentally naive (ages 11-12, mean weight 10.3 kg) male monkeys served as controls. Each monkey was fitted with an aluminum collar (Primate Products, Redwood City, CA) and trained to sit in a primate restraint chair (Primate Products). Monkeys were weighed weekly and fed enough food daily (Purina Monkey Chow and fresh fruit) to maintain ~95% free-feeding body weight; water was available *ad libitum* in their homecage, which measured 0.71 x 0.84 x 0.84 m (Allentown Caging Inc., Allentown, NJ). Environmental enrichment was provided as outlined in the Institutional Animal Care and Use Committee’s Non-human Primate Environmental Enrichment Plan.

All monkeys were surgically implanted, under ketamine anesthesia and aseptic conditions, with indwelling vascular access ports and intravenous catheters (Access Technologies, Skokie, IL) into a major vein (see Czoty et al., 2007). Catheters were flushed with 3 ml of heparinized saline (100 U/ml) to minimize clotting and to maintain catheter patency.
Table 1. Subject information and drug history

<table>
<thead>
<tr>
<th>ID</th>
<th>AGE</th>
<th>CUMULATIVE COCAINE INTAKE (mg/kg)</th>
<th>DURATION BTWN ID/ED TASKS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ID/ED 1</td>
<td>ID/ED 2</td>
</tr>
<tr>
<td>Cocaine SA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1374</td>
<td>13</td>
<td>1770.9</td>
<td>1888.7</td>
</tr>
<tr>
<td>1375</td>
<td>14</td>
<td>785.0</td>
<td>905.1</td>
</tr>
<tr>
<td>1377</td>
<td>14</td>
<td>1439.6</td>
<td>1560.1</td>
</tr>
<tr>
<td>1381</td>
<td>13</td>
<td>1172.2</td>
<td>1224.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine naive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1681</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1682</td>
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<td>0</td>
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</tr>
<tr>
<td>1696</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Operant Behavior

All animals underwent two daily operant behavioral sessions (5-7 days/week) consisting of cognitive testing between 7:00-10:00 AM and either food (controls) or cocaine self-administration during afternoon sessions between 1:00-4:00 PM (see below). A minimum of 2 hours elapsed between morning and afternoon sessions during which monkeys were returned to their home cages.

Self-administration sessions

Operant panels were located in sound-attenuating chambers (1.5 x 0.74 x 0.76 m; Med Associates, East Fairfield, VT) and contained two photo-optic switches (Model 117-1007; Stewart Ergonomics, Inc., Furlong, PA) with white stimulus lights above each, spaced equidistant from a central food receptacle. Illumination of the white light over the right or left photo-optic switch signaled the initiation of each trial. A response was registered by breaking the infrared beam within a circular recess of the switch. Responding was maintained by delivery of either 1-g banana-flavored sucrose pellets (control monkeys) via a pellet dispenser (Gerbrands Corp., Arlington MA), or an intravenous cocaine infusion (cocaine SA group) (~ 1.5 ml over 10 sec) using a peristaltic infusion pump (Cole-Parmer, Inc., Chicago, IL).

Prior to cocaine SA sessions, the area surrounding the vascular access port was shaven and cleaned using betadine followed by a 95% ethanol scrub. The port was connected to an infusion pump (outside the chamber) via a 20-guage Huber Point Needle (Access Technologies) and tubing. A 3-second infusion filled the internal catheter with cocaine hydrochloride. Cocaine (0.1 mg/kg/injection) was available under a fixed-ratio (FR) 30 schedule of reinforcement followed by a 30-sec timeout. Control monkeys
responded under identical conditions, except responding was maintained by 1-g banana-flavored pellets. Session length was 120 minutes or until 15 reinforcers were received, whichever occurred first.

**Cognition sessions**

Cognitive performance was assessed using the Cambridge Neuropsychological Test Automated Battery (CANTAB) apparatus (Lafayette Instruments, Lafayette, IN). Each CANTAB cognition station (0.38 x 0.56 x 0.31 m) was located in sound-attenuating, ventilated chambers (0.8 x 0.8 x 1.32 m) and included a touch-sensitive computer screen (0.3 x 0.23 m) with a stimulus light, non-retractable response lever, and a pellet receptacle located to the right side of the front panel. Sessions began by illumination of a panel light that remained lit throughout correct trials and reinforcement, but was extinguished following incorrect responses. A correct response resulted in delivery of a 190-mg sucrose pellet and illumination of a background light within the receptacle that extinguished upon pellet retrieval. With a maximum of 200 trials per session, maximal reinforcement could only be 38 grams, therefore minimizing satiation (average total chow intake ~200 grams/day). Monkeys were initially trained to respond on the touch-sensitive computer screen when the entire screen appeared purple. Responding anywhere on the screen resulted in a correct response and pellet delivery. Four consecutive correct trials resulted in a decrease in the size of the response area (i.e. purple square). In this manner, monkeys were trained to touch a purple square that eventually shrank to 3.8 x 3.8 cm square that was then randomly distributed in different
locations across the screen. All animals responded to stimuli with their left hand and used their right hand for pellet retrieval.

Once each monkey acquired this simple visual-motor response task (responding to >95% of trials three days in a row) monkeys began the intra/extra-dimensional shifting task. The version utilized was slightly modified from that described by Dias et al. (1996a,b) and Weed et al. (1999). In the first stage, (simple discrimination; SD), two shapes were presented, touching one of these stimuli was reinforced (S+) and followed by a 5-sec timeout while touching the other was not reinforced (S-) and resulted in trial termination and a 15-sec timeout. Within each trial the two shapes were distributed on the left or right side of the screen in a pseudo-random fashion. Acquisition criterion for each stage was defined as 6 consecutive correct responses. Upon acquisition of each stage, monkeys progressed immediately to the next stage within the same session. If a monkey failed to complete a stage within one session, the next day’s session began at the same stage, with the same stimuli and contingencies from the end of the previous day’s session. A maximum of 200 trials were available each session. Failure to respond within 10 seconds on any trial resulted in termination of the trial and was followed by a 15-sec timeout before the next trial began. The second stage of this task was a compound discrimination (CD) that involved the same contingencies and shapes from stage 1, but also included a line pattern overlaying the two shapes. The shapes continued to function as S+ and S- while the lines served only as distracter images. All four images (shapes and lines) were pseudo-randomly distributed independently of each other between the left and right sides of the screen. In the third stage (intra-dimensional shift; IDS), two new shapes and two new lines were presented; shapes remained the attentional set associated with
reinforcement contingencies and lines continued to be distracter images. This stage established a focused attentional set (shapes) that monkeys learned to attend to despite presentation of new stimuli. In stage 4 (extra-dimensional shift; EDS), two new shapes and two new lines were presented. In this stage, lines were now the response-contingent attentional set (S+ and S-) and shapes were distracters. In this stage, monkeys had to learn a new attentional set, shifting from shapes to lines. Upon acquisition of the EDS the task was completed, and the session ended for the day. Stimuli used for this task included the first 6 shapes and lines from the “University of Cambridge ID/ED stimulus sets” that were programmed with the CANTAB apparatus. As learning may occur to frequent repeated exposures to this task, a minimum of ~ 2 months passed between the first exposure (described above) and the second exposure coinciding with FDG-PET (see below; Table 1). During this duration monkeys began training 5-7 days/week on a delay match-to-sample task to assess working memory (data not presented in current study).

**FDG-PET imaging**

T1- weighted magnetic resonance images (MRIs) were acquired for later coregistration with the PET data on all monkeys using a 3.0T MR scanner (GE Medical Systems) under ketamine-induction (10 mg/kg) and 1.5% isoflurane-maintained anesthesia. Each animal underwent two FDG-PET studies associated with CANTAB sessions: a baseline session to control for visual-motor activity and during EDS, with the order counterbalanced within groups and separated by a minimum of 2 weeks. For the baseline condition, the cognitive session consisted of trials during which a single purple square (3.8 x 3.8 cm) was presented in random locations across the computer screen and
responding within the square resulted in delivery of a 190 mg sucrose pellet. Each trial was followed by a 30 sec timeout. The EDS condition used for the other FDG scan was identical to the above description with one exception. Upon acquisition of the IDS, instead of immediately progressing to the EDS, monkeys continued to respond on the contingencies associated with the IDS for ~150 additional trials following acquisition in order to allow for the scheduling of PET scans. The additional responding under the IDS further solidified the attentional set so each group of monkeys performed at equal accuracies prior to the FDG scan (Table 2).

On the day of a PET study, the monkey was seated in a primate chair and a 0.5 ml blood sample was collected for baseline glucose analysis (using a handheld Glucometer; Accu-Chek Advantage, Roche Diagnostics) prior to being placed in the CANTAB chamber. Approximately 1.0 ml $[^{18}\text{F}]$FDG (range= 5.5-5.9 mCi) was injected into the vascular access port followed by a 3 ml flush of sterile saline and the cognitive session was immediately started. After 40 minutes, the session was terminated and the monkey was anesthetized with an intramuscular (i.m.) injection of a cocktail containing 0.04 mg/kg Dexmedetomidine (Dexdomitor, Pfizer Pharmaceuticals) and 5.0 mg/kg ketamine HCl. While sedated, ~1.5 ml of venous blood was collected at 45 min post FDG administration via the saphenous vein to determine glucose levels (see above) and the blood concentration of FDG (using an automated gamma-well counter). Monkeys were then transported to the Wake Forest University School of Medicine PET Center. PET scans were acquired using a General Electric 64-slice PET/CT Discovery VCT scanner (GE Medical Systems, Milwaukee, WI, United States) with ~5-6-mm resolution (see Teras et al., 2007). An initial low dose CTAC scan was acquired, followed by a 10-min
3D emission scan. The 3D data were corrected for attenuation and reconstructed transaxially using OSEM VUE point (28 subsets; 2 iterations) with a 3-mm FWHM filter resulting in a 128 x 128 matrix. Heart rate, blood pressure, and blood oxygen saturation were constantly monitored throughout the 15-min PET scan and during recovery. At the end of the study, the monkey was returned to his homecage and was administered 0.2 mg/kg atipamezole i.m. (Anteseden, Pfizer Pharmaceuticals), to reverse the anesthetic effects of dexmedetomidine. Monkeys were monitored until they regained sternal recumbency (typically within 15 min of administration of atipamezole).

Data analysis

Cognitive assessment. The primary dependent variables were number of trials completed and number of errors committed before acquiring each stage of the ID/ED task. Trials in which no response was made (omitted trials) were rare, and were excluded from analysis. Individual-subject data were normalized by square-root transformation prior to statistical analysis. In addition, response latency (time to respond on a given stimulus within a trial) and pellet retrieval latencies were also examined. Two-way analyses of variance (ANOVAs) were conducted using group (cocaine SA vs cocaine-naive) and stage (SD, CD, ID, ED) as factors to analyze the first exposure to the complete ID/ED task. Significant main effects were followed by post hoc Holm-Sidak t-tests. A pre-planned t-test was conducted between groups in the ED component, as this was our primary variable of interest. A two-way repeated-measures ANOVA was conducted to assess potential differences in acquisition performance between the first and second exposure to the SD, CD, and ID stages of the ID/ED task (EDS excluded due to procedural
differences associated with FDG administration and sedation). Additional two-way ANOVAs were conducted to compare dependent variables between the two FDG sessions and included number of trials completed, and number of pellets ingested. $t$-tests were conducted to compare % accuracy between groups during the EDS FDG session (by design, since every response in the BL condition was “correct” no comparison of accuracy between conditions could be made). Additional $t$-tests were conducted to compare number of trials completed and % accuracy between groups in the post-acquisition ID stage to assess potential differences in training leading up to the ED shift that might influence behavior or underlying glucose utilization. For all analyses, significance threshold was $p<0.05$.

**PET imaging.**

Using the method of Porrino et al (Porrino et al., 2005; Deadwyler et al., 2007), the concentration of FDG in blood over time for each subject (blood input function) was determined by scaling a population-averaged FDG blood curve by the subject’s blood FDG concentration at $T=45$ minutes. The individual blood input curves and glucose values were then applied to respective PET data to generate images of cerebral metabolic rates of glucose (MRGlu) using the “MRGlu (FDG Autorad)” model based on Huang et al., (1980) implemented in the pixel-wise modeling tool in the PMOD image analysis software (PMOD Technologies, Zurich, Switzerland). Subsequent manipulations and analyses were conducted on the PET MRGlu data using the Statistical Parametric Mapping (SPM5) software (University College London, London, United Kingdom; [http://www.fil.ion.ucl.ac.uk/spm/](http://www.fil.ion.ucl.ac.uk/spm/)) in conjunction with MATLAB (MathWorks, Natick,
MA, United States). Using this software, PET MRGlu data were co-registered to respective individual structural MR images and then normalized to a standard rhesus macaque template (Black et al., 2004). Proportional normalization and grand mean scaling were applied to account for differences in global activity. Finally, images were smoothed using a 2 mm isotropic Gaussian kernel with a voxel size of 1 x 1 x 1 mm.

Whole brain analyses of PET MRGlu data were performed for the following comparisons: cocaine-naive monkeys, extra-dimensional shift versus baseline condition; cocaine SA monkeys, extra-dimensional shift versus baseline condition; and baseline condition, cocaine-naive monkeys versus cocaine SA monkeys. For each within-group comparison a statistical parametric map was created by applying a paired \( t \)-test factorial design matrix. A non-paired \( t \)-test factorial design matrix was applied for the baseline comparison between groups. An initial voxel height threshold of \( p<0.005 \) (uncorrected) and minimum cluster size of 25 contiguous voxels were set to establish clusters; of the identified clusters, only those with a \( p<0.05 \) value (corrected for search volume) were considered significant. Areas of activation are displayed on a T1 MR template (Black et al., 2004) and the associated brain regions were identified using a rhesus monkey brain atlas (Saleem and Logothetis, 2007).

**RESULTS**

**Afternoon operant behavioral sessions**

For the cocaine-naive monkeys responding under the FR 30 schedule of food presentation, the group mean (± SEM) response rate during the 5 days preceding the EDS and FDG administration was 0.77 (± 0.38) responses/sec. For cocaine SA monkeys, the
group mean (± SEM) response rate during the 5 days preceding the EDS and FDG administration was 0.17 (± 0.02) responses/sec. For both groups of monkeys, response rates and reinforcement frequency did not significantly change over the course of the study; all monkeys received the maximum number of reinforcers (i.e., 15 pellets or 15 injections).

**Cognitive performance**

**ID/ED: Initial Effects.** There was no main effect of group when comparing the number of trials to acquisition between cocaine-naive and cocaine SA monkeys. However, pre-planned comparisons showed a significant difference between groups in the number of trials to acquire the EDS, with cocaine SA monkeys requiring significantly more trials ($t_6=3.759$, $p=0.009$; Fig. 1A, 1B). The number of errors committed was significantly different between groups ($F_{1,24}=4.424$, $p=0.046$) with the cocaine SA monkeys making significantly more errors during the EDS compared to cocaine-naïve control monkeys ($t_6=2.383$, $p=0.025$; Fig. 1C, 1D).

There was an overall main effect of group ($F_{1,24}=4.428$, $p=0.046$) on average response latency. The mean (± SEM) group response latencies across all stages were 2.13 ± 0.29 sec vs. 1.60 ± 0.35 sec for the cocaine-naive and cocaine SA groups, respectively. Similarly, there were significant group differences in average pellet retrieval latency ($F_{1,24}=30.196$, $p<0.001$), with cocaine-naive monkeys taking 1.17 ± 0.04 sec compared to 0.62 ± 0.19 sec for cocaine SA monkeys. *Post-hoc* testing showed significant differences between groups in pellet retrieval latency across all stages (SD, CD, ID, ED, all $p<0.05$, data not shown).
Figure 1. Performance on an ID/ED task in cocaine-naive and cocaine SA monkeys.

A, C show group and B, D show individual data for the number of trials completed and errors committed, respectively, to acquire each stage of the task. Open bars and symbols represent cocaine-naive monkeys, filled bars and symbols represent cocaine SA monkeys; * denotes significance at p<0.05.
Behavior prior to and during FDG uptake. There were no group differences in the total number of trials completed (or pellets received) during the baseline FDG study (Table 2). There were no group differences between the number of trials completed, number of errors committed, or % accuracy during the SD, CD, ID, the extended IDS trials (see Table 2 “ID (post ACQ)”), or the abbreviated EDS stage during FDG incorporation. Furthermore, there was not a significant difference in % accuracy on day 1 from day 2 on the post acquisition IDS trials between or within groups (all p>0.05), suggesting retention of the established attentional set over time.

There were significantly greater numbers of trials completed (F_{1,12}=31.227, p<0.001; Table 2) yet fewer number of reinforcer earned (e.g. pellets ingested; F_{1,12}=17.819, p<0.05) in the EDS compared to BL session during FDG incorporation. These effects were present in both groups (number of trials, cocaine-naive, t_{1}=3.258, p<0.01; cocaine SA, t_{1}=4.645, p<0.001; number of reinforcers, cocaine-naive, t_{1}=3.707, p=0.003; cocaine SA, t_{1}=2.263, p=0.043; Table 2), but neither group was different from the other in either condition.
Table 2. Task performance prior to and during FDG incorporation

<table>
<thead>
<tr>
<th>MONKEY</th>
<th>PURPLE BOX</th>
<th>SD</th>
<th>CD</th>
<th>ID</th>
<th>ID (POST ACQ)</th>
<th>% CORRECT</th>
<th>ED (40 MIN)</th>
<th>% CORRECT</th>
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<tbody>
<tr>
<td>Cocaine SA</td>
<td>1374</td>
<td>195</td>
<td>9 / 3</td>
<td>46 / 18</td>
<td>144 / 6</td>
<td>95.9</td>
<td>222 / 84</td>
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<tr>
<td></td>
<td>1375</td>
<td>182</td>
<td>243 / 102</td>
<td>14 / 4</td>
<td>134 / 37</td>
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<td>175</td>
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<td>89 / 40</td>
<td>196 / 64</td>
<td>67.3</td>
<td>249 / 73</td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>1381</td>
<td>144</td>
<td>144 / 73</td>
<td>315 / 161</td>
<td>178 / 87</td>
<td>50.8</td>
<td>223 / 108</td>
<td>115</td>
</tr>
<tr>
<td>MEAN</td>
<td>174</td>
<td>126 / 58.3</td>
<td>116 / 55.8</td>
<td>61.3 / 29.8</td>
<td>163 / 48.5</td>
<td>71.6</td>
<td>229.3* / 87.8</td>
<td>141.5*</td>
</tr>
</tbody>
</table>

| Cocaine naive | 1681     | 150       | 67 / 34   | 268 / 131 | 187 / 81      | 56.7      | 207 / 98    | 109       | 52.7      |
|               | 1682     | 185       | 37 / 14   | 30 / 8    | 149 / 50      | 73.2      | 212 / 82    | 130       | 61.3      |
|               | 1683     | 171       | 58 / 20   | 118 / 49  | 148 / 29      | 80.4      | 224 / 93    | 131       | 58.5      |
|               | 1696     | 199       | 258 / 127 | 73 / 31   | 140 / 41      | 70.7      | 217 / 95    | 122       | 56.2      |
| MEAN         | 176.3    | 105 / 48.8| 122.3 / 72| 153.8 / 62| 156 / 50.3    | 70.3      | 215* / 92   | 123.0*    | 57.2      |

¹Behavior during FDG incorporation; * significantly more trials were completed yet fewer reinforcers earned (SR; e.g. correct responses) by both groups during the ED session compared to their respective baseline (BL) sessions but there were no differences between groups. Note: trials completed and number of reinforcers earned are identical in the BL condition, no “incorrect” response could be made.
PET imaging

A between-group comparison showed that cocaine-naive monkeys had greater glucose metabolism compared to cocaine SA monkeys during the baseline condition in the left pre/post-central gyrus. Cocaine SA monkeys showed greater glucose metabolism in the left inferior occipital/fusiform gyrus compared to the cocaine-naive monkeys (Table 3).

Table 3. Differences in relative glucose utilization between cocaine-naive monkeys and cocaine SA monkeys (n=4/group).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Brain Region</th>
<th>Hemisphere</th>
<th>Standard z-score(^1)</th>
<th>Cluster size (# of voxels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine-naive &gt; Cocaine SA</td>
<td>precentral/ postcentral gyrus</td>
<td>LF</td>
<td>3.32</td>
<td>87</td>
</tr>
<tr>
<td>Cocaine SA &gt; Cocaine-naive</td>
<td>inferior occipital/ fusiform gyrus</td>
<td>LF</td>
<td>3.63</td>
<td>150</td>
</tr>
</tbody>
</table>

\(^1\) p< 0.05; corrected for search volume
During the EDS, both cocaine-naive and cocaine SA monkeys showed higher glucose utilization in the right lateral orbital gyrus and right posterior cingulate/postcentral gyrus, compared to their respective BL metabolic activity (Table 4, Fig. 2). In contrast, only cocaine-naive monkeys showed increased glucose utilization in the anterior cingulate/frontal gyrus, midcingulate/prefrontal gyrus, right caudate, right globus pallidus, left inferior temporal, lingual gyrus, hippocampus, and the precuneus, while cocaine SA monkeys showed greater glucose utilization in the left superior occipital gyrus (Table 4, Fig. 2).

Relative to the BL condition, cocaine-naive monkeys showed less glucose utilization during the EDS in the left fronto-orbital gyrus and left precentral gyrus whereas the cocaine SA monkeys showed lower glucose utilization in the right thalamus, right inferior occipital gyrus, and right cuneus (Table 4).
Table 4. Glucose utilization between a baseline motor task (BL) and an extradimensional shift (EDS) in cocaine-naive monkeys and cocaine SA monkeys (n=4/group).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Brain Region</th>
<th>Hemisphere</th>
<th>Standard z-score&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Cluster size (# of voxels)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ED shift &gt; BL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cocaine-naive</strong></td>
<td>lateral orbital gyrus</td>
<td>RT</td>
<td>3.63</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>ant. cingulate/ sup. frontal gyrus</td>
<td>Both</td>
<td>3.94</td>
<td>359</td>
</tr>
<tr>
<td></td>
<td>ant. cingulate/ sup. frontal gyrus&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Both</td>
<td>3.74</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ant. cingulate gyrus&lt;sup&gt;2&lt;/sup&gt;</td>
<td>RT</td>
<td>3.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mid-cingulate/precentral gyrus</td>
<td>Both</td>
<td>4.27</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>post. cingulate/postcentral gyrus</td>
<td>RT</td>
<td>3.32</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>caudate nucleus</td>
<td>RT</td>
<td>3.30</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>globus pallidus&lt;sup&gt;2&lt;/sup&gt;</td>
<td>RT</td>
<td>3.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>inf. temporal gyrus</td>
<td>LF</td>
<td>3.72</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Hippocampus</td>
<td>LF</td>
<td>4.43</td>
<td>183</td>
</tr>
<tr>
<td></td>
<td>lingual gyrus&lt;sup&gt;2&lt;/sup&gt;</td>
<td>LF</td>
<td>2.86</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Precuneus</td>
<td>Both</td>
<td>4.12</td>
<td>151</td>
</tr>
<tr>
<td><strong>Cocaine SA</strong></td>
<td>lateral orbital gyrus</td>
<td>RT</td>
<td>4.05</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>post. cingulate/postcentral gyrus</td>
<td>RT</td>
<td>3.07</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>sup. occipital gyrus</td>
<td>LF</td>
<td>3.91</td>
<td>26</td>
</tr>
<tr>
<td><strong>BL &gt; ED shift</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cocaine-naive</strong></td>
<td>fronto-orbital gyrus</td>
<td>LF</td>
<td>3.83</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>precentral/postcentral gyrus</td>
<td>LF</td>
<td>4.52</td>
<td>78</td>
</tr>
<tr>
<td><strong>Cocaine SA</strong></td>
<td>Thalamus</td>
<td>RT</td>
<td>3.89</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>inf. occipital gyrus</td>
<td>RT</td>
<td>3.72</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>cuneus</td>
<td>RT</td>
<td>3.67</td>
<td>30</td>
</tr>
</tbody>
</table>

<sup>1</sup> p< 0.05; corrected for search volume

<sup>2</sup> significant region within the larger cluster directly above it in the table
Figure 2. Areas of increased glucose utilization during an extradimensional shift. Group data from whole-brain analyses showing areas where cocaine-naive monkeys (top) and cocaine SA monkeys (bottom) showed significantly greater glucose utilization during the initial response to an EDS compared to their respective baseline measures. The saggital slices (far left) show the location (blue lines) of respective coronal slices in a rostrocaudal direction. Cluster threshold p<0.05, corrected for search volume; scale bar represents t score. orbPFC- orbital prefrontal cortex; Cd- caudate nucleus; STG- superior temporal gyrus; ACC- anterior cingulate gyrus; MCC-mid-cingulate/precentral gyrus; PCC- posterior cingulate gyrus.
The present study examined cognitive performance and underlying neural activity using a set-shifting task in cocaine-naive monkeys and monkeys with an extensive and current cocaine self-administration history, modelling long-term, current cocaine users. As hypothesized, monkeys with a cocaine self-administration history required more trials to acquire the EDS and made more errors compared to cocaine-naive monkeys. Cognitive differences were also associated with different patterns of glucose metabolism as determined using PET and \(^{18}\text{F}\)-FDG. The current behavioral data add to studies demonstrating cocaine-associated cognitive impairments in rodents (Schoenbaum et al., 2004; Briand et al., 2008; George et al., 2008) and monkeys (Jentsch et al., 2002; Liu et al., 2008, 2009; Porter et al., 2011) and PET data extend lesioning studies (e.g. Dias et al., 1996a,b; Birrell and Brown, 2000) to implicate multiple brain regions mediating intact cognitive control and cocaine-associated deficits in behavioral flexibility.

In cocaine-naive monkeys, there were increases in glucose metabolism during the EDS, compared to baseline, across mesolimbic and cortical regions. Increases in frontal lobe activity included the orbitofrontal (orbPFC), medial frontal (mPFC), ACC and superior frontal gyrus, areas that are interconnected and integral in executive function (Gehring and Knight, 2000; Bush and Posner, 2000). In the parietal lobe increased activity included the posterior cingulate, precentral gyrus and precuneus. In the temporal cortex the inferior and superior temporal, lingual gyrus and hippocampus also showed increased activity. These areas relay sensory information through direct connections to the PFC (see Cavanna and Trimble, 2006; Aron and Paulus, 2007 for reviews). The caudate nucleus also showed greater activity in the EDS. The striatum has been
implicated in mediating behavioral flexibility by implementing efficient behavioral strategies such as updating and establishing stimulus-reward associations through PFC circuits (Bar-Gad et al., 2000; Volkow and Fowler, 2000; Leblois et al., 2006; Nagano-Saito et al., 2008) and simultaneously activating relevant or inhibiting irrelevant posterior cortical circuits specific to sensory modalities (e.g. shapes vs lines, objects vs faces; see van Schouwenburg et al., 2010). Taken together, areas of increased activation in response to the EDS in cocaine-naive monkeys comprised frontal-cingulate, parietal-frontal, and striato-cortical circuits similar to activation patterns seen in human fMRI studies examining shifting between attentional domains (e.g. Hampshire and Owens, 1996; Kondo et al., 2004a,b; Kubler et al., 2005; Simard et al., 2011).

Strikingly, both cocaine-naive and cocaine SA monkeys showed increased activity in nearly identical regions of the orbPFC during the EDS compared to baseline. Region-specific lesioning studies in rodents, monkeys and humans have classically associated intact orbPFC activity with accurate reversal learning and dlPFC function with set-shifting behavior (Owen et al. 1991; Dias et al., 1996a, 1997; McAlonan and Brown, 2003; Kazama and Bachevalier, 2009). Moreover, hypofunction of the orbPFC has been demonstrated in cocaine users compared to control subjects under baseline metabolic assessment using FDG-PET (Volkow et al., 1993). However, the apparently discordant results between PET, fMRI, lesioning studies and the current data are reconcilable. First, the orbPFC receives projections from cortical sensory and limbic reward-related areas and projects to limbic (striatum, ACC) and cortical (dlPFC, mPFC) regions (Carmichael and Price, 1995a,b; see Wallis et al., 2004 for review). Functionally, the orbPFC is implicated in maintaining stimulus-reinforcement associations based on feedback from
these sensory and reward-related inputs and integrating relevant information to other PFC
regions such as the dIPFC and ACC (for reviews see Volkow et al., 2000; Wallis, 2007;
Liu et al., 2011). Second, glucose utilization as measured via FDG-PET is thought to
represent primarily axonal activity (Schwartz et al., 1979). The current data may
therefore reflect activity of neuronal inputs to the orbPFC (via cortico-cortical, and
striato-cortical circuits). However, the lack of metabolic activity in cocaine SA monkeys
in other regions integral to error-detection and stimulus-reward feedback (e.g. ACC,
caudate) may reflect hypofunctional orbPFC activity. Similar activation patterns using
$[^{15}\text{O}]\text{H}_2\text{O}$ and PET have been reported during a decision-making task such that both
chronic cocaine users and control groups showed increased activity in the orbPFC, but
cocaine users showed less activity in other PFC areas (dIPFC, mPFC) compared to
controls (Bolla et al., 2003).

While data from the cocaine-naive monkeys are in agreement with previous
studies implicating the integrative function of both anterior (PFC, ACC) and posterior
(PCC) attentional networks underlying successful set shifting behavior (Posner and
Petersen 1990; Nagahama et al., 1999), data from the cocaine SA monkeys suggests
cognitive impairments may be caused by dysregulations primarily in the anterior
attentional network. Through connections with other PFC regions (including the
orbPFC), the ACC responds to task-specific errors to re-direct goal-oriented behaviors
and is postulated to be at the center of this attentional network (e.g. Aron and Paulus,
2007; Liu et al., 2011). Similar to the current study, cocaine users compared to control
groups showed hypoactivity in the ACC regardless of the cognitive domain examined
(e.g. Bolla et al., 2004; Hester and Garavan, 2004; Kubler et al., 2005; Tomasi et al.,
Further, the unaltered ability to evaluate consequences (mediated by the posterior attentional network) fits with clinical literature such that addicts recognize deleterious drug-related behaviors yet are unable to redirect or adapt to advantageous behavioral strategies, thus contributing to compulsive drug use or relapse.

A comparison between cocaine-naive and cocaine SA monkeys revealed few differences in baseline metabolic activity. Cocaine-naive monkeys showed relatively greater glucose utilization in the pre/postcentral gyrus compared to the cocaine SA monkeys. Although chronic cocaine use has been associated with deficits in fine motor performance (Bolla et al., 1999; Hanlon et al., 2011), there were no signs of gross motor impairments in our cocaine SA monkeys as evidenced by significantly quicker response latencies than cocaine-naive monkeys. The cocaine SA group showed relatively greater glucose metabolism compared to the cocaine-naive group in the inferior occipital/fusiform gyrus that may suggest greater activity in the visual system in the cocaine SA monkeys. Regardless, similar cognitive performance across SD, CD, and IDS components suggest that potential neuronal compensation for motoric or visual deficiencies would not affect EDS performance nor would such neuronal activity affect the conclusions based on glucose metabolism during the EDS performance as our measurements were relative to each group’s baseline performance.

It is necessary to comment briefly on the study design. While a 40 minute FDG uptake period lacks the temporal refinement to parse distinct attentional domains involved in adapting to and altering behavior in response to shifting attentional sets (e.g. reaction to error, adapting to changing contingencies, strategy development, new stimuli-reinforcement association, suppression of irrelevant stimuli), this study provided a
summation of all brain activity underlying cognitive performance between cocaine-naive and cocaine SA monkeys. Unlike typical human studies, the shift between attentional sets (shapes to lines) occurred at the beginning of, not midway through a test session. Therefore, it was imperative to demonstrate the retention of a stimulus set over a 24-hour period. “Post ID acquisition” trials extended over a 2-3 day period. Cocaine SA and cocaine-naive monkeys showed equal retention of the stimulus-reinforcement contingencies within the attentional set (see Table 2). Such retention is important due to one inconsistency between our results and existing literature. Neither cocaine SA nor cocaine-naive monkeys showed significant differences in the acquisition of the EDS compared to an IDS. Typically a larger number of trials/errors to complete the EDS compared to IDS represents an established attentional set that must be broken prior to acquisition of an EDS (Dias et al., 1996a,b, 1997; Birrell and Brown, 2000). Although this inconsistency may be a result of the current acquisition criteria (6 correct responses in a row), opposed to a serial acquisition criteria (e.g. 16 or 18 correct responses out of 20 consecutive trials; Dias et al., 1996a,b, 1997; Weed et al., 2008), preliminary studies within our laboratory showed that acquisition based on both criteria occurred nearly simultaneously (i.e. 6 correct consecutive responses occurred for the first time within a set of 16 correct responses within 20 consecutive trials; data not shown). The current acquisition criterion resulted in significant differences in the EDS acquisition between groups in the current study and demonstrated adequate difficulty in other studies using rhesus monkeys (Decamp and Schneider, 2004, 2006).

Cocaine-associated executive dysfunction is hypothesized to be a result of neurobiological deficits in the dopaminergic system. An extensive literature details the
involvement of DA neurotransmission in mediating cognition including striatal DA for cognitive flexibility and DA in the PFC for memory (for reviews see van Schouwenburg et al., 2010; Cools, 2011). Using PET imaging in monkeys, an inverse relationship has been shown between DA D2-like receptor availability in the striatum and performance on reversal learning tasks, another measure of behavioral flexibility (Grosman et al., 2011). Further, cocaine self-administration in monkeys induced reductions in striatal D2-like receptor availability (Nader et al., 2006) and noncontingent cocaine administration induced deficits in reversal learning (Jentsch et al., 2002). Similarly, in humans, recent cocaine users showed lower DA D2-like receptor availability in the striatum compared to controls and a direct correlation between D2-like receptor availability and basal glucose metabolism in the orbPFC and ACC (Volkow et al., 1993). Functional MRI studies in recent cocaine users showed impairments in PFC activity underlying impaired cognitive processes such as decision-making, set shifting, behavioral inhibition, and working memory (e.g. Hester and Garavan, 2004; Kubler et al., 2005; Tomasi et al., 2007a,b; Goldstein et al., 2010). Lastly, functional and cognitive deficits associated with cocaine use are similar to those present in patients with Parkinson’s Disease, schizophrenia and ADHD, other conditions associated with hypodopaminergic function (for review see Chudasama and Robbins 2006). Similar to fMRI studies in human cocaine users wherein stimulant drugs (e.g. methylphenidate) have normalized areas of hypofunctionality and improved cognitive performance on an attentional task (Goldstein et al., 2010), results from the present study have established functional deficits in a monkey model to allow future assessment of novel pharmacotherapeutic agents aimed to improve neural function and enhance cognitive performance.
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REFERENCES


CHAPTER IV

EFFECTS OF NICOTINIC ACETYLCHOLINE RECEPTOR STIMULATION ON COGNITION IN RHESUS MONKEYS WITH A CHRONIC COCAINE SELF-ADMINISTRATION HISTORY

Robert W. Gould, Pradeep K. Garg, Sudha Garg, Michael A. Nader

The following manuscript is in preparation to be submitted to Neuropsychopharmacology in October, 2011. Stylistic variations are due to the requirements of the journal. Robert W. Gould performed the experiments, analyzed the data and prepared the manuscript. Sudha Garg and Pradeep K. Garg conducted positron emission tomography studies. Michael A. Nader acted in an advisory and editorial capacity.
ABSTRACT

Cocaine use is associated with impaired cognitive function, which may negatively impact treatment outcomes. One pharmacological strategy to improve cognition involves nicotinic acetylcholine receptor (nAChR) stimulation. However, the effects of chronic cocaine exposure on nAChR distribution and function have not been characterized. One goal of the present study was to examine nAChR availability in rhesus macaques with an extensive cocaine self-administration history (n=4; > 5 years, mean intake, 1463 mg/kg) compared to age-matched, drug-naive monkeys (n=5). A second goal was to examine the effects of nAChR agonists on working memory using a delayed match-to-sample (DMS) task in the same monkeys during periods of abstinence from cocaine. Using [11C]-nicotine and positron emission tomography (PET) imaging, cocaine-experienced monkeys showed significantly greater receptor occupancy in the hippocampus compared to cocaine-naive monkeys. When administered acutely, the nonselective high-efficacy agonist nicotine, the low-efficacy α4β2* subtype-selective agonist varenicline and the high-efficacy α7 subtype-selective agonist, PNU-282987 significantly improved performance in both cocaine-naive monkeys and monkeys with a cocaine self-administration history. These data demonstrate differential receptor availability as a function of cocaine self-administration. Importantly, nAChR agonists enhanced working memory during periods of abstinence from cocaine suggesting that long-term cocaine exposure does not ameliorate the efficacy of pharmacotherapies targeting cognitive enhancement.

Key Words: cocaine, DMS, nAChR, nicotine, nonhuman primates, PET, PNU-282987, varenicline
INTRODUCTION

Chronic cocaine use continues to be a significant public health concern worldwide (SAMHSA, 2009). Cocaine binds to the dopamine (DA), serotonin (5-HT), and norepinephrine (NE) transporters (DAT, SERT, and NET, respectively; Ritz et al., 1987) and induces numerous neurobiological changes throughout mesocorticolimbic regions that disrupt executive function (Volkow et al., 1993; Tomasi et al., 2009; Moeller et al., 2010). For example, compared to control groups, chronic cocaine users showed lower neuronal function measured via fMRI or positron emission tomography (PET) in regions underlying cognitive function and showed impaired performance on tasks measuring response inhibition, behavioral flexibility, impulsivity, and working memory (Volkow et al., 1991, 1992; Fillmore and Rush 2002; Bolla et al., 2004; Hester and Garavan, 2004; Tomasi et al., 2007a,b; Goldstein et al., 2007, 2010; Moeller et al., 2010).

Currently, there are no FDA-approved treatments for cocaine dependence (Karila et al., 2008). Behavioral treatment strategies, aimed at modifying deleterious, drug-related behaviors to more goal-directed positive behaviors have proven moderately successful (see Vocci and Montoya, 2009 for review). Moreover, success rates of behavioral treatments are directly correlated with neuropsychological measures upon treatment initiation (Teichner et al., 2001; Aharonovich et al., 2006; Turner et al., 2009; Schmitz et al., 2009; Moeller et al., 2010). Cognitive enhancement may increase retention and success of behavioral treatments, thus improving overall abstinence from cocaine (e.g., Sofuoglu 2010; Perry et al., 2011).
The acetylcholine (ACh) neurotransmitter system has been extensively studied as a mechanism to improve cognitive deficits associated with depression and Parkinson’s Disease, and other neuropathologies like cocaine dependence associated with hypodopaminergic function (for reviews see Rezvani and Levin 2001; Forgacs and Bodis-Wollner, 2004; Cincotta et al., 2008). Nicotine, a high efficacy agonist that nonselectively binds at all nicotinic acetylcholine receptor (nAChR) subtypes indirectly stimulates dopamine release (e.g. Rollema et al., 2007, 2009) and has shown cognitive-enhancing effects on measures of attention and memory in rodent, monkey and human studies (see Rezvani and Levin, 2001 for review). However, the high abuse liability of nicotine and its facilitatory effects on other drug use (e.g. Schorling et al., 1994; Roll et al., 1996) precludes its use clinically as a cognitive enhancer.

Subtype-specific nAChR agonists have produced cognitive enhancing effects in animal models (e.g. Hahn et al., 2003; Bitner et al., 2007; Howe et al., 2010; Castner et al., 2011) and may have lower abuse liability. Nicotinic AChRs are comprised of 5 subunits of homogenous (primarily containing α7 subunits) or heterogenous (α1-10; β1-4) makeup with subtype composition affecting binding affinity and efficacy (Corringer et al., 2000; Le et al., 2002). The two primary subtypes distributed within the CNS are α7 and α4β2* nAChRs (* denotes the presence of additional accessory subunits; e.g. Livingstone and Wonnacott, 2009). For example, varenicline (Chantix©), an FDA-approved medication with success as a smoking cessation agent (Gonzales et al., 2006; Jorenby et al., 2006), has high affinity and low efficacy at α4β2* receptors and low affinity and high efficacy at α7 receptors (Coe et al., 2005; Mihalak et al., 2006). Further, animal studies and limited clinical data suggest that varenicline improves cognition.
across multiple domains (see Rollema et al., 2009 for review) and has limited abuse liability (Rollema et al., 2007, McColl et al., 2008; Gould et al., 2011).

However, the direct effects of cocaine on nAChR distribution and function are not clear, due to a lack of animal studies, and the high co-abuse of nicotine and cocaine. Cocaine self-administration in animal models produces parallel neurobiological and cognitive deficits to those seen in human cocaine users (see Beveridge et al., 2008 for review) including impairments on attention, memory, impulsivity and behavioral flexibility in rodents (Dalley et al., 2005; Briand et al., 2008; George et al., 2008; Winstanley et al., 2007, 2009) and monkeys (Liu et al, 2008, 2009; Porter et al., 2011). Therefore, one goal of the current study was to examine nAChR availability, using $[^{11}C]$-nicotine and PET imaging in rhesus monkeys with an extensive cocaine self-administration history compared to age-matched drug-naive monkeys. The second goal of this study was to examine the effects of subtype-selective nAChR agonists of various efficacies including nicotine, varenicline, and PNU-282987, a novel high efficacy $\alpha_7$-selective nAChR agonist (Bodnar et al., 2005) on working memory in monkeys trained to perform a delayed match-to-sample task. We hypothesized that, similar to the effects of smoking in humans and chronic nicotine exposure in animals (e.g. Breese et al., 1997; Mugnaini et al., 2002; Muhkin et al., 2008), nAChR availability would be higher in monkeys with a cocaine self-administration history. Further, we hypothesized that nAChR agonists would improve working memory in all monkeys, but that nicotine would be more efficacious in monkeys with a cocaine self-administration history.
MATERIALS AND METHODS

Subjects. Nine singly housed adult male rhesus macaques (*Macaca mulatta*) of Indian origin served as subjects. Four monkeys (13-14 years old) had an extensive cocaine self-administration history (~6 yrs; mean 1463 mg/kg cumulative cocaine intake; see Table 1) at the initiation of this study (Czoty et al., 2007). Five additional monkeys were age-matched, and cocaine-naive (11-14 years old). Each monkey was fitted with an aluminum collar (Primate Products, Redwood City, CA) and trained to sit in a primate restraint chair (Primate Products). All monkeys (except R-1756) were surgically implanted, under ketamine anesthesia and aseptic conditions, with indwelling vascular access ports and intravenous catheters (Access Technologies, Skokie, Il) into a major vein (see Czoty et al., 2007). A 3 ml bolus of heparinized saline (100 U/ml) was injected into the port following each cocaine self-administration session (cocaine SA group) and once weekly (cocaine-naive group) to maintain catheter patency. Monkeys were weighed weekly and fed enough food daily (Purina Monkey Chow and fresh fruit) to maintain ~95% free-feeding body weight; water was available ad libitum in their homecage, which measured 0.71 x 0.84 x 0.84 m (Allentown Caging Inc., Allentown, NJ). All experimental procedures were performed in accordance with the 2003 National Research Council *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* and were approved by the Wake Forest University Institutional Animal Care and Use Committee. Environmental enrichment was provided as outlined in the Institutional Animal Care and Use Committee’s Nonhuman Primate Environmental Enrichment Plan.

Apparatus. During training, all animals underwent two daily operant behavioral sessions (5-7 days/week) consisting of cognitive testing between 7:00-10:00 AM and respective
afternoon sessions between 1:00-4:00 PM in which responding was maintained by intravenous cocaine (cocaine-experienced group) or food pellets (control group). A minimum of 2 hours elapsed between sessions during which monkeys were returned to their home cages. Cognitive testing was conducted using the Cambridge Neuropsychological Test Automated Battery (CANTAB; Lafayette Instruments, Lafayette, IN) apparatus. Each CANTAB cognition station (0.38 x 0.56 x 0.31 m) was located in sound-attenuating, ventilated chambers (0.8 x 0.8 x 1.32 m) and included a touch-sensitive computer screen (0.3 x 0.23 m) with a stimulus light, a non-retractable response lever, and a pellet receptacle located to the right side of the front panel. Operant panels, for the afternoon sessions, were located in separate sound-attenuating chambers (1.5 x 0.74 x 0.76 m; Med Associates, East Fairfield, VT) and contained two photo-optic switches (Model 117-1007; Stewart Ergonomics, Inc., Furlong, PA) with white stimulus lights above each, spaced equidistant from a central food receptacle. Illumination of a white light over the right or left photo-optic switch signaled the initiation of each trial. A response was registered by breaking the infrared beam within a circular recess of the switch. Responding was maintained by delivery of either 1-g banana-flavored sucrose pellets (drug-naive monkeys) via a pellet dispenser (Gerbrands Corp., Arlington MA), or an intravenous cocaine infusion (cocaine self-administration group) (~ 1.5 ml over 10 sec) using a peristaltic infusion pump (Cole-Parmer, Inc., Chicago, IL).

Cognition. Monkeys were trained on a delayed match-to-sample (DMS) task. At the start of each trial the house light was illuminated for 5 seconds followed by the appearance of a “target” stimulus in the center of the computer screen (sample phase). A response on
this stimulus was followed by a delay (see below) and the presentation of 2 or more shapes around the edges of the screen (match phase) that remained on the screen for a maximum of 10 seconds or until a response was registered. One stimulus was the previously shown image and the others were distracter images. Responding on the matching image during this phase resulted in delivery of 2, 190-mg sucrose pellets, whereas a response on any distracter image resulted in trial termination. The house light remained illuminated during each delay and remained lit throughout post-trial timeouts (5 sec) following correct trials but was extinguished following incorrect responses. If no response was emitted within 10 second during the sample or match phase, the house light was extinguished and the trial was terminated. Three delays were randomly distributed throughout a total of 60 trials per session (20 trials/delay). Delay values were individualized such that short, mid and long delays (0-150 sec) produced delay-dependent reductions in % accuracy (short delay, >80% accuracy; middle delay, 55-80%; long delay, 30-55%). As learning continued to occur with consistent repetition of the task, delays and the number of distracter images were periodically increased to retain delay-dependent decreases following these criteria on an individual basis.

Operant Behavior. Prior to cocaine self-administration sessions, the area surrounding the vascular access port was shaven and cleaned using betadine followed by a 95% ethanol scrub. The port was connected to an infusion pump (outside the chamber) via a 20-gauge Huber Point Needle (Access Technologies) and tubing. A 3-second infusion filled the internal catheter with cocaine hydrochloride. Cocaine (0.1 mg/kg/injection) was available under a fixed-ratio (FR) 30 schedule of reinforcement followed by a 30-sec timeout.
Previous studies with these monkeys found that this dose was at the peak of the descending limb of the cocaine dose-response curve (Blaylock et al., 2011). Self-administration sessions occurred 5 days/week, in the afternoon, and lasted for 120 minutes or until 15 total reinforcers were received, whichever occurred first. Cocaine-naive monkeys also completed operant behavioral sessions in the afternoon under identical parameters as the cocaine-experienced group, with the only difference being that responding was maintained by delivery of a 1-gram banana flavored pellets.

Experiment 1. Effects of cocaine self-administration on nAChR availability. PET studies occurred prior to testing nAChR agonists on working memory performance. T1-weighted magnetic resonance images (MRIs) were acquired for later co-registration with the PET data on all monkeys using a 3.0T MR scanner (GE Medical Systems) under ketamine-induction (10 mg/kg) and 1.5% isoflurane-maintained anesthesia. On the day of the PET study (~16 hours after the last self-administration session in the cocaine-experienced monkeys), monkeys were anesthetized with 10 mg/kg ketamine hydrochloride and transported to the WFUSM PET Center. Monkeys were intubated and anesthesia was maintained by inhaled isoflurane (1.5%) for the duration of the 90-minute scan. A 22-ga catheter was placed into the saphenous vein by percutaneous stick for intravenous administration of $[^{11}C]$-nicotine at the start of the scan, and for delivery of NaCl solution to the monkey throughout the study. A paralytic (0.07 mg/kg vecuronium bromide, i.v.) was administered and respiration was maintained by a respirator. $[^{11}C]$-nicotine was injected at the start of the scan, followed by a 3 ml flush of saline. Heart rate, blood pressure, and blood oxygen saturation were monitored throughout the scan.
and during recovery. At the end of the study, neostigmine (0.07 mg/kg) and glycopyrulate (20μg/kg) were administered to reverse the effects of the paralytic.

Data from PET scans were acquired using a General Electric 64-slice PET/CT Discovery VCT scanner (GE Medical Systems, Milwaukee, WI, United States) with ~5-6-mm resolution (see Teras et al., 2007). An initial low dose CTAC scan was acquired, followed by a 90 min 3D emission scan consisting of 36 sequential frames of the following dimensions (modified from Sihver et al., 1999): 10 x 6 sec (0-1 min), 5 x 1 min (1-6 min), 7 x 2 min (6-20 min), 14 x 5 min (20-90 min). Initiation of each PET scan coincided with intravenous injection of $[^{11}C]$-nicotine (average 9.3 and 9.4 mCi for the cocaine-naive and cocaine-experienced groups, respectively). The 3D data were corrected for attenuation and reconstructed transaxially using OSEM VUE point (28 subsets; 2 iterations) with a 3-mm FWHM filter resulting in a 128 x 128 matrix.

Experiment 2. Effects of nicotine, varenicline, and PNU-282987 on DMS performance.

Following 5 days of cocaine self-administration, PM operant sessions were suspended until completion of a nAChR drug’s dose-response curve. Acute doses of nicotine tartrate (0.0003-0.56 mg/kg, base), varenicline dihydrochloride (0.0003-0.3, salt) and PNU-282987 (0.001-0.56 mg/kg, salt) were administered intramuscularly prior to DMS sessions. Nicotine was administered 5 min prior to each test session. Based on preliminary assessment within our laboratory, PNU-282987 was administered 5 min prior to each test session and due to the slow $T_{\text{max}}$ (Obach et al., 2006) and previous studies (Gould et al., 2011) varenicline was administered 60 minutes prior to each test session. A minimum of four doses of each drug, separated by half-log units, were tested twice in
each monkey, in random order; if the percent change from the previous session varied by
greater than 50% between determinations, a third determination was conducted. Drugs
were tested in the following order: nicotine, varenicline, PNU-282987. A minimum of 2
days separated test sessions with nicotine or PNU-282987 and 3 days with varenicline
due to its ~24 hr half life in monkeys (Obach et al., 2006). A 5-day washout period
occurred between switching from one test drug to another during which monkeys with a
cocaine history self-administered 0.1 mg/kg/inj (max 15 injections) in afternoon sessions.

Data Analysis

Experiment 1: Effects of cocaine self-administration on nAChR availability. PET data
were coregistered to individual T1-weighted MRs using PMOD Biomedical Image
Quantification Software (version 3.1; PMOD Technologies, Zurich, Switzerland).
Spherical regions of interest (ROIs) were drawn directly on each individual MR image
and were subdivided into three groups. Areas associated with reward and the mesolimbic
dopamine system included bilateral caudate nucleus (2.5 mm radii), bilateral putamen
(2.5 mm radii), bilateral nucleus accumbens (2.0 mm radii), bilateral amygdala (2.5 mm
radii), and unilateral thalamus (3.0 mm radius). Areas associated with executive function
included bilateral anterior cingulate cortex (2.5 mm radii), bilateral dorsolateral prefrontal
cortex (PFC; 2.5 mm radii), bilateral orbital PFC (2.5 mm radii), bilateral hippocampus
(2.5 mm radii) and unilateral dorsomedial PFC (5.0 mm radius). Lastly, regions were
selected based on differences in glucose metabolism during an attentional set-shifting
task between these two groups of monkeys (Gould et al., under review) and included
unilateral midcingulate cortex (2.5 mm radius), unilateral posterior cingulate cortex (2.5
mm radius) and unilateral precuneus (4.0 mm radius), and regions known to be associated with nAChR distribution including bilateral insular cortex (2.5 mm radii) and bilateral cerebellum (4.0 mm radii).

Individual tissue time activity curves were calculated for each ROI. Values from minutes 30-50 post $[^{11}C]$-nicotine injection were averaged (e.g. Sihver et al., 1999) and expressed as uptake values for each ROI. The uptake value for each ROI was divided by the uptake value from the reference region, the centrum semiovalus, a large white matter tract, to generate a normalized ratio of uptake (Sihver et al., 1999). Recent studies have used white matter tracts as a measure for nondisplaceable binding when the receptor of interest is distributed throughout grey matter (e.g. Giovacchini et al., 2009), including the $\alpha_4\beta_2^*$ subtype-selective nAChR PET tracer 2-FA (Kendziorra et al., 2010). Further, a ratio analysis method has been shown to minimize the effects of blood flow with other PET tracers (Logan et al., 1994). For analysis, a two-way ANOVA was conducted using region and group as factors followed by post-hoc Bonferroni t-tests.

Experiment 2: Effects of nicotine, varenicline, and PNU-282987 on DMS performance.
The primary dependent variables were percent accuracy and response latencies. Percent accuracy was determined by dividing the number of correct responses by the total number of trials completed at each delay (short, mid, long). Trials were omitted if no response was made during the ‘sample’ or ‘match’ phase. The effects of all drug pretreatments were compared to the previous day’s session (baseline) in which greater than 75% of trials were completed and there was a delay-dependent reduction in percent accuracy such that percent accuracies were $>80\%$, between 55-80%, and $<55\%$ for
corresponding short, middle, and long delays. The percent change following a drug pretreatment was determined for each individual dose by dividing the percent accuracy at each delay by the previous day’s percent accuracy at each corresponding delay. From each individual dose-response curve, the dose of drug that engendered the greatest percent increase in accuracy at the longest delay was used for a ‘best-dose’ group analysis. Two-way repeated measure ANOVAs were conducted comparing percent accuracy between group (cocaine-naive versus cocaine-experienced) and drug treatment (‘best dose’ versus previous day’s session) at each delay value. In addition to assessing acute effects, separate ANOVAs examined sustained effects by comparing the day after drug treatment to baseline (nicotine and PNU-282987, 24 hrs; varenicline, 25 hrs post administration). A two-way repeated-measures ANOVA was also used to compare the maximal percent increase in accuracy from baseline using drug (saline, nicotine, varenicline, PNU-282987) and group (cocaine-naive, cocaine-experienced) at the longest delay. One-way repeated measure ANOVAs were conducted to examine effects of the ‘best dose’ of each drug on phase 1 (target), phase 2 (match) and pellet retrieval latencies compared to when saline was administered, using percent change in response rate from the previous day’s session. When appropriate, Bonferoni post-hoc testing was conducted; p<0.05 was considered significant.

Drugs. (-)Cocaine HCl (National Institute on Drug Abuse, Bethesda, MD), nicotine hydrogen tartrate (Sigma- Aldrich, St. Louis, MO), varenicline ditartrate and PNU-282987 (National Institute on Drug Abuse, RTI, Durham, NC) were dissolved in sterile 0.9% saline. NaOH was added to varenicline and nicotine to reach a pH range of 5-8.
The $^{11}$C labeled (S)-nicotine was prepared from the precursor (S)-nor-nicotine with slight modifications made to a previously published methods (Halldin et al 2004, Rose et al 2010). Briefly, a solution of (S)-nor-nicotine (0.25 - 0.5 mg) in acetonitrile was reacted with C-11 methyl triflate in the presence of 1,2,2,6,6-pentamethylpiperidine (10 µL of 5% solution in acetonitrile) at room temperature for 3 min. The desired product $[^{11}\text{C}]$-nicotine was separated from the crude reaction mixture using HPLC purification in 24 ± 11% radiochemical yields and >98% radiochemical purity. The specific activity of $[^{11}\text{C}]$-nicotine used in these experiment ranged from 839 mCi/µmole to 2621 mCi/µmole (1503 ± 778 mCi/µmole).

RESULTS

*Experiment 1. Effects of cocaine self-administration on nAChR availability.* PET imaging of $[^{11}\text{C}]$-nicotine demonstrated a rapid uptake in rhesus monkey brain with peaks in the time-activity curves within 6 min, and rapid washout that became linear around minute 30, demonstrating a similar pharmacokinetic profile as reported by Sihver et al. (1999). Similar to previous $[^{11}\text{C}]$-nicotine PET studies, highest uptake was seen in cortical and subcortical regions, with lesser binding in the cerebellum and lowest binding in white matter (e.g. Sihver et al., 1999). There were no significant differences between the ratio of uptake in the left or right side of any regions where bilateral ROIs were examined and were therefore averaged. There was a main effect of drug history ($F_{1,105}=7.75$, $p<0.01$) and region ($F_{14,105}=6.04$, $p<0.001$); post-hoc testing showed a significant difference in the hippocampus between the cocaine-experienced and cocaine-naive monkeys (Table 1, Fig.1; $p<0.05$).
Table 1. Individual and mean (±SEM) ratios of $[^{11}C]$-nicotine uptake for each region of analysis

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Cd, caudate nucleus; Pt, putamen; NAc, nucleus accumbens; Thal, thalamus; Am, amygdala; Hp, hippocampus; dmPFC, dorsomedial prefrontal cortex; dIPFC, dorsolateral PFC; orbPFC, orbital PFC; ACC, anterior cingulate cortex; mCC, mid-cingulate cortex; PCC, posterior cingulate cortex; Prcn, precuneus; Cb, cerebellum; * p<0.01.
Figure 1. Group mean ratio of [11C]nicotine uptake (±SEM) for cocaine naive (Coc-Naive, open bars) and monkeys with a cocaine SA history (Coc-Experienced, filled bars) for each region of interest. Cd, caudate nucleus; Pt, putamen; NAc, nucleus accumbens; Thal, thalamus; Am, amygdala; Hp, hippocampus; dmPFC, dorsomedial prefrontal cortex; dlPFC, dorsolateral PFC; orbPFC, orbital PFC; ACC, anterior cingulate cortex; mCC, midcingulate cortex; PCC, posterior cingulate cortex; Prcn, precuneus; Cb, cerebellum; *p<0.01.
Experiment 2. Effects of nicotine, varenicline, and PNU-282987 on DMS performance.

Individualized short-, mid- and long-delay values resulted in delay-dependent reductions in percent accuracy (see Table 2 for individualized delays). Baseline performance on days preceding test sessions were not different between groups. The average number of sessions to complete each dose-response curve and the number of days of abstinence from cocaine were 52.4 ± 4.8, and 63.9 ± 6.8 days for cocaine-experienced and cocaine-naive groups, respectively. Based on visual inspection, individualized delays and number of distracters necessary to produce delay-dependent reductions were not different between groups at the end of the study suggesting improvements as a function of repetition were not different over time (Table 2). At the longest delay, pretreatment with nicotine, varenicline, and PNU-282987 produced inverted U-shaped curves in percent change from baseline in all monkeys (see Fig. 2 for representative curves). Based on visual inspection of the data, DMS performance at the longest delays was most sensitive to drug-induced increases in performance (Fig. 2).

Table 2. Individual baseline parameters that produced similar delay-dependent effects on DMS performance; dist., distracter images.

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</table>
Figure 2. Representative dose-response curves following acute nicotine (5 min PT; left), varenicline (60 min PT; middle) and PNU-282987 (5 min PT; right panels) expressed as a percent of the previous day’s baseline in one cocaine-naive monkey (R-1681, top) and one monkey with a cocaine SA history (R-1377, bottom); NR, no responding, greater than 15% of trials were omitted.
Best-Dose Analysis. Individual differences in sensitivity to the cognitive enhancing effects of nAChR agonists precluded group analysis across the full dose-response curves, so data were arranged based on the dose that produced maximal effects in each monkey. There was a main effect of nicotine (F1,5=49.26, p<0.001), varenicline (F1,5=57.61, p<0.001), and PNU-282987 (F1,5=91.79, p<0.001) on percent accuracy following acute administration, but no effect of drug history at the longest delay. Post-hoc tests showed that at the longest delay, percent accuracy was significantly different from baseline following nicotine (t=4.92, p<0.005; t=5.03, p<0.005), varenicline (t=6.26, p<0.05; t=4.62; p<0.01) and PNU-282987 (t=6.40, p<0.005, t=7.14, p<0.001) for cocaine-naive and cocaine-experienced groups, respectively (Fig. 3). There were no significant effects of any treatment on performance during the short- or mid-delay values. There was a main effect of varenicline treatment (F1,5=11.46, p<0.05) on percent accuracy 25 hrs after drug administration, only at the longest delay such that percent accuracy was still improved by 23.4 ± 9.4 and 19.4 ± 3.0 (mean ± SEM, cocaine-naive and cocaine-experienced, respectively), but only the cocaine-naive group was significantly different from baseline (t=2.92; p<0.05) and this effect was not apparent 24 hrs later.
Figure 3. Delay-effect curves for cocaine naive (Coc-Naive, triangles) and monkeys with a cocaine SA history (Coc-Exp, squares) under baseline conditions (open symbols) and ‘best-dose’ analysis (filled symbols) for nicotine (top), varenicline (middle) and PNU-282987 (bottom). Curves are offset slightly for clarity; *p<0.05 compared to baseline at the respective delay.
When comparing maximal increases in percent accuracy, there was a main effect of drug treatment but not group \((F_{3,15}=26.46, p<0.001)\) such that nicotine \((t=4.94, p<0.001; t=7.35, p<0.001)\), varenicline \((t=4.04, p<0.01; t=3.42, p<0.05)\) and PNU-282987 \((t=3.26, p<0.05; t=4.54, p<0.005)\) each improved percent accuracy to a greater extent that saline in cocaine-naive and cocaine-experienced monkeys, respectively (Fig. 4; Table 3). In the cocaine-experienced group only, there was a significant difference in the percent maximal increase in accuracy between nicotine and varenicline administration \((t=3.92, p<0.01)\). At the highest doses tested (see Fig. 2 for examples) each drug disrupted responding such that less than 75% of trials were completed. However, no significant effects on response or pellet retrieval latencies resulted from administration of the dose the engendered the greatest pro-cognitive effects. Saline did not have a significant effect on percent accuracy in any monkey.

Table 3. Maximal effect of nicotine (Nic) varenicline (Var), or PNU-282987 (PNU) administration on DMS performance (and dose), expressed as a percent of the previous session’s baseline performance.

<table>
<thead>
<tr>
<th>Cum Coc Intake (mg/kg)</th>
<th>Maximal effect, % of baseline (dose, mg/kg)</th>
<th>Nic</th>
<th>Var</th>
<th>PNU</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cocaine-Naive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1681</td>
<td>0</td>
<td>126 (0.0003)</td>
<td>140 (0.01)</td>
<td>131 (0.01)</td>
</tr>
<tr>
<td>1682</td>
<td>0</td>
<td>141 (0.001)</td>
<td>135 (0.003)</td>
<td>137 (0.1)</td>
</tr>
<tr>
<td>1696</td>
<td>0</td>
<td>137 (0.1)</td>
<td>137 (0.001)</td>
<td>126 (0.03)</td>
</tr>
<tr>
<td>1756</td>
<td>0</td>
<td>179 (0.03)</td>
<td>143 (0.001)</td>
<td>136 (0.003)</td>
</tr>
<tr>
<td>AVE</td>
<td>16.2</td>
<td>145.8</td>
<td>138.6</td>
<td>132.4</td>
</tr>
<tr>
<td><strong>Cocaine-Experienced</strong></td>
<td></td>
<td>(SEM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1374</td>
<td>2108</td>
<td>201 (0.1)</td>
<td>153 (0.03)</td>
<td>155 (0.003)</td>
</tr>
<tr>
<td>1375</td>
<td>1077</td>
<td>150 (0.1)</td>
<td>121 (0.001)</td>
<td>123 (0.3)</td>
</tr>
<tr>
<td>1377</td>
<td>1750</td>
<td>152 (0.1)</td>
<td>120 (0.03)</td>
<td>147 (0.1)</td>
</tr>
<tr>
<td>AVE</td>
<td>20.6</td>
<td>167.6</td>
<td>131.3</td>
<td>141.6</td>
</tr>
<tr>
<td>(SEM)</td>
<td></td>
<td>(SEM)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 4. Group means (±SEM) for the ‘best-dose’ of nicotine (Nic), varenicline (Var), PNU-282987 (PNU) and saline (Sal) at the longest delay only in cocaine-naive (white bars) and monkeys with a cocaine SA history (black bars), expressed as a percent of the previous session’s baseline (BL); *p<0.05, † p<0.01; ‡ p<0.005 significantly different from respective saline administration; § p<0.05 significant difference between Nic and Var of cocaine SA history monkeys.
DISCUSSION

The present study examined nicotinic acetylcholine receptor availability and the influence of nAChR activation on cognition in rhesus monkeys with a chronic cocaine self-administration history compared to cocaine-naive monkeys. Using PET imaging, monkeys with a cocaine self-administration history showed higher $[^{11}\text{C}]-\text{nicotine}$ uptake in the hippocampus compared to cocaine-naive monkeys. When administered acutely, the nonselective nAChR agonist nicotine, the low-efficacy $\alpha_4\beta_2^*$ subtype-selective nAChR agonist varenicline, and the high-efficacy, potent $\alpha_7$-selective nAChR agonist PNU-282987 significantly improved working memory performance in both groups of monkeys. These data extend previous studies in rodents, monkeys and humans showing that nAChR agonists enhance cognitive performance in healthy cohorts (e.g. Rezvani and Levin, 2001; Hahn et al., 2003; Katner et al., 2004; Castner et al., 2011) and demonstrate similar cognitive-enhancing effects in a monkey model of cocaine abuse, supporting nAChR modulation as a mechanism to pursue cognitive enhancement in treatment-seeking cocaine users.

To the best of our knowledge, this is the first study to examine the effects of cocaine self-administration on nAChR availability \textit{in vivo}. Across the 15 brain regions assessed, the only region where nAChR availability was different between cocaine-experienced and cocaine-naive monkeys was in the hippocampus. Similar to nAChR upregulation following nicotine exposure in animals and higher binding in chronic smokers, overall receptor availability was higher across most brain regions in the cocaine-experienced group. Post-mortem tissue from long-term smokers showed higher nAChR binding sites in the thalamus and hippocampus using $[^3\text{H}]-\text{nicotine}$ compared to
controls (Breese et al., 1997), suggesting similarities between chronic nicotine and chronic cocaine exposure. In rats, chronic nicotine exposure resulted in greater α4β2* subtype-selective nAChR binding measured via autoradiography in cortical and subcortical regions (Mugnaini et al., 2002; Nguyen et al., 2003; Metaxas et al., 2010), whereas higher α7-subunit binding was highly restricted to the ACC and VTA (Mugnaini et al., 2002). Using the selective nAChR PET tracer 2[18F]F-A-85380 (2FA), Muhkin and colleagues (2008) reported higher α4β2*-subunit binding in frontal, striatal and midbrain sites in smokers compared to non-smokers. Using [11C]-nicotine in the present study, we cannot speculate as to whether differences in receptor availability between groups were specific to α4β2*- or α7- nAChRs. Given the strong interactions between DA and ACh neurotransmitter systems (e.g. reviews Lester et al., 2010; Maskos et al., 2010) and similar effects of cocaine and nicotine on mesolimbic reward systems it is not unexpected to see similar alterations in the nAChR system. For example, chronic smoking is associated with lower DA D2-like receptor availability as measured by PET (Fehr et al., 2008), similar to chronic cocaine use and other drugs that elevate DA (see Volkow et al., 2004 for review).

Chronic cocaine use is associated with hypodopaminergic function (see Volkow et al., 1999, 2004 for reviews). Nicotinic AChR agonists can stimulate DA release in both striatal and cortical brain regions (e.g. Chan et al., 2007; Livingstone et al., 2009; Rollema et al., 2009) and have shown cognitive enhancing effects in healthy cohorts across species (e.g. Rezvani and Levin, 2001; Hahn et al., 2003; Katner et al., 2004; Castner et al., 2011) as well as animal models of Parkinson’s Disease (e.g. Decamp and Schneider, 2006), a neuropathology associated with hypodopaminergic activity. Thus, we
hypothesized that nAChR activation would stimulate a hypodopaminergic system in the cocaine-experienced monkeys to produce greater cognitive improvements compared to control monkeys. Acute nicotine administration improved working memory performance at the longest delay values, similar to other studies in drug-naive rhesus monkeys (Hironaka et al., 1992; Katner et al., 2004). However, contrary to our hypothesis, nicotine was equally effective in cocaine-experienced monkeys and cocaine-naive monkeys. Despite similar efficacy, there were differences in the potency of nicotine to improve working memory between groups. The doses of nicotine that engendered the maximal increase in accuracy in 3 of 4 cocaine-naive monkeys were lower than the doses that produced maximal cognitive-enhancing effects in the cocaine-experienced monkeys and the range extended 2.5 log-units (Table 3). nAChR availability was higher and nicotine was a less potent cognitive enhancer in the cocaine-experienced monkeys, suggesting differences in ACh neurotransmitter function, or other neurotransmitter systems that are implicated in working memory and indirectly stimulated by ACh activity (e.g. DA). Marks and colleagues (1993) showed that chronic nicotine exposure resulted in higher nAChR binding, yet lower nAChR function. Alternately, DA agonist administration can improve working memory in healthy and DA-depleted individuals (see Cools and D’Esposito for review). However, the relationship between DA function and cognition has been described as an inverted U-shaped curve such that doses necessary to enhance memory in a hypofunctional DA state may impair cognition in healthy individuals (Cools and D’Esposito, 2011). In monkeys with a cocaine self-administration history, greater stimulation of the DA system may be necessary to reach peak cognitive performance.
Further assessment of DA and ACh function in monkeys with a chronic cocaine self-administration is warranted.

This is also the first cognitive assessment of varenicline and PNU-282987 conducted in monkeys. Both drugs produced similar enhancements in working memory performance at the long delays in cocaine-naive and cocaine-experienced monkeys, although increases in percent accuracy were less than those produced by nicotine. In rodents, varenicline improved attention assessed via a sustained attention task, and declarative memory assessed via a novel object recognition test (Rollema et al., 2009). In chronic smokers, varenicline increased working memory performance during acute abstinence (3 days; Patterson et al., 2009; Loughead et al., 2010). PNU-282987 improved working and recognition memory in rodents (Chan et al., 2007; Redrobe et al., 2009; Vicens et al., 2011), similar to the effects of other α7-selective nAChR agonists (see Thomsen et al., 2010).

The current study modeled a critical period of abstinence, the first several weeks when behavioral modification treatment is initiated and when deficits in working memory have been reported (Hoff et al., 1996; Tomasi et al., 2007b; Hanlon et al., 2011). Abstinence from cocaine was imposed for ~55 days while each drug was being tested. Neurobiological deficits including reduced metabolic activity and reduced D2-like receptor availability have been shown to persist for at least three months into abstinence (Strickland et al., 1993; Volkow et al., 1993; Nader et al., 2006). Although the direct effects of 5 days of cocaine self-administration following extended abstinence are not well characterized, in previously cocaine-naive monkeys this duration of self-administration is associated with changes in glucose metabolism (Porrino et al., 2004).
and models a lapse back to drug use following extended periods of abstinence. Although future imaging studies will be needed to confirm this, it is unlikely that the order of testing and the exposure to repeated abstinence periods confounded our results.

Acute nicotine, varenicline and PNU-282987 enhanced working memory to a similar degree in both groups of monkeys. However, studies in monkeys examining chronic dosing regimens may produce divergent effects on cognition. Although tolerance developed to unconditioned behavioral and physiological effects of nicotine following repeated dosing (Marks et al., 1993), tolerance to the pro-cognitive effects of nicotine have not been observed (e.g. see Buccafusco et al., 2005 for review). In fact, repeated dosing of nicotine and some, (e.g. Castner et al., 2011) but not all (e.g. current study; Gatto et al., 2004; Buccafusco et al., 2007) nAChR agonists showed pro-cognitive effects that extended beyond their pharmacokinetic profile (see Buccafusco et al., 2005; Thomsen et al., 2010 for reviews). In the present study, the pro-cognitive effects of varenicline but not nicotine or PNU-282987 extended 25 hrs post administration. Further studies are necessary to determine if this effect was related to pharmacokinetics or intracellular adaptations. However, in rats tolerance developed to the cognitive enhancing effects of varenicline following 6 days of dosing (King et al., 2011) but not following 15 days of PNU-282987 administration (McLean et al., 2011). Further, Maloku and colleagues (2011) showed that 5-day varenicline or PNU-282987 treatment differentially altered protein levels in mice, supporting distinct epigenetic modulation between α4β2*-selective agonists and α7-selective nAChR agonists (see Buccafusco et al., 2005; Thomsen et al., 2010). Such differential effects may be attributable to receptor affinity, efficacy, or intracellular mechanisms that may not fully occur within an acute treatment
regimen. The current results warrant further investigation into the cognitive enhancing effects of subtype-selective nAChR agonists and their potential to improve executive function in treatment-seeking cocaine users.

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CHAPTER V

EFFECTS OF VARENICLINE ON THE REINFORCING AND DISCRIMINATIVE STIMULUS EFFECTS OF COCAINE IN RHESUS MONKEYS

Robert W. Gould, Paul W. Czoty, Susan H. Nader, Michael A. Nader

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ABSTRACT

Varenicline is a low-efficacy α4β2* subtype-selective nicotinic acetylcholine receptor (nAChR) agonist that has shown success in smoking cessation and promise in preclinical assessments relating to other drugs of abuse. The primary goal of the present study was to examine the effects of varenicline on cocaine self-administration and cocaine discrimination and to compare these effects to those of the nAChR agonist nicotine and antagonist mecamylamine. One limitation of agonist treatments is the potential for abuse. Thus, a second goal was to examine the abuse potential of varenicline in rhesus monkeys. In the first experiment, rhesus monkeys (n=3) were trained to self-administer cocaine (saline, 0.01-0.56 mg/kg) under a progressive-ratio schedule of reinforcement; monkeys also earned all their food by responding on another lever under a fixed-ratio 50 schedule of reinforcement. Chronic administration of varenicline (0.01-0.56 mg/kg, p.o., salt) potentiated the reinforcing effects of cocaine, while mecamylamine (0.3-1.7 mg/kg, p.o., i.m., i.v., salt) had no significant effects on cocaine self-administration up to doses that disrupted food-maintained responding. Neither varenicline (0.01-0.17 mg/kg, salt) nor nicotine (0.01-0.1 mg/kg, base) functioned as reinforcers when substituted for cocaine. Finally, in monkeys trained to discriminate self-administered 0.3 mg/kg cocaine, varenicline (0.1-0.3 mg/kg, i.v.) did not substitute for cocaine but, along with mecamylamine (0.3-1.7 mg/kg, i.v.) and nicotine (0.03-0.1 mg/kg, i.v.), potentiated the discriminative stimulus effects of cocaine. These results suggest that varenicline has low abuse liability in monkey models of cocaine abuse, but would not be an effective medication for cocaine addiction.
INTRODUCTION

The effects of cocaine on dopamine (DA) neurotransmission continue to be a primary target for pharmacotherapy development (e.g., Grabowski et al., 2004). More recently, researchers have examined neurotransmitter systems that indirectly modulate DA activity (e.g. Karila et al., 2008). For example, interactions between the acetylcholine (ACh) and DA neurotransmitter systems have implications for drug development to treat addiction (Lester et al., 2010). Consistent with the observation that nicotinic ACh receptor (nAChR) agonists can increase DA release (Rollema et al., 2007, 2010), nicotine, a high-efficacy agonist that binds non-selectively to all nAChR subtypes, fully substituted for cocaine in monkeys and rodents (de La Garza and Johanson, 1983; Desai et al., 2003) and d-methamphetamine in rodents (Desai and Bergman, 2010), in discrimination studies. Moreover, nicotine and cocaine combinations resulted in greater self-administration than cocaine alone in monkeys (Freeman and Woolverton, 2009). Conversely, mecamylamine, a noncompetitive antagonist at all nAChR subtypes, selectively attenuated cocaine self-administration in rodents (Levin et al., 2000). In cocaine-dependent, non-treatment-seeking individuals, acute nicotine enhanced and mecamylamine diminished cue-induced craving for cocaine (Reid et al., 1998,1999). These studies support nAChR blockade as a mechanism to reduce cocaine-related behaviors.

However, one clinical trial examined a single low dose of mecamylamine in treatment-seeking cocaine-dependent patients compared to a placebo-control group, and reported no differences in cocaine intake, rates of abstinence or self-reports of cocaine craving, and severity of dependence was higher in the mecamylamine-treated group (Reid et al., 2005). Increased withdrawal symptoms or negative side effects are common
drawbacks associated with an antagonist treatment approach and often result in poor compliance (see Grabowski et al., 2004). Therefore, a drug with both agonist and antagonist effects, such as a low-efficacy agonist, may be more successful in reducing craving and drug use than an antagonist alone and possess lower abuse liability than higher efficacy agonists (for review see Rahman, 2011). In support of this view, mecamylamine-nicotine combinations and low-efficacy nAChR agonists have been shown to increase rates of abstinence and reduce nicotine craving compared to placebo-controlled groups in smoking cessation trials (Rose et al., 1994; Gonzales et al., 2006).

Varenicline (Chantix©), an FDA-approved medication for smoking cessation, has high affinity (>4000 and 5000-fold compared to α3β4* or α7*) and potency (>24 and 8-fold compared to α3β4* or α7*) at α4β2* nAChRs (* denotes the presence of additional accessory subunits; Coe et al., 2005; Mihalak et al., 2006). Consistent with a partial agonist profile, in vitro studies showed that varenicline had low efficacy (~15%) at α4β2* but high efficacy (~93% and 75%) at α3β4* or α7* receptors compared to the full agonist ACh (Mihalak et al., 2006). In vivo, varenicline elevated DA levels in the nucleus accumbens but to a lesser degree (~35%) than nicotine and attenuated nicotine-induced DA release and nicotine self-administration in rodents (Rollema et al., 2007). In clinical trials, varenicline reduced nicotine craving and rates of relapse to cigarette smoking (Gonzales et al., 2006). Varenicline treatment decreased ethanol intake in rodents (Steensland et al., 2007) and alcohol consumption in moderate smokers (McKee et al., 2009). Additionally, low doses of varenicline attenuated cue- and cocaine-induced reinstatement of cocaine seeking in rodents (Guillem and Peoples, 2010), although in one
published trial, varenicline did not affect cocaine use in treatment-seeking individuals (Poling et al., 2010).

In measures of abuse liability, varenicline has been shown to substitute for nicotine in drug discrimination and functioned as a reinforcer when substituted for nicotine on a progressive-ratio schedule in rats (Rollema et al., 2007). In a reinstatement model in rats previously trained to self-administer cocaine, a high dose of varenicline increased cue-induced reinstatement (Guillem and Peoples, 2010). However, Desai and Bergman (2010) recently found that while nicotine completely substituted for the methamphetamine stimulus, varenicline did not substitute and did not attenuate the discriminative stimulus effects of the training dose of methamphetamine. One goal of the current study was to extend the assessment of abuse liability of varenicline to monkey models of cocaine abuse, using intravenous cocaine self-administration and cocaine discrimination. A second goal was to examine the efficacy of varenicline on cocaine self-administration and cocaine discrimination. For the self-administration studies, a dosing regimen was employed in which treatment occurred every day, but access to cocaine was only studied weekly, in order to better model the human treatment conditions (Czoty et al., 2011). For drug discrimination studies, monkeys self-administered the drug or vehicle, rather than investigator administered (Martelle and Nader, 2009). In most studies, we also compared the effects of varenicline with those of the high-efficacy agonist nicotine and the nonselective antagonist mecamylamine.
MATERIALS AND METHODS

Subjects. Eight adult male rhesus monkeys (Macaca mulatta) served as subjects. All subjects had a history of cocaine self-administration and/or drug discrimination including experimental treatment with various dopaminergic drugs. All were previously surgically implanted with chronic indwelling femoral or jugular catheters that were connected to vascular access ports (Access Technologies, Skokie, IL, USA); surgical procedures have previously been described (Czoty et al., 2006). Five monkeys (R-1268, R-1425, R-1427, R-1429 and R-1526) were involved in drug self-administration studies; all but R-1526 also responded on a lever and earned their daily food in the form of 1-g food pellets (Bio-Serv, Frenchtown, NJ, USA). Three monkeys (R-1524, R-1525, and R-1430) were used in cocaine discrimination studies and, along with R-1526, were fed LabDiet Monkey chow in addition to food pellets. The total daily ration of pellets was calculated to maintain target weights, determined monthly, at approximately 95% of free-feeding weights (range 110-185 pellets). When monkeys earned fewer than the requisite number of food pellets, supplementary food (LabDiet Monkey Chow) was given at 7:30 a.m. to raise the total food intake to the necessary level. Monkeys received fruits or vegetables at least three times per week. Water was available ad libitum. Experimental and enrichment protocols were approved by the Wake Forest University Animal Care and Use Committee. All studies were carried out in accordance with the 2003 National Research Council Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research as adopted by the National Institutes of Health.

Apparatus. Monkeys were individually housed in sound-attenuating, ventilated cubicles (91 cm x 91 cm x 91 cm, Plas Labs, Lansing, MI, USA) which also served as their

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experimental chamber. The front of each panel was made of clear Plexiglas to allow visual access to other monkeys in the room. Each monkey was fitted with a stainless steel harness (Restorations Unlimited, Chicago, IL, USA). A metal spring arm (Restorations Unlimited) connected the harness to the back wall of the cubicle. A catheter was connected to an infusion pump outside the cage, threaded through the spring arm and connected to a vascular access port via a 22-gauge Huber Point Needle (Model PG20-125, Access Technologies). To maintain catheter patency, the catheter was filled with heparinized saline (100 U/ml) between sessions. Two metal levers (PRL-001, BRS/LVE, Beltsville, MD, USA) were mounted 23 cm apart on the Plexiglas door with four stimulus lights, alternating white and red, above each lever. In addition, cubicles of monkeys R-1430, R-1524 and R-1525 (cocaine discrimination study) had a photo-optic switch (Stewart Ergonomics, Furlong, PA, USA) mounted centrally on the door, ~30 cm above and equidistant between the two response levers. A yellow stimulus light was mounted above the switch. A food-pellet dispenser (G5210, Model A, Gerbrands, Arlington, MA, USA) was mounted on the top of the door and connected by tygon tubing to a food receptacle located between the two levers. An infusion pump (Cole-Parmer, Chicago, IL, USA) was located on the top of the chamber to deliver drug solutions intravenously over 10 sec at a rate of approximately 1.5 ml/10 sec. Self-administration doses were studied by changing the drug concentration delivered intravenously.

Cocaine Self-Administration

Procedure. For five monkeys, responding on the left lever was maintained by food pellet presentation under a fixed-ratio (FR) 50 schedule and on the right lever by injections of
cocaine under a progressive-ratio (PR) schedule (Czoty et al., 2011). Food availability began each morning at 8:00 a.m. and lasted 23 hr or until all pellets were received which under baseline conditions typically occurred within 2 hrs. Cocaine availability under the PR schedule began at 3:00 p.m. each afternoon and lasted 16 hrs or until 2 hrs expired during which no injection was received. Thus, after 3:00 p.m. food and cocaine were concurrently available if all food had not been obtained. The response requirement for each drug injection followed the equation established by Richardson and Roberts (1996): ratio = [5e^((injection # x 0.2)) – 5. The first response requirement corresponded with the 12\textsuperscript{th} value from the equation (50 responses) and was followed by 62, 77, 95, 117, etc.

Availability of each reinforcer was signified by illumination of 2 white lights above the respective lever. Upon completion of a ratio the white lights were extinguished and 2 red lights above the lever were illuminated for 2 seconds (pellet delivery) or 10 seconds (drug infusion). A timeout period followed, during which all lights above that lever were extinguished and responding on that lever had no consequences but was recorded; the timeout period lasted 10 seconds after pellet delivery or 10 minutes after drug delivery.

Initially, to determine baseline dose-effect curves, saline and each dose of cocaine (0.003-0.56 mg/kg) were made available for a minimum of five days and until the number of injections received varied less than 20\% from the mean of 3 consecutive days with no apparent trend. For the drug substitution and the subsequent studies involving chronic varenicline or mecamylamine treatment, a cocaine maintenance dose was chosen for each monkey (R-1268, R-1425, 0.1 mg/kg/inj; R-1427, 0.017 mg/kg/inj; R-1526, 0.03 mg/kg/inj cocaine) from the ascending limb of the dose-response curve.
Experiment 1: Assessment of the reinforcing effects of varenicline and nicotine. In three monkeys (R-1268, R1425 and R-1427) doses of varenicline dihydrochloride (0.01-0.17 mg/kg/injection, salt) and nicotine bitartrate (0.01-0.1 mg/kg/injection, free base) were substituted for the maintenance dose of cocaine for a minimum of 5 consecutive sessions and until responding was deemed stable according to the aforementioned criteria. Varenicline was tested first in all monkeys and all doses were tested in random order.

Experiment 2: Effects of chronic varenicline and mecamylamine treatment on cocaine- and food-maintained responding. Following redetermination of a cocaine dose-response curve, cocaine self-administration sessions were discontinued; food-maintained responding continued to be studied daily. Initially, a low dose of varenicline (0.03 mg/kg, salt, p.o.) was administered once per day approximately 30 minutes before food availability began (i.e., 7:30 a.m.). Seven days later, the maintenance dose of cocaine was made available for one PR self-administration session in the evening. If the number of cocaine injections from this session deviated (± 3 injections) from the baseline, treatment was maintained at this dose and a randomly selected cocaine dose (0.01-0.56 mg/kg/injection) was tested every third day until the reinforcing strength of each cocaine dose was determined twice in the presence of varenicline. If no effect of treatment was noted after the first test session, the varenicline dose was increased and a PR self-administration session was conducted seven days later. The first increase in varenicline dose was from once- to twice-daily oral treatment with 0.03 mg/kg varenicline (7:30 a.m. and 5:00 p.m.). Further increases in varenicline dose were accomplished by maintaining twice-daily administration and increasing the dose in quarter- or half-log increments up to
0.56 mg/kg (b.i.d.). All oral dosing was achieved by injecting a small volume of drug into palatable food items (piece of banana, peanut butter, etc). In monkeys, the half-life of varenicline is ~24 hour (Obach et al., 2006). Therefore, steady-state plasma levels were attained prior to initial cocaine self-administration sessions. After the cocaine dose-effect curve had been re-determined during 0.56 mg/kg varenicline treatment, chronic varenicline treatment was discontinued. Two weeks later, post-treatment cocaine dose-response curves were determined under a similar regimen such that a random dose of cocaine (saline, 0.003-0.56 mg/kg) was made available every third day under the PR schedule.

Following redetermination of the cocaine dose-effect curve, the effects of chronic mecamylamine HCl treatment were examined in a manner similar to varenicline. Treatment was initiated with 0.3 mg/kg (p.o.) at 7:30 a.m. and 4:30 p.m. and a cocaine self-administration session was conducted on Day 7 of treatment. If a lack of effect was observed, the mecamylamine dose was raised in quarter- or half-log increments up to 1.7 mg/kg (p.o.), b.i.d. If this dose was found to be ineffective in altering cocaine self-administration, the route of administration was changed first to intramuscular injection, then to intravenous administration, in an effort to maximize potential behavioral effects without drawing on dose ranges outside of a therapeutic window. For example, 1.7 mg/kg mecamylamine has been shown to produce behavioral effects when administered i.m. (e.g. Katner et al., 2004). When administered intravenously, mecamylamine was flushed directly through the intravenous access catheter with 3 ml of saline; afternoon doses were infused at 2:30 p.m. so as not to disrupt self-administration probe sessions.
Cocaine Discrimination

Procedure. R-1524, R-1525, and R-1430 had previously been trained to discriminate response-contingent intravenous cocaine from saline (Martelle and Nader, 2009). For the present studies, each monkey was trained to discriminate 0.3 mg/kg cocaine from saline. Briefly, prior to each session the catheter was filled with saline or 0.3 mg/kg cocaine. Each session began with the illumination of a yellow light over the centrally-located switch. Completion of an FR 50 (R-1430 and R-1524) or FR 100 (R-1525) resulted in extinction of the yellow light and simultaneous illumination of the red lights above both levers for 10 sec, during which the stimulus to be discriminated (cocaine or saline) was infused over 10 sec. This infusion was followed by a 15-min timeout during which all lights were extinguished and responding across all manipulanda had no consequence. Following the timeout, the discrimination component began, signaled by illumination of the white stimulus lights above each lever. Completion of the FR on the injection-appropriate lever resulted in delivery of a 1-g pellet; responding on the opposite lever was not reinforced and reset the response requirement on the correct lever. Training occurred under a CCSSCSSCCS (C, cocaine; S, saline) schedule 5-7 days per week. Cocaine- and saline-associated levers were counterbalanced across monkeys. Once each monkey reliably acquired the discrimination (5 consecutive days on which the 1st fixed-ratio was completed on the correct lever and > 80% of total responses were emitted on the drug-appropriate lever), test (T) sessions were implemented following a CSTSCT schedule. Test sessions were only conducted if criteria were met for responding in the previous C and S training sessions. Test sessions were identical to training sessions except that completion of an FR on either lever resulted in pellet delivery. Sessions lasted 45 min or
until all 10 pellets had been received, whichever came first. Thirty minutes following the completion of the discrimination component (training or test session), a 30-second infusion of heparinized saline (100 U/ml) was delivered via a second infusion pump to maintain catheter patency and expel any residual drug in the catheter.

**Experiment 3: Effects of nAChR compounds on cocaine discrimination.** Varenicline (0.1 and 0.3 mg/kg, salt) and mecamylamine (0.3-1.7 mg/kg, salt) were administered non-contingently 1 hr before monkeys self-administered the dose of cocaine or saline to be discriminated; nicotine (0.03 and 0.1 mg/kg, base) was administered 15 minutes before the start of the session. Haloperidol (0.003-0.03 mg/kg) was tested in combination with the training dose of cocaine and was administered intravenously 5 minutes before the start of the session. Order of testing for all monkeys was as follows: varenicline, nicotine, mecamylamine, haloperidol. Before testing another drug and following completion of all studies, the discriminative stimulus effects of selected doses of cocaine were re-evaluated to confirm that the shape of the cocaine dose-response curve was not altered.

**Data Analysis**

For the self-administration studies, the primary dependent variable was the number of injections received. For self-administration dose-response curves (Experiment 1), data were analyzed using a one-way analysis of variance (ANOVA) using 3-day means for each substituted dose. *Post-hoc* Holm-Sidak t-tests compared the number of injections received for each dose to the number of injections of saline received. Drug doses were considered reinforcing if the 3-day mean was significantly greater than the average
number of injections received when saline was available, with significance accepted when p<0.05.

For Experiment 2, individual differences in dose sensitivity of the treatments precluded group statistics. To examine the effects of chronic treatment on cocaine self-administration, two-way analyses of variance (ANOVA)s were conducted for each individual monkey using treatment dose (varenicline or mecamylamine) and cocaine dose as factors. Additional one-way ANOVA[s] were conducted for each monkey examining the percentage of total food pellets earned in the first two hours of availability. For this analysis, sessions were divided into weekly bins of 7 sessions each (corresponding with the minimum 7-day treatment per dose). *Post-hoc* Holm-Sidak tests were used to compare specific doses between treatment conditions and to compare average weekly percentage of food pellets earned to baseline levels when a main effect was observed. For mecamylamine treatment, because doses delivered via various routes produced similar reductions in food-maintained responding without altering the reinforcing strength of cocaine, data were pooled across routes of administration and a two-way ANOVA was conducted using mecamylamine dose and cocaine dose as factors. Since treatment durations were shorter than chronic varenicline treatments, food-maintained responding could not be separated into weekly bins for statistical comparison across weeks of treatment.

For cocaine discrimination data (Experiment 3), the primary dependent variables were % cocaine-appropriate responding and response rates (responses per second). The discriminative stimulus effects of cocaine (in the absence of drug pretreatment) did not change throughout the duration of the study, so mean cocaine dose-response curve data
were used for all treatment drugs. Doses of drugs or drug combinations with the training dose that resulted in <50% cocaine-appropriate responding were considered significantly different from the training dose effects. Doses of drugs or drug combinations with any dose of cocaine that resulted in 50-80% cocaine-appropriate responding were considered to partially substitute for cocaine. Doses of drugs or drug combinations that resulted in >80% cocaine-appropriate responding were considered to fully substitute for the cocaine discriminative stimulus. In addition, ED<sub>50</sub> values (interpolated doses that resulted in 50% cocaine-appropriate lever responding) were generated for R-1430 and R-1524 for each treatment and dose. ED<sub>50</sub> values could not be calculated for monkey R-1525 because pretreatment with nicotine, varenicline, and mecamylamine all engendered >50% cocaine-appropriate responding when administered prior to the lowest dose of cocaine tested.

**Drugs**

(-)Cocaine HCl (National Institute on Drug Abuse, Bethesda, MD) was dissolved in sterile 0.9% saline. Varenicline (National Institute on Drug Abuse, RTI, Durham, NC), nicotine bitartrate (Sigma- Aldrich, St. Louis, MO) and mecamylamine HCl (US Pharmacopeia, Rockville, MD) were dissolved in sterile saline. Sodium hydroxide was added to varenicline and nicotine to reach a stable pH range of 5-8. For drug self-administration studies, different doses were studied by changing the concentration of drug available. Haloperidol (McNeil Pharmaceuticals, Raritan, NJ) was dissolved in sterile saline immediately before testing.
RESULTS

Self-Administration

Experiment 1: Assessment of the reinforcing effects of varenicline and nicotine.

When substituted for the training dose, cocaine dose-dependently increased the number of injections self-administered under a PR schedule (F5,9=10.55, p<0.005) with all cocaine doses except 0.01 mg/kg resulting in significantly higher numbers of injections compared to when saline was available (all p<0.05; Fig.1). When substituted for the baseline dose of cocaine, no dose of varenicline or nicotine resulted in significantly higher number of injections compared to when saline was available (Fig. 1).

Figure 1. Mean (±SEM) number of injections of self-administered cocaine (Coc), varenicline (Var) or nicotine (Nic). Data are means of the last three sessions of availability in three monkeys (except cocaine 0.03 mg/kg; n=2). * significantly different from saline, p<0.05.
Experiment 2: Effects of chronic varenicline and mecamylamine treatment on cocaine- and food-maintained responding. Effects of varenicline on cocaine self-administration. For R-1425, R-1429 and R-1526, number of self-administered cocaine injections varied significantly as a function of dose (F$_{5,25}$=55.62, F$_{6,28}$=51.71, F$_{6,29}$=65.91, respectively, all p<0.001; Fig. 2A-C). After varenicline treatment (Fig. 2), there was a significant main effect of varenicline dose (F$_{2,25}$=13.77, F$_{2,29}$=14.80, both at p<0.001 in R-1425 and R-1526) and/or a significant interaction (F$_{12,28}$=4.49, F$_{12,29}$=2.16, p<0.05 in R-1429 and R-1526) on number of cocaine injections. Low doses of varenicline (0.03 and 0.1 mg/kg) did not alter the number of self-administered cocaine injections. Post-hoc analysis for data from R-1425 revealed that treatment with both 0.3 and 0.56 mg/kg b.i.d. varenicline resulted in significant increases in the number of cocaine injections when 0.01 mg/kg and 0.56 mg/kg cocaine were available compared to baseline (p <0.001; Fig. 2A). In R-1429, post-hoc testing indicated that 0.3 and 0.56 mg/kg b.i.d. varenicline resulted in significant increases in numbers of injections from the baseline cocaine dose-response curve when 0.003 mg/kg and significant decreases when 0.1 mg/kg cocaine was available (all p<0.05; Fig. 2B). In R-1526, post-hoc tests indicated that 0.3 mg/kg b.i.d. varenicline treatment resulted in significant increases in the number of injections from the baseline cocaine dose-response curve when saline and 0.003 mg/kg cocaine were available (p<0.05), while 0.56 mg/kg b.i.d. varenicline treatment resulted in significant increases in the number of 0.56 mg/kg cocaine injections received (p<0.001; Fig. 2C).

Effects of varenicline on food-maintained responding. Under baseline conditions, R-1425 and R-1429 earned all their food allotment within approximately 2 hrs by responding
under an FR schedule (Fig. 2, D and E). In these two monkeys, cocaine reinforcement in
the afternoon sessions did not influence number of pellets or the time to obtain all food
reinforcement on subsequent days (e.g., all pellets were received within the first 2 hours
of the session; food-maintained responding was not studied in R-1526). In R-1425, there
was a significant effect of treatment on the percent of pellets received within 2 hrs when
examined in weekly bins ($F_{23,135}=13.97$, $p<0.001$) such that weeks 10, 11, 12 and 14 were
significantly lower than baseline ($p<0.001$; Fig. 2D). On days 2-14 of 0.56 mg/kg
varenicline treatment, the number of food reinforcers earned averaged less than half of
the total number of reinforcers allotted. Tolerance to the rate-decreasing effect of 0.56
mg/kg varenicline was apparent by day 15 such that all reinforcers were earned within the
allotted time period (data not shown). In R-1429, there was a significant effect of
varenicline dose on the percent of pellets received within the first 2 hrs when examined in
weekly bins ($F_{24,144}=1.79$, $p<0.05$) such that week 16 was significantly different from
baseline ($p<0.05$; Fig. 2E). All pellets were still earned within the 23-hr time period.
Figure 2. Effects of varenicline (Var) on cocaine (Coc) and food self-administration. A-C, mean ± SEM number of cocaine injections received in three monkeys. D and E, percentage of total daily food reinforcers (SRs) earned during the first 2 hours of availability in weekly bins. * significantly different from baseline (BL). For food-maintained responding, † represents 0.1 mg/kg once-daily varenicline, and ◊ represents 0.1 mg/kg b.i.d. varenicline; WO, washout; S, saline.
Effects of mecamylamine on cocaine self-administration and food-maintained responding. In contrast to the effects of varenicline, no dose of mecamylamine regardless of route of administration altered the baseline cocaine dose-response curve, so data were combined across routes to compare across cocaine doses (Fig. 3, A-C). At the highest dose tested (1.7 mg/kg mecamylamine, b.i.d.), food-maintained responding was reduced >50% when administered i.m. or i.v., but not p.o. (Fig. 3, D and E). Although both monkeys showed reduced rates of food-maintained responding, only R-1425 failed to earn all food pellets and this only occurred on 6 of 48 days during 1.7 mg/kg mecamylamine treatment (not shown).
Figure 3. Effects of mecamylamine (Mec) on cocaine (Coc) and food self-administration. A-C, mean ± SEM number of cocaine injections received in three monkeys. D and E, percentage of total daily food reinforcers (SRs) earned during the first 2 hours of availability each day of treatment. BL, baseline; WO, washout; S, saline. Points without error bars were not double-determined.
Cocaine Discrimination

Experiment 3: Effects of nAChR compounds on cocaine discrimination. In all monkeys, cocaine engendered dose-dependent increases in cocaine-appropriate responding with approximately 100% response allocation on the cocaine-associated lever when the training dose was available (Fig. 4, filled symbols). Mean rates of responding were 1.49 ± 0.44 and 1.40 ± 0.52 responses/sec following saline and 0.3 mg/kg cocaine, respectively; these response rates were not significantly different. Nicotine partially substituted for the cocaine stimulus in one of three monkeys (R-1525; Fig. 4, top panels, points above S). In all three monkeys, 0.1 mg/kg nicotine pretreatment significantly increased cocaine-appropriate responding when combined with low doses of cocaine. In R-1525, a lower dose of nicotine, 0.03 mg/kg, increased % cocaine-appropriate responding when combined with 0.01 mg/kg cocaine, but decreased cocaine-like responding when combined with 0.1 mg/kg cocaine. A higher nicotine dose (0.1 mg/kg) only increased percent cocaine-appropriate responding for 0.01 and 0.03 mg/kg cocaine (Fig. 4, top, right panel). In both R-1430 and R-1524, 0.1 mg/kg nicotine shifted the ED50 from 0.10 and 0.12 mg/kg, respectively, to 0.02 mg/kg. There were no significant effects of nicotine on response rates (data not shown).

In R-1430, 0.1 mg/kg varenicline alone occasioned nearly 50% cocaine-appropriate responding, indicating partial substitution (Fig. 4, middle left panel, data above S). Varenicline pretreatment also increased cocaine-appropriate responding when combined with low cocaine doses in all three monkeys. For two monkeys, 0.3 mg/kg varenicline increased cocaine-appropriate responding at two doses, which caused a leftward shift in the cocaine dose-response curve. For R-1525, varenicline pretreatment increased cocaine-
appropriate responding when combined with 0.01 mg/kg cocaine which, when tested alone, occasioned 0% cocaine-lever responding (Fig. 4, middle right panel). In R-1430 and R-1524, 0.3 mg/kg varenicline shifted the ED$_{50}$ from 0.10 and 0.12 mg/kg to 0.03 and 0.05 mg/kg, respectively. There were no significant effects of varenicline on response rates (data not shown).

As was seen with nicotine, mecamylamine (0.3 mg/kg) alone partially substituted for the cocaine stimulus in R-1525. Mecamylamine pretreatment significantly increased cocaine-appropriate responding when combined with low cocaine doses (Fig. 4, bottom panels). For R-1430 this occurred following the highest dose of mecamylamine (1.7 mg/kg), which shifted the cocaine dose-response curve to the left. For R-1524 and R-1525, mecamylamine pretreatments increased cocaine-appropriate responding following one dose of cocaine. In R-1430 and R-1524, 1.7 mg/kg mecamylamine shifted the ED$_{50}$ from 0.10 and 0.12 mg/kg to 0.03 and 0.06 mg/kg, respectively. There were no significant effects of mecamylamine on response rates (data not shown). As a positive control, haloperidol was tested with the training dose of cocaine and decreased percent cocaine-appropriate responding by >50% in all three monkeys; in two monkeys, haloperidol also decreased response rates (Table 1).
Figure 4. Effects of nicotine (top panel), varenicline (middle panel) and mecamylamine (bottom panel) on the discriminative stimulus effects of cocaine. Different symbols represent different doses: cocaine alone: + 0.03 mg/kg; + 0.1 mg/kg; + 0.3 mg/kg; ◊ + 1.0 mg/kg; ▲ + 1.7 mg/kg. S, saline.
Table 1. Effects of haloperidol on the discriminative stimulus effects of cocaine (Coc: 0.3 mg/kg, i.v.)

<table>
<thead>
<tr>
<th>Monkey</th>
<th>R-1430</th>
<th>R-1524</th>
<th>R-1525</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Cocaine Response (± SD)</td>
<td>Response Rate (± SD)</td>
<td>% Cocaine Response (± SD)</td>
</tr>
<tr>
<td>Haloperidol (mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coc</td>
<td>99.94 (0.11)</td>
<td>2.09 (0.07)</td>
<td>96.91 (5.34)</td>
</tr>
<tr>
<td>Coc + 0.003 Haloperidol</td>
<td>75.00 (7.07)</td>
<td>1.99 (0.06)</td>
<td>59.7 (56.14)</td>
</tr>
<tr>
<td>Coc + 0.0056 Haloperidol</td>
<td>N.T.</td>
<td>N.T.</td>
<td>12.7 (10.32)</td>
</tr>
<tr>
<td>Coc + 0.01 Haloperidol</td>
<td>86.67 (21.21)</td>
<td>1.91 (0.01)</td>
<td>N.T.</td>
</tr>
<tr>
<td>Coc + 0.017 Haloperidol</td>
<td>90.2 (11.74)</td>
<td>0.1 (0.08)</td>
<td>N.T.</td>
</tr>
<tr>
<td>Coc + 0.03 Haloperidol</td>
<td>43.5^</td>
<td>0.81 (1.15)</td>
<td>N.T.</td>
</tr>
</tbody>
</table>

N.T., not tested.

Each point is the mean (± SD) of two determination except where noted (^).

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DISCUSSION

The effects of varenicline, a low-efficacy α4β2* subtype-selective nicotinic acetylcholine receptor agonist, the non-selective high-efficacy agonist nicotine and the non-selective antagonist mecamylamine were evaluated for their ability to influence the reinforcing strength and discriminative stimulus effects of cocaine in nonhuman primates. In contrast to our hypothesis, chronic administration of varenicline increased the reinforcing strength of cocaine in all monkeys tested; varenicline also decreased food-maintained responding, but tolerance developed to that effect. In contrast to the potentiation in cocaine self-administration by varenicline, chronic mecamylamine did not significantly affect cocaine break points, but did decrease food-maintained responding. When substituted for cocaine using a PR schedule, neither varenicline nor nicotine maintained responding above levels observed when saline was available, consistent with low abuse potential in humans (McColl et al., 2008). Similarly, neither varenicline nor nicotine fully substituted for cocaine in monkeys trained to discriminate cocaine from saline. While chronic varenicline increased cocaine self-administration, acute administration of varenicline potentiated the discriminative stimulus effects of cocaine, as did acute administration of nicotine and mecamylamine. Overall, the abuse liability of varenicline in monkey models of cocaine abuse appears minimal, but the ability of varenicline to potentiate the reinforcing and discriminative-stimulus effects of cocaine suggests that varenicline would not be an effective pharmacotherapy for cocaine dependence.

Other investigators have shown that varenicline can have reinforcing effects (e.g. Rollema et al., 2007). However, in monkeys trained to self-administer cocaine under a
PR schedule, breakpoints and number of injections of varenicline were not different from saline, indicating that varenicline did not function as a reinforcer under these conditions. One caveat to these findings is that nicotine also did not result in breakpoints or number of injections that were significantly different from saline, suggesting the PR contingencies used in this study may have been too demanding to show reinforcing effects of full and partial nicotine agonists. The infusion rate, one factor that is deemed important in establishing intravenous nicotine self-administration in animals (see Freeman and Woolverton, 2009), was identical to the rate of delivery (10 sec) in studies where nicotine was reinforcing (albeit a weak reinforcer) under a PR schedule in rhesus monkeys (Freeman and Woolverton, 2009). In monkeys trained to discriminate cocaine, varenicline, nicotine and mecamylamine all showed partial substitution in one of three monkeys. The lack of robust substitution following nicotine is at odds with other monkey (de La Garza and Johanson, 1983) and rodent studies (Desai et al., 2003). One possible reason for the lack of cocaine-like discriminative stimulus effects may be a methodological consideration in the present drug discrimination paradigm. As described recently (Martelle and Nader, 2009), these monkeys self-administered the stimulus that was evaluated in drug discrimination. It may be that the discriminative stimulus effects of self-administered drugs, while having greater face validity than non-contingent drug administration, yield different substitution profiles. Additionally, route of drug pretreatment precluded testing higher doses that may have engendered greater substitution. Although additional research is needed using this model, the data from the cocaine discrimination and cocaine self-administration studies reported here suggest that varenicline has low abuse potential.
The ability of varenicline to potentiate rather than attenuate the reinforcing strength of cocaine resembles the effects of acute nicotine (Freeman and Woolverton, 2009) and direct DA agonists (Rowlett et al., 2007) on cocaine self-administration under PR schedules of reinforcement in monkeys. This enhancement of cocaine self-administration is opposite to the effects seen when chronic dL-amphetamine, a DA transporter blocker/DA releaser was administered under similar conditions in monkeys (e.g., Negus and Mello, 2003; Czoty et al., 2011). Interestingly, varenicline increased breakpoints at both the ascending and descending limbs of the curve, an effect that may be unique to partial agonists in that agonist- and antagonist-like effects may occur at different points of the dose-response curve based on endogenous neurotransmitter activity.

Recent evidence suggested that blockade of both α4β2* and α7* nAChR subtypes may be necessary to reduce cocaine-related behaviors (Zanetti et al., 2006). However, in the present study, chronic administration of the non-selective nAChR antagonist mecamylamine did not attenuate the reinforcing strength of cocaine. These data are consistent with previous results in nonhuman primates in which acute mecamylamine did not affect cocaine-maintained responding (Spealman and Goldberg, 1982) but contrast with results of rodent studies (e.g. Levin et al., 2000). Such discrepancies may be due to species differences in receptor subtype distribution or density (Han, 2000; Quik et al., 2000), schedules of reinforcement used or drug history of the subjects. The animals in the current study had an extensive cocaine self-administration history. In addition, mecamylamine was administered chronically in this study (up to 80 consecutive days) in which access to cocaine was studied intermittently. A growing literature describes the
important implications of dosing regimen for nAChR compounds including acute versus chronic treatment, use of mini-pump administration or frequent, repeated dosing (current study) versus once daily injections (e.g., Levin et al., 2000), and the effects of each regimen on receptor activation versus desensitization (see Matta et al., 2007; Picciotto et al., 2008 for reviews).

Varenicline and mecamylamine administration resulted in dose-dependent reductions in food-maintained responding under an FR schedule. Our dose escalation procedure modeled a titration regimen prescribed for varenicline for smoking cessation to minimize unwanted side effects. Humans taking varenicline or mecamylamine report constipation, taste perversion and nausea attributed to parasympathetic system inhibition (see Young et al., 2001; Gonzales et al., 2006). All monkeys readily ingested supplemental fruit and enrichment between sessions suggesting that the rate-decreasing effects on food-maintained responding, whether centrally or peripherally mediated, were not the result of anorexia or satiation. Regardless, tolerance developed to the rate-decreasing effects of both varenicline and mecamylamine (Figs. 2 and 3, D and E). No unconditioned behavioral effects of chronic oral varenicline were noted. Also, our implementation of intermittent access to cocaine self-administration models chronic drug treatment followed by lapses in abstinence often observed in human drug users (see Czoty et al., 2011 for discussion).

Acute varenicline pretreatment potentiated the discriminative stimulus effects of cocaine. In two of three monkeys, varenicline, nicotine and mecamylamine shifted the cocaine dose-response curve upward or leftward. The behavioral effects of all three nAChR agents resembled the effects of direct and indirect DA agonists (for review see
Callahan et al., 1997) in that they potentiated the discriminative stimulus effects of cocaine, supporting the hypothesis that the current findings are a result of nAChR-mediated DA release (Desai et al., 2003; Rollema et al., 2007, 2010). As a positive control, the DA D2-like receptor antagonist haloperidol attenuated the discriminative stimulus effects of cocaine in all three monkeys. It is possible that higher doses of varenicline or mecamylamine would have decreased cocaine-appropriate responding. However, while there were no effects of these drugs on response rates, higher doses were not tested because intravenous administration produced unwanted side effects (nicotine and varenicline: hypersalivation, panting, pupil dilation, flushing of the face, or scratching that subsided within 1 minute of administration; mecamylamine: sedative effect lasting several hours).

Previous studies in rats have shown that mecamylamine does not alter the discriminative stimulus effects of cocaine (Desai et al., 2003). Unexpectedly, the current results showed that mecamylamine potentiated the discriminative stimulus effects of cocaine in all three monkeys tested. Such discrepancies may be due to species-specific receptor distribution, route of administration, pretreatment time or drug history. An ongoing focus regarding nAChR-mediated effects is the uncertain behavioral consequences of receptor activation versus desensitization. *In vitro* studies showed that both activation and desensitization of α4β2* receptors resulted in nAChR-mediated DA release (for review see Picciotto et al., 2008). Further, nicotine and varenicline can both activate and desensitize α4β2* nAChRs in a brain region-specific manner depending on dose and duration of administration (Rollema et al., 2010). In theory, receptor desensitization may produce similar behavioral effects to functional antagonism (for
Similar effects of nicotine and mecamylamine on measures of monoaminergic release (Kenny et al., 2000) and cognition (for review see Young et al., 2001) have also been documented.

In the present study, varenicline increased the reinforcing strength and discriminative stimulus effects of cocaine similar to the effects of the full agonist nicotine in monkeys (Freeman and Woolverton, 2009) suggesting that even low efficacy at α4β2* nAChRs may stimulate DA release sufficiently to potentiate the reinforcing strength and discriminative stimulus effects of cocaine. Alternatively, varenicline is a high-efficacy agonist with low affinity for α7* and α3β4* nAChRs (Coe et al., 2005; Mihalak et al., 2006). Administration of high doses of varenicline can activate α7* nAChRs and simultaneously desensitize α4β2* subtype-receptors in a region-specific manner (for review see Dani and Bertrand, 2007; Picciotto et al., 2008). Future studies with more selective compounds of varying efficacy for nAChR subtypes will help elucidate the respective contributions of α4β2* and α7* nAChR subtypes and the importance of activation versus desensitization mediating ACh effects on DA neurons. The current behavioral data do not support the use of varenicline as a treatment for cocaine dependence.

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**FOOTNOTES**

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CHAPTER VI
DISCUSSION

The overarching goal of the work presented in this dissertation was to complement classic behavioral pharmacology paradigms with a novel strategy to examine potential pharmacotherapies to treat cocaine dependence. At the time this research was conducted, the most successful strategies to aid treatment-seeking cocaine users were behavioral modification strategies, including cognitive behavioral therapy (CBT) and contingency management (CM). Cocaine users show deficits in executive function across multiple domains integral in facilitating behavioral modification, including behavioral flexibility and memory (e.g. Kubler et al., 2005; Tomasi et al., 2007a,b; Moeller et al., 2010). Cognitive performance at the time of treatment initiation has correlated with treatment success (e.g. Teichner et al., 2001; Aharonovich et al., 2006; Turner et al., 2009). Thus cognition, a state variable, influences likelihood to continue treatment and maintain abstinence. Cognitive enhancement has been proposed as a strategy to improve behavioral modification practices (Sofuoglu, 2010). The work described within this dissertation validated a nonhuman primate model of cocaine-related neurobiological and cognitive deficits with strong face validity to the human condition, providing a behavioral platform in which to assess putative cognitive enhancers. Further studies assessed the abuse liability of cognitive enhancing compounds and their potential to influence further abuse potential of cocaine. Drug interactions within the CNS are complex and therapeutic potential represents a balance between all positive and negative drug effects. Critical evaluation of compounds across multiple behavioral assays will provide greater translational utility than any one assay alone.
Hypothesis: Monkeys with a chronic cocaine SA history will demonstrate impaired working memory performance compared to age-matched, cocaine-naive monkeys. High-dose cocaine SA will further disrupt working memory performance in monkeys. During initial abstinence, disruptions in cognitive performance will be exacerbated, but improvements in working memory will be apparent following extended durations of abstinence.

Chronic cocaine use is associated with cognitive deficits, including impaired working memory, compared to drug-naive groups that persist into periods of abstinence. Some studies suggest acute abstinence (~72 hrs) unmasks further deficits in working memory (Woicik et al., 2009). Following extended durations of abstinence, working memory is often still impaired when compared directly to drug-naive groups, but a few studies showed that working memory is better during abstinence than in current cocaine users (e.g. Hanlon et al., 2011). These studies reiterate the need for within-subject assessments to characterize the effect of extended cocaine SA and varying durations of abstinence on working memory.

The studies in Chapter II were designed to compare working memory between rhesus monkeys with a ~6 yr cocaine SA history that continued to self-administer 0.1 mg/kg cocaine ~5 days/week and age-matched, cocaine-naive monkeys. Surprisingly, initial assessment of working memory using a delayed match-to-sample task revealed
similar delay-dependent reductions in working memory in cocaine-naive and cocaine-experienced monkeys. When the cocaine dose available for self-administration was increased to 0.3 mg/kg cocaine, working memory was disrupted in all three monkeys. Importantly, these cognitive assessments occurred ~16 hrs after cocaine SA, such that cocaine was no longer in the organism. However, tolerance developed to the disruptive effects of high-dose cocaine self-administration within 10 sessions. The current results are similar to a study by Porter and colleagues (2011) such that initial cocaine self-administration disrupted working memory but tolerance developed to the disruptive effects within several sessions. These studies demonstrate working memory deficits on days following high dose cocaine SA, validating a nonhuman primate model of cocaine-induced cognitive disruptions.

Following 10 days of high-dose cocaine SA, afternoon SA sessions were discontinued and working memory was assessed during 30 consecutive days of abstinence. Working memory was not disrupted during acute abstinence and performance improved significantly by day 30. These data are consistent with the few human studies that have conducted repeated assessments in abstinent cocaine users (e.g. Di Sclafani et al., 2002; Pace-Schott et al., 2008) demonstrating face validity to the human condition. These findings are encouraging considering treatment goals are first to remain abstinent but secondly to successfully integrate into a social and work setting, both requiring high levels of cognitive function. Despite improvement in cognition by day 30, the first month of abstinence is a critical period during which a large proportion of patients relapse (Gawin and Kleber, 1985, 1986). Therefore, it is imperative to understand neurobiological alterations associated with chronic cocaine use that underly poor
executive function and then target these regions to improve CNS function and cognitive performance in a more expedient manner, in an attempt to facilitate behavioral modification strategies.

**BEHAVIORAL FLEXIBILITY: COCAINE-RELATED DEFICITS IN SET SHIFTING AND METABOLIC ACTIVITY**

*Hypothesis:* Monkeys with a cocaine SA history will show impaired performance on a measure of behavioral flexibility, set shifting and lesser metabolic activity, a measure of neuronal activity, in brain regions implicated in mediating learning, reinforcement, and executive function compared to cocaine-naive monkeys.

In Chapter III, a set-shifting task was utilized to examine behavioral flexibility between monkeys with a cocaine SA history and cocaine-naive monkeys. Throughout this study, the cocaine SA history group continued to self-administer 0.1 mg/kg cocaine in afternoon sessions. There were no differences between groups across the simple, compound or intradimensional components of this task, suggesting no cocaine-related impairments on learning or retention of simple tasks, further supported by the results from studies in Chapter II. However, when the focus of attention was switched between two sets (shapes to lines), cocaine SA monkeys required a greater number of trials and committed a greater number of errors to acquire this extra-dimensional shift. FDG-PET was used to examine patterns of glucose utilization underlying these cognitive differences. While both groups of monkeys showed significantly greater glucose
utilization in the orbital PFC and posterior cingulate cortex, only cocaine-naive monkeys showed increased activity in the anterior cingulate cortex, caudate nucleus, and hippocampus. Together, the activation pattern in cocaine-naive monkeys represents regions implicated in learning, establishing and adapting to stimulus-reinforcement associations, and activating/inhibiting neuronal pathways dependent on relevant stimuli (e.g. Hampshire and Owens, 1996; Kondo et al., 2004a,b). The lack of higher glucose utilization in these regions in cocaine SA monkeys suggests neurobiological deficits underlying impaired cognitive function. Moreover, these deficits are similar to those seen in human cocaine users during tasks measuring attention, behavioral flexibility and working memory (e.g. Hester and Garavan, 2004; Kubler et al., 2005; Tomasi et al., 2007a,b).

The regions associated with hypoactivity in response to the EDS are areas known to be influenced by dopaminergic function (see Beaulieu and Gainetdinov, 2011 for review). It is hypothesized that a hypodopaminergic state underlies impaired executive function in cocaine users (see Bolla et al., 1998; Volkow et al., 1999, 2004). For example, cocaine self-administration is associated with decreased DA D2-like receptor availability (Volkow et al., 1993; Nader et al., 2006) and density (Moore et al., 1998; Nader et al., 2002) and increased DAT density (Tella et al., 1996; Letchworth et al., 2001). During abstinence from cocaine following chronic use, increased DA uptake and fewer D2-like receptors results in lower circulating DA levels and less DA binding at receptors to produce an effect (see Volkow et al., 1999, 2004 for reviews). Recently, Groman and colleagues (2011) showed a direct correlation between DA D2-like receptor availability in the striatum and reversal learning performance in monkeys. Further, both
noncontingent and self-administered cocaine impaired reversal learning in monkeys (Jentsch et al., 2002; Porter et al., 2010). In the current group of monkeys, an initial assessment showed that monkeys with a cocaine SA history also committed a greater number of errors to acquire a reversal learning task (Figure 1A). Therefore, we would hypothesize that cocaine SA in the current monkeys reduced DA D2-like receptor availability that would correspond to deficits in measures of behavioral flexibility, including reversal learning and set shifting behavior.

Indeed, using [18F]-Fluoroclebopride (FCP) and PET imaging, the four monkeys with a ~6 year cocaine SA history in our lab showed lower DA D2-like receptor availability following the first 6 months of SA (Table 1). The average reduction in receptor availability present at 6 months was ~18%. The maximum reduction documented in our laboratory is ~20%, which occurred within the first month of SA and persisted without further reductions following up to one year of SA (Nader et al., 2006). Therefore, it is reasonable to assume that reductions in D2-like receptor availability would persist as a result of continued cocaine SA throughout the five years since initial assessment. There was a strong correlation between the percent reduction in DA D2-like receptor availability in the caudate nucleus and the number of errors to acquire both the reversal learning component \( (r^2 = 0.91, p<0.05) \) and the first EDS \( (r^2 = 0.98, p<0.05; \) Figure 1B), but no correlation between DVRs and the SD, CD, or ID components across either task (data not shown). Due to the small sample size these data are merely speculative. However, these data closely align with a hypodopaminergic hypothesis for cocaine-associated cognitive disruptions, and are similar to correlations between DA D2-
like receptor availability and reversal learning performance in cocaine-naive monkeys (Groman et al., 2011).

Table 1. Individual baseline (BL) DVRs for DA D2-like receptor availability and percent reductions from BL following 6 months of cocaine SA and 30 days of abstinence (unpublished data)

<table>
<thead>
<tr>
<th></th>
<th>BL DVR</th>
<th>% of Baseline</th>
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<tbody>
<tr>
<td></td>
<td>Cd Pt</td>
<td>6 months Coc SA</td>
<td>30 Days Abst</td>
<td></td>
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<tr>
<td>Cd Pt Cd Pt Cd Pt Cd Pt</td>
<td>Cd Pt Cd Pt Cd Pt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1374</td>
<td>2.74 2.99</td>
<td>88.2 84.4</td>
<td>86.4 105.6</td>
<td></td>
</tr>
<tr>
<td>1375</td>
<td>3.56 3.34</td>
<td>79.8 89.7</td>
<td>66.9 72.1</td>
<td></td>
</tr>
<tr>
<td>1377</td>
<td>3.02 3.3</td>
<td>86.1 77.9</td>
<td>94.4 85.5</td>
<td></td>
</tr>
<tr>
<td>1381</td>
<td>3.02 3.77</td>
<td>80.0 74.9</td>
<td>81.5 82.2</td>
<td></td>
</tr>
<tr>
<td>AVE</td>
<td>3.09 3.35</td>
<td>83.5 81.6</td>
<td>82.2 86.32</td>
<td></td>
</tr>
<tr>
<td>SEM</td>
<td>0.20 0.19</td>
<td>2.48 3.83</td>
<td>6.66 8.09</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. A, Monkeys with a cocaine SA history committed significantly greater number of errors (filled bars) than naive monkeys (open bars) to acquire a reversal learning task; B, Percent reduction from baseline DVRs in the caudate nucleus directly correlated with the number of errors committed to acquire a reversal learning task and the extradimensional shift of a set-shifting task; * p<0.05.
Interestingly, there was not a correlation between cumulative cocaine intake and cognitive performance across any of the measures in either Chapters II or III. In fact, the monkey with the most extensive lifetime cocaine intake (R-1374) committed fewer errors than the other cocaine SA monkeys across all components of the discrimination and set-shifting tasks. The only part of the task where this monkey showed any difference in performance from the cocaine-naive monkeys was the EDS. In addition, R-1374 was least affected by the high-dose cocaine condition (Chapter II, Figure 3). This monkey also had the lowest baseline DVRs in the caudate nucleus and putamen and showed the lowest percent change following the initial 6 months of cocaine SA, suggesting a possible genetic or metabolic effect that appears to be protective despite an extensive cocaine SA history. Clearly, more research is needed to elucidate genetic or metabolic factors influencing susceptibility to the cognitive disruptive effects of cocaine.

It is also worth noting that following the first year of cocaine SA, DA D2-like availability was re-assessed after 30 days of abstinence (Table 1). Although DA D2-like availability can recover over extended durations of abstinence, and at different individual rates (e.g. Nader et al., 2006), little recovery occurred in these particular monkeys during the first 30 days of abstinence. These data suggest that spontaneous recovery of DA D2-like receptor availability following 30 days of abstinence, in these 4 monkeys would not be responsible for the improvements in working memory that were shown in Chapter II, over 30 days of abstinence. This provides a rationale for other pharmacological treatment strategies, including the one utilized in this dissertation with nicotinic acetylcholine compounds.
The data from Chapters II and III demonstrated deficits in two cognitive domains, working memory and behavioral flexibility, in monkeys with a cocaine SA history compared to cocaine-naive monkeys. Further, in Chapter III neurobiological substrates including the striatum, ACC and hippocampus were identified as regions that did not increase glucose utilization in the cocaine SA monkeys during set shifting, thus providing anatomical targets for pharmacotherapeutic development to improve neural activity and cognitive function. These regions are associated with DA neurotransmission (see Beaulieu and Gainetdinov, 2011 for review). Therefore, we would hypothesize that administration of a dopamine agonist would increase DA function and enhance cognitive performance. Although direct-acting DA agonists can improve cognitive function in human conditions associated with low DA function (e.g. Costa et al., 2009; Ersche et al., 2011; see Cools and D’Esposito et al., 2011 for review), other effects of direct-acting DA agonists on cocaine-related behaviors (e.g. increased abuse liability, likelihood to trigger relapse; see Grabowski et al., 2004 for review) suggest an indirect mechanism to stimulate DA release may be a more successful approach for therapeutic development.

**NICOTINIC ACETYLCHOLINE RECEPTORS AND EFFECTS OF AGONISTS ON COGNITION**

*Hypothesis:* Nicotinic acetylcholine receptor (nAChR) availability would be higher in monkeys with a cocaine SA history compared to cocaine-naive monkeys, similar to the effects of smoking, and that nAChR agonists will improve working memory.
performance to a greater extent in cocaine-experienced monkeys compared to cocaine-naive monkeys.

One goal of Chapter IV was to examine nAChR agonists on working memory in both cocaine-naive monkeys and monkeys with a cocaine SA history. Importantly, cocaine SA sessions were suspended to examine the effects of nAChR agonists during abstinence from cocaine, when cognitive enhancement would be most beneficial. Nicotine the nonselective nAChR agonist, varenicline a low-efficacy \( \alpha_4\beta_2^* \) subtype-selective agonist, and PNU-282987 a high-efficacy \( \alpha_7 \) subtype-selective agonist each improved working memory performance at the long delay only, in both groups of monkeys. Nicotine produced the greatest maximal effect while both varenicline and PNU-282987 produced similar effects, which may suggest that stimulation of both \( \alpha_4\beta_2^* \) and \( \alpha_7 \) subtypes would produce a greater effect than either subtype-selective agonist alone. A high-efficacy \( \alpha_4\beta_2^* \) subtype-selective agonist may have produced a more robust effect than varenicline, the low-efficacy agonist. Additional studies assessing a selective, high-efficacy \( \alpha_4\beta_2^* \) subtype-selective agonist as well as combination studies administering both \( \alpha_7 \) and \( \alpha_4\beta_2^* \) subtype-selective compounds simultaneously would be necessary to address this question and is outside the scope of the current research. By targeting subtype-selective drugs, our goal is to identify a cognitive enhancer that lacks effects that would hinder therapeutic efficacy, such as abuse liability.

Interestingly, while maximal efficacy was similar between the two groups of monkeys, the dose that engendered the maximal pro-cognitive effect of nicotine in 3 of 4 cocaine-naive monkeys was lower than in the cocaine-experienced monkeys. This may
suggest 1) changes in cholinergic receptor availability or function as a result of cocaine SA, or 2) that a greater neuromodulatory effect on other neurotransmitter systems (e.g. DA) is needed to engender similar pro-cognitive effects. For example, the relationship between DA function and cognition has been described as an inverted-U-shaped curve, such that the cognitive performance of healthy individuals falls within a “natural” or “peak” range and that dopamine depletion/hypofunction as well as hyperfunction hinders cognition (for review see Cools and D’Esposito, 2011). Thus, in monkeys with a cocaine SA history and a hypofunctional DA state, greater stimulation may be necessary to return cognitive function to peak levels (Figure 2). Additional studies are necessary to determine the direct relationship between basal DA function and cognition in these monkeys. One such study would be to examine the effects of a direct-acting DA agonist.

**Figure 2. Cognitive performance related to basal DA function.** Cocaine use is associated with low DA function which is hypothesized to underly deficits in executive function. Higher doses of drugs that stimulate DA release may be needed to produce peak cognitive performance in cocaine users than healthy controls. Optimal cognitive enhancing doses will vary per individual based on basal DA function (figure modified from Cools and D’Esposito, 2011).
One phenomenon associated with nAChR pharmacology is the development of tolerance or sensitization to an effect of repeated nicotine administration. Chronic nicotine exposure produced tolerance to the locomotor-depressant effects and physiological-depressant effects of nicotine (e.g. Marks et al., 1993) and sensitization to the locomotor-stimulated effects (e.g. Govind et al., 2009). Interestingly, chronic treatment has shown both sensitization and tolerance to nicotine-stimulated DA release (e.g. Marks et al., 1993; see Balfour et al., 1998 for review) and may depend on dose, route and frequency of nicotine administration (see Matta et al., 2007 for review). However, tolerance infrequently has been shown to develop to the pro-cognitive response following repeated nicotine dosing (see Buccafusco et al., 2005 for review). Although a sensitized response to the pro-cognitive effects of nicotine has not been shown per se, chronic nicotine administration can result in an extended pro-cognitive profile lasting days to weeks after the last nicotine administration suggesting intracellular changes following repeated exposure (see Buccafusco et al., 2005 for review). A similar pro-cognitive response extending outside the pharmacokinetic profile has been shown following administration of some but not all nAChR agonists (see Buccafusco et al., 2005).

Therefore, future studies examining the long-term effects of varenicline and PNU-282987 on cognition in monkeys are warranted. In rodents tolerance developed to repeated varenicline administrations such that the BOLD response, a measure of brain activity via fMRI, and the cognitive enhancing effects on spatial working memory were attenuated on day 6 compared to day 1 (King et al., 2011). This may be due to the longer
half-life of varenicline (~17 hr in human, 24 hr in monkey, ~4 hr in rat; Obach et al., 2004), or an effect of its high-affinity, low-efficacy agonist profile for α4β2*-subtype receptors in that varenicline can desensitize receptors with a minimal activation profile (e.g. Rollema et al., 2009b). In contrast, PNU-282987 is a highly selective, high-affinity, high-efficacy α7 subtype-selective agonist (Bodnar et al., 2005, Hajos et al., 2005) with a similar half-life as varenicline (McLean et al., 2011). In cognition studies, PNU-282987 attenuated PCP-induced deficits in working memory on day 1 and following 15 days of daily dosing suggesting that neither tolerance nor sensitization developed to this effect (McLean et al., 2011). Further, the cognitive enhancing effects were not present on subsequent days after treatment cessation, suggesting that effects of PNU-282987 are attributed to the active compound being present, and not a result of long-term changes within the organism (McLean et al., 2011). Maloku and colleagues (2011) showed that 5-day varenicline or PNU-282987 treatment differentially altered mRNA and protein levels in mice, suggesting distinct epigenetic alterations, implicating distinct therapeutic potential.

Additionally, differences in nAChR availability or function may explain the differences in potency of nicotine to enhance working memory between the cocaine-experienced and cocaine-naive monkeys. A second goal of Chapter IV was to examine nAChR availability using [11C]-nicotine and PET imaging. Similar to rodent and human literature examining nAChR distribution in chronic smokers (e.g., Breese et al., 1997; Muhkin et al., 2008), monkeys with a cocaine SA history showed greater overall receptor availability, but the only region significantly different from cocaine-naive monkeys was the hippocampus. This is important from a therapeutic perspective. A reduction in
availability would have inhibited the potential for drugs targeting this receptor system to produce a behavioral effect. Further, differences in the hippocampus suggest implications for influencing learning and memory. Although counterintuitive, nAChR upregulation has been shown in chronic smokers and animals following chronic nicotine exposure potentially as a compensatory mechanism following reduced function from receptor desensitization (e.g. Marks et al, 1993). However, there were no correlations between nAChR availability in any brain region and either the maximal percent increase in accuracy or the dose of nicotine that engendered this maximal effect. These data suggest that differences in receptor availability are not the cause of subtle differences in potency, and that other factors such as upregulation of specific nAChR subtypes, nAChR function or influence of other neurotransmitters (e.g. DA) are important. The influence of individual receptor-subtypes could not be determined using the nonselective radiolabelled nicotine. Although we examined nAChR agonist activity on cognition, we did not directly measure nAChR function. A direct assessment such as nicotine-stimulated ACh or DA release measured via microdialysis would more directly assess nAChR function. Lastly, PET studies utilizing α4β2* or α7 subtype-selective radioligands would be important for future studies assessing subtype-selective compounds and their influence on cognition.

Although [11C]-nicotine was used frequently in the early 1980’s and 1990’s to examine nAChR availability, it has largely been replaced by more selective radiotracers with better pharmacokinetic and pharmacodynamic profiles. Several criteria for designing optimal radiotracers include good blood-brain-penetration, proven receptor saturation and displacement by the non-radiolabelled molecule, establishment of kinetic modeling of the
molecular flow through different tissue compartments (e.g. blood to brain tissue, tissue to bound receptor, and the reverse sequence), and lastly, examination of the effects of radiotracer on cardio- and cerebro-vascular effects (see Sedvall et al., 1986; Sihver et al., 2000). Despite a high rate of blood-brain penetration, animal and human studies have questioned the utility of $[^{11}C]$-nicotine for assessing changes in neurobiology (e.g. Nordberg et al., 1989; Nyback et al., 1994). For example, the ability of non-radiolabelled nicotine administration prior to or during $[^{11}C]$-nicotine PET scans has produced small but significant reductions (e.g., Nordberg et al., 1989), or equivocal effects (e.g. Nyback et al., 1994) on $[^{11}C]$-nicotine binding in receptor-rich brain regions leading some investigators to conclude that $[^{11}C]$-nicotine may not accurately reflect specific binding to receptors versus circulating, nonbound radioactivity within the CNS (e.g. Nyback et al., 1994, Sihver et al., 1999). Secondly, the effects of nicotine in the CNS are blood-flow dependent (e.g. Nyback et al., 1994), such that incorporating a measure of blood flow into the kinetic model using shorter acting radioactive molecules such as $[^{15}O]$.H$_2$.O or $[^{11}C]$-butanol is ideal (e.g. Nordberg et al., 1995).

However, results from early studies using $[^{11}C]$-nicotine to compare nAChR availability between smokers and nonsmokers, or Parkinson’s disease patients with healthy controls have been validated using complex kinetic models or more selective radiotracers. For example, using $[^{11}C]$-nicotine long-term smokers showed higher nAChR availability than nonsmokers (Nyback et al., 1994). In this study, Nyback and colleagues (1994) used an analytical method similar to the one employed in Chapter IV, comparing tissue time activity curves between groups. Recently a higher distribution of $\alpha$4$\beta$2* nAChR subtypes (Muhkin et al., 2008) was reported in smokers compared to nonsmokers
using a much more rigorous kinetic model and a radiotracer with better pharmacokinetic profile. Further, receptor binding studies using autoradiographic techniques corroborated PET results using $^{[11]C}$-nicotine. For example using $[^3H]$-nicotine, post-mortem tissue from long-term smokers showed higher nAChR binding in cortical and subcortical regions, including the hippocampus and thalamus compared to non-smoking controls (Benwell et al., 1988; Breese et al., 1997). Similar conclusions regarding initial results with $^{[11]C}$-nicotine and novel radiotracers have been confirmed regarding decreased nAChR, notably $\alpha_4\beta_2^*$ receptor subtypes in patients with Parkinson’s Disease (e.g. Guan et al., 2002; Kas et al., 2009). Therefore, although novel, selective radiotracers with more optimal profiles for PET imaging exist, their use corroborates early studies using $^{[11]C}$-nicotine, suggesting that $^{[11]C}$-nicotine can be used to assess nAChR availability.

The results from Chapter IV extend previous studies in animals and humans showing that nAChR agonists can enhance cognitive function by demonstrating similar cognitive enhancing effects in a NHP model of cocaine abuse. These data support further investigation of subtype-selective nAChR agonists as pharmacological adjuncts to aid behavioral modification strategies in treatment-seeking cocaine users. However, further studies are necessary to examine the effects of nAChR agonists on other drug-related behaviors. For example, cognitive enhancement will not be sufficient to maintain abstinence from cocaine if, at similar doses the proposed drug increases likelihood to relapse or possesses abuse ability of its own. Therefore, it is necessary to examine the effects of putative therapies across multiple behavioral assays before concluding the overall therapeutic potential of a drug.
Hypothesis: Acute administration of low- and high-efficacy nAChR agonists will potentiate, while antagonism will attenuate or have no effect on the discriminative stimulus effects of cocaine. Chronic administration of low-efficacy agonists or antagonists will attenuate the reinforcing strength of cocaine.

The studies in Chapter V were designed to examine the pharmacotherapeutic potential of varenicline to aid in treating cocaine dependence by assessing its effects on several cocaine-related behaviors. Nicotine increased DA release in vitro and in vivo (e.g. Zhou et al., 2001; Rollema et al., 2007, 2010) and potentiated various cocaine-related behaviors in rodents, monkeys and humans (e.g. Reid et al., 1998; Desai et al., 2003; Freeman and Woolverton, 2009) whereas the nonselective antagonist mecamylamine reduced or had no effect on similar behaviors (e.g. Spealman and Goldberg, 1982; Reid et al., 1999; Levin et al., 2000). These studies support the use of partial agonists that may engender positive effects similar to an indirect agonist treatment approach but with minimal abuse liability. Consistent with the effects of nicotine, varenicline potentiated the discriminative stimulus effects of cocaine, but did not substitute for cocaine in drug discrimination studies. Surprisingly, mecamylamine also potentiated the discriminative stimulus effects of cocaine. Similar behavioral effects resulting from nAChR agonist and antagonist administration have previously been reported (e.g. Kenny et al., 2000; Young
et al., 2001), and will be discussed in further detail (see below). When administered chronically, varenicline potentiated the reinforcing strength of cocaine, similar to the effects of acute nicotine administration (Freeman and Woolverton, 2009; Mello and Newman, 2011), whereas mecamylamine had no effect on the reinforcing strength of cocaine, also consistent with acute administration (Spealman and Goldberg, 1982). Although varenicline did not maintain responding at levels greater than when saline was substituted for cocaine, suggesting low abuse liability in subjects with a cocaine history, its ability to potentiate both the discriminative stimulus and reinforcing strength of cocaine suggest that varenicline is not an ideal pharmacotherapy for reducing cocaine-related behaviors in cocaine-dependent populations, nor in reducing intake during relapse.

As described in Chapter I, ACh plays a neuromodulatory role on DA function. However, ACh influence on dopaminergic function is neither a simple nor singular mechanism as different receptor subtypes (e.g. \( \alpha 4\beta 2^* \), \( \alpha 7 \)) are localized pre- or postsynaptically on DA, GABA, and glutamate neurons in a brain region-specific manner. Therefore, nAChR agonists can differentially affect neurotransmitter release or action potential gradients, influencing the net \textit{in vivo} effects of multiple neurotransmitter systems (see Livingstone and Wonnacott, 2009 for review). Purported nAChR agonists and antagonists defined from \textit{in vitro} assays may produce similar \textit{in vivo} results based on subunit composition and localization. Initial exposure to nicotine can \textit{activate} \( \beta 2 \)-containing nAChRs directly on DA neurons and \( \alpha 7 \)-subtype nAChRs on glutamatergic inputs to DA neurons in the VTA, to stimulate DA release. At the same time, non- \( \alpha 7 \) subtype receptors (primarily \( \alpha 4\beta 2^* \)-subtype receptors) on GABA neurons projecting to
DA neurons are initially activated followed by rapid desensitization (seconds to minutes depending on dose delivered) resulting in reduced inhibition on postsynaptic DA neurons and increased net excitation of DAergic tone (Mansvelder and McGehee, 2000; Mansvelder et al., 2002; Keath et al., 2007; for review, see Dani and Bertrand, 2007). This phenomenon is specific to the VTA. In the striatum, tonically active ACh neurons are primarily under control of β2-subtype receptors. Desensitization of these receptors in the striatum reduced tonic DA release, but increased DA burst firing in response to primary or conditioned reinforcement (Zhou et al., 2001; Rice and Cragg, 2004, Zhang and Sulzer, 2004; for review see Picciotto et al., 2008). Net effects of nAChR function on DA release are dependent on brain region and receptor subtype, tonic or phasic endogenous activity, and effects of receptor activation, desensitization, or blockade (for reviews, see Dani and Bertrand, 2007; Exley and Cragg, 2008; Picciotto et al., 2008).

While such mechanisms could not be directly examined in the current behavioral studies, it provides a plausible explanation for a similar behavioral effect following pretreatment with an agonist and antagonist in cocaine discrimination studies. Similar effects of nicotine and mecamylamine have been reported on other measures of monoaminergic release (Kenny et al., 2000) and cognition (see Young et al., 2001; Buccafusco et al., 2005 for reviews). Our initial hypothesis was that the nAChR partial agonist varenicline might produce a different net effect on DAergic tone compared to nicotine. As a partial agonist, varenicline could potentially increase inhibitory tone on DA neurons via α4β2*-nAChRs activation located on GABA cells (e.g. Dani and Harris, 2005) without affecting α7-nAChRs on glutamatergic inputs. However, as a potent full agonist with low affinity at α7-nAChRs (Rollema et al., 2007), at higher doses
varenicline may activate α7-subtype receptors and potentially desensitize α4β2-subtype receptors resulting in a net behavioral profile similar to nicotine (see above). Based on the latter hypothesis, mecamylamine was examined with the hypothesis that blockade of both α4β2- and α7-subtype nAChRs were necessary to evoke a behavioral effect opposite that of nicotine (Corigall et al., 1994, 1999; Zanetti et al., 2006). However, the acute and chronic effects of varenicline were similar to the acute effects of nicotine in that each potentiated the discriminative stimulus effects and reinforcing strength of cocaine. Mecamylamine engendered similar effects on cocaine discrimination when administered acutely, but did not have any effect on the reinforcing strength of cocaine up to doses that disrupted food-maintained responding.

THERAPEUTIC POTENTIAL OF VARENICLINE

Historically, preclinical success of a pharmacotherapeutic agent was measured in terms of reducing or blocking the reinforcing effects of a drug. However, the lack of successful treatments for psychostimulant abuse has caused a re-examination of preclinical models with a translatable focus towards relapse prevention (see O’Brien, 2008). Clinical strategies to aid in relapse prevention include behavioral modification and cognitive enhancement (reviewed in Chapter I). Acute, low dose varenicline administration improved working memory equally, in monkeys during abstinence following a chronic cocaine SA history and in cocaine-naive monkeys (Chapter IV). Rodent studies showed that acute varenicline administration improved cognitive performance and increased brain function, measured via fMRI (King et al., 2011), in regions known to be hypoactive in monkey models (Chapter III) and human cocaine
users (e.g. Volkow et al., 1993; Tomasi et al., 2007a,b; Moeller et al., 2010) including the hippocampus, ACC, and striatal regions. Varenicline did not substitute for cocaine in drug discrimination studies, and was not self-administered when substituted for cocaine under a PR schedule of reinforcement (Chapter V). In rodents, varenicline blocked cue- and cocaine-induced reinstatement of previously extinguished cocaine-maintained behavior at low doses in rodents (Guillem and Peoples, 2010). Varenicline moderately stimulated dopamine release but to a lesser degree than nicotine (Rollema et al., 2007), possibly a mechanism to curb craving or depressive-like symptoms associated with initial periods of abstinence (Rollema et al., 2009a,b). Further, long-term treatment with varenicline resulted in increased D2-like receptor density in drug-naive rats measured via autoradiography (Crunelle et al., 2009, 2011), similar to increased D2-like receptor availability following treatment with DA antagonists in drug-naive monkeys assessed via PET imaging (e.g. Czoty et al., 2005a). Reversal of cocaine-induced neuroadaptations, such as increasing DA D2-like receptor availability, has been a proposed treatment strategy (for reviews see, Nader et al., 2008; Stairs and Bardo, 2009; Vokow et al., 2009) given that higher D2-like receptor availability is associated with reduced vulnerability to the reinforcing effects of cocaine in rodents, monkeys, and humans (Volkow et al., 1999; Morgan et al., 2002; Czoty et al., 2005b; Dalley et al., 2007).

However, varenicline potentiated the reinforcing strength of cocaine (Chapter V) and therefore, by historical assessments would be discounted for future pharmacotherapeutic potential. It must be noted that varenicline potentiated the reinforcing strength of cocaine at doses that also produced disruptions in food-maintained responding (0.3 and 0.56 mg/kg), but had no effect on the reinforcing strength of cocaine.
at lower doses. Further, varenicline potentiated but did not substitute for the
discriminative stimulus effects of cocaine (Chapter V). Despite the inability of
varenicline to attenuate the reinforcing strength or discriminative stimulus effects of
cocaine, varenicline may possess pharmacotherapeutic potential in aiding treatment-
seeking cocaine users, not by reducing cocaine’s reinforcing effects during potential
relapse, but through restoring underlying neurobiology to pre-addictive states, improving
mood, cognitive control and, thus relapse prevention (O’Brien, 2008; Rollema et al.,
2009a,b).

Two preliminary clinical trials, each with small sample sizes, have evaluated the
effects of varenicline as a treatment for cocaine dependence. The first clinical trial
evaluated varenicline versus saline in methadone-maintained cocaine users that were also
heavy smokers, in conjunction with once weekly CBT (Poling et al., 2010). Interestingly
the rate of smoking decreased, but the number of cocaine-positive urines was not
different from the control group. Although not significantly different, the retention rate
over the 12-week period was 92% for the varenicline group compared to 78% in the
placebo group. In this same study, depression scores were lower in the varenicline
treatment group compared to placebo. The second clinical trial also compared varenicline
versus saline treatment in cocaine users only, that were not methadone-maintained and
employed CM strategies (Plebani et al., 2011). Participants receiving placebo were two-
times more likely to use cocaine over the course of an 8-week treatment period. The
group receiving varenicline reported decreased rates of cocaine reward, assessed by a
questionnaire rating preference between different monetary values and doses of cocaine.
Notably, in the study by Plebani and colleagues (2011) likelihood of cocaine use and
rating of reward value decreased, opposite the cocaine SA and drug discrimination results from Chapter V. These discrepancies may be a result of inherent differences in preclinical versus clinical studies (see Chapter I) or an effect of the doses of varenicline tested (see below). These preliminary assessments warrant further examination on a larger scale, and reiterate, as discussed in Chapter I, the utility of pharmacological + behavioral treatment strategies. Furthermore, extending initial success of smoking cessation (e.g. Gonzalez et al., 2006; Jorenby et al., 2006) to recent success in reduction of ethanol intake (McKee et al., 2009) and cigarette smoking in cocaine users (Poling et al., 2010), varenicline may enhance success of abstinence from psychostimulant use by minimizing polydrug use. An ongoing dilemma in polydrug users is that alcohol and cigarette smoking often facilitates cocaine use (e.g. Schorling et al., 1994; Roll et al., 1996).

Interestingly, the dose range of varenicline that produced maximal cognitive enhancing effects in the cocaine-experienced monkeys in Chapter IV was 0.001-0.03 mg/kg varenicline. In Chapter V, doses that potentiated the discriminative stimulus effects of cocaine were 0.1 and 0.3 mg/kg and doses administered chronically that potentiated the reinforcing efficacy of cocaine were 0.3 and 0.56 mg/kg varenicline. Notably, doses that increased cocaine-related behaviors in Chapter V also reduced food-maintained responding and were almost a log unit higher than doses that enhanced cognition. Low doses of varenicline that acutely improved working memory did not affect the discriminative stimulus effects or reinforcing strength of cocaine in Chapter V. Doses that potentiated the discriminative stimulus effects and reinforcing strength of cocaine in Chapter V were on the descending limb of the dose-response curve relative to working memory performance, or in some monkeys disrupted food-maintained
responding during cognitive assessment (data not shown). Similarly, in the rodent studies above, cognitive enhancing effects were reported from 0.04-0.4 mg/kg varenicline (King et al., 2011), 0.1 and 0.3 mg/kg varenicline reduced cue- and cocaine-induced reinstatement of cocaine-seeking behavior, and only higher doses of varenicline (2.0 mg/kg) potentiated cue-induced reinstatement of cocaine seeking behavior (Guillem and Peoples, 2010).

Together these preclinical assessments suggest that at low doses, varenicline may have beneficial effects on cognition and on models of relapse, although at high doses varenicline may produce unwanted effects in models of cocaine abuse, highlighting an interesting point in terms of therapeutic efficacy. A drug can have multiple effects, including cognitive-enhancing or -disrupting effects, discriminative stimulus effects, reinforcing effects, aversive effects, and other unconditioned effects; these effects most likely occur at different dose ranges. Although nicotine, varenicline, and PNU-282987 each improved working memory, these same doses may not have similar effects on other cognitive domains. For example, working memory is a cognitive domain more reliant on PFC and temporal brain regions whereas measures of behavioral flexibility require more cortico-striatal involvement. In the hypothetical example below (Figure 3) cognitive function is characterized as an inverted-U-shaped curve. Cognitive performance (y-axis) can be influenced by varying drug doses (x-axis). One drug may differentially affect cortical versus subcortical activity such that a single dose (a) may enhance cognitive performance on a single cognitive domain (Task A) without affecting a second task (Task B). However, at higher doses, performance on both tasks may be enhanced or (b) performance of Task A may be impaired while Task B performance is enhanced. At even
higher doses (c) the drug may impair cognitive performance across both tasks and have reinforcing effects. Even higher doses may engender effects such as sedative or aversive physiological effects that reduce SA (d). Dose (d) could also represent a drug dose that reduces the reinforcing effects of cocaine at doses that impair cognition.

Figure 3. Overlapping effects on behaviors relevant to pharmacotherapeutic development for cocaine dependence. In this hypothetical example, a low dose of drug (a) will improve cognition on task A; a moderate dose of the same drug (b) will inhibit performance of Task A but enhance performance at Task B. At higher doses (c), the same drug may be reinforcing, but only at doses that impair cognition (Tasks A and B); and even higher doses (d) may be associated with aversive effects, or a reduction in the reinforcing effects of a drug at doses that impair cognition; figure modified from Cools and D’Esposito, 2011.
Assessing multiple behavioral effects will provide more information than any one behavioral assay alone when evaluating a potential pharmacotherapy. Under ideal situations, the cognitive enhancing effects of a drug should overlap across multiple cognitive domains. This same drug treatment should not maintain responding when substituted for the drug of abuse suggesting low or no abuse liability (similar to varenicline in Chapter V). The effects of a drug treatment in combination with the substance of abuse (e.g. cocaine) should attenuate cocaine-related effects. However, relapse to drug use is often associated with medication non-compliance, or high dose self-administration may overcome beneficial effects of a medication. Therefore, relapse prevention may be more important for successful treatment than pharmacological approaches to reduce drug-related effects. From this perspective, a drug profile similar to varenicline in which the potentiation of cocaine’s effects occur only at high doses (far right side of Figure 3), possibly outside the therapeutic window, is worth further investigation. For comparison, another indirect DA agonist, modafinil has shown positive signals both in preclinical and preliminary clinical studies (see Chapter I) despite demonstrating abuse liability in monkeys at extremely high doses (Gold and Balster, 1996).

We did not assess PNU-289287 for its ability to influence the discriminative stimulus effects or reinforcing strength of cocaine. PNU-282987 and varenicline represent two divergent approaches to therapeutic development. Varenicline is an FDA-approved drug with a well-established mechanism of action. Although we have focused on the ability of varenicline to stimulate DA release, varenicline also increased NE and histamine release in the PFC an effect hypothesized to be at doses that activated α7-
nAChRs (Rollema et al., 2009b) and is an agonist at the 5HT3 receptor (Lummis et al., 2011). PNU-282987 is a newly synthesized potent, α7-selective nAChR agonist that is inactive at monoamine, glutamate, muscarine, and GABA receptors and, only at extremely high doses is a low affinity antagonist at 5HT3 receptors (Bodnar et al., 2005; Hajos et al., 2005). However, the in vivo profile including abuse potential or the ability to influence psychostimulant-related behaviors of this compound is largely unknown. The highly selective effects of PNU-282987 need to be further characterized and compared to the in vivo effects of other nAChR agonists such as varenicline whose net effects spread across neurotransmitter systems.

CONCLUSION

The research conducted in this dissertation established a nonhuman primate model of cocaine-associated cognitive deficits, demonstrating deficits in measures of behavioral flexibility and working memory and underlying neurobiological deficits similar to those reported in human cocaine users. Nicotinic acetylcholine receptor agonists were examined for their cognitive enhancing effects, one pharmacological approach to aid treatment of cocaine dependence through improving the success of behavioral modification strategies. Nicotine enhanced working memory, although its abuse liability precludes its therapeutic utility. Varenicline enhanced working memory, possessed low abuse liability, but potentiated the reinforcing and discriminative stimulus effects of cocaine. Thus, varenicline may have therapeutic utility in enhancing cognition that may aid behavioral modification to prevent relapse within a low dose range, but will not be efficacious in reducing cocaine-related behaviors directly. PNU-282987 produced similar
cognitive enhancing effects; its full therapeutic potential is yet to be determined.

Nicotinic acetylcholine receptor agonists appear to be a promising target for cognitive enhancement. By combining classic behavioral pharmacology with cognitive assessment and PET imaging, the current research provides a better understanding of the effect of cocaine on neurobiology and cognition, and the therapeutic utility of nAChR agonists as a mechanism for further development to treat cocaine dependence.
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HONORS AND AWARDS

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2010  Selected as WFUHS graduate student representative to apply for US delegation to attend the annual meeting of Nobel Laureates in Lindau, Germany (10/2010)

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**Gould RW.** Effects of chronic cocaine self-administration on neurobiology and cognition in monkeys. Student Seminar Series, Dept. Physiology/Pharmacology, Wake Forest University School of Medicine, Winston Salem, NC; Nov. 22, 2010.


**Gould RW.** Effects of cocaine self-administration on cognitive performance in monkeys. Student Seminar Series, Dept. Physiology/Pharmacology, Wake Forest University School of Medicine, Winston Salem, NC; March 1, 2010.

**Gould RW.** Effects of chronic cocaine self-administration on cognitive performance in monkeys. 2009 WFU/Emory University annual lab exchange: Atlanta, GA; Sept. 18, 2009.


**Gould RW.** Nicotinic acetylcholine receptors as targets of pharmacotherapies for cocaine dependence. Student Seminar Series, Dept. Physiology/Pharmacology, Wake Forest University School of Medicine, Winston Salem, NC; March 9, 2009.
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COMMUNITY ACTIVITIES AND SERVICE

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