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Debra A. Cooper Date

Effects of pharmacological dopamine β-hydroxylase inhibition on cocaine-induced behavior and neurochemistry

By

Debra Andrea Cooper

Doctor of Philosophy

Graduate Division of Biological and Biomedical Science

Neuroscience

_____
Leonard Howell
Advisor

_____
David Weinshenker
Advisor

_____
Heather Kimmel
Committee Member

_____
Robert Malison
Committee Member

_____
Yoland Smith
Committee Member

Accepted:

_____
Lisa A. Tedesco, Ph.D. Dean of the James T. Laney School of Graduate Studies

_____ Date

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By

Debra Andrea Cooper

B.S., Duke University, 2006

Advisor: Leonard L. Howell, Ph.D.

Advisor: David Weinshenker, Ph.D.

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Abstract

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Disulfiram has shown promise as a pharmacotherapy for cocaine dependence in clinical settings, though it has many molecular targets and the behavioral and molecular mechanisms underlying its efficacy are unclear. One of many biochemical actions of disulfiram is inhibition of dopamine beta-hydroxylase (DBH), the enzyme that converts dopamine to norepinephrine (NE) in noradrenergic neurons. Thus, disulfiram simultaneously reduces NE and elevates DA levels in the brain. Because we know that relapse-like behavior in animal models depends on NE signaling, we hypothesized that DBH inhibition and the subsequent reduction in NE levels mediates the therapeutic effects of disulfiram. Indeed, we found that disulfiram decreased brain NE levels and blocked cocaine-primed reinstatement of drug seeking in rats (a commonly used model of relapse), consistent with clinical results. Furthermore, nepicastat, a selective DBH inhibitor that lacks disulfiram's target promiscuity and adverse side effects, also blocks cocaine-primed reinstatement in rats, supporting the use of DBH inhibitors for the treatment of cocaine dependence. We next assessed the ability of DBH inhibitors to reduce cocaine seeking in non-human primates. Squirrel monkeys trained to self-administer cocaine under a second-order schedule were pretreated with disulfiram or nepicastat prior to cocaine-primed reinstatement sessions. Neither pretreatment altered cocaine-primed reinstatement. Unexpectedly, when administered alone, nepicastat was sufficient to induce a modest reinstatement effect. To investigate the neurochemical mechanisms underlying the behavioral results, the effects of DBH inhibition on extracellular DA were analyzed in the nucleus accumbens (NAc) using *in vivo* microdialysis in squirrel monkeys. Nepicastat had no effect on basal extracellular DA levels in the NAc, but attenuated cocaine-induced DA overflow. These results suggest that there may be species or methodological differences between rats and non-human primates that influence the behavioral and neurochemical discrepancies. Understanding the discrepancies between the animal models will ultimately be instrumental in influencing the translation of these therapies to a human population and determining under what specific circumstances DBH inhibition is a suitable treatment for preventing relapse to cocaine abuse.

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LIST OF ABBREVIATIONS

5-HT	5-hydroxytryptamine; serotonin
α1AR	α1 adrenergic receptor
α2AR	α2 adrenergic receptor
ALDH	aldehyde dehydrogenase
aCSF	artificial cerebrospinal fluid
ANOVA	analysis of variance
βAR	β-adrenergic receptor
BNST	bed nucleus of the stria terminalis
BOLD	blood oxygen level-dependent
CPP	conditioned place preference
CRF	corticotrophin releasing factor
DA	dopamine
DAT	dopamine reuptake transporter
DBH	dopamine β-hydroxylase
Dbh –/–	dopamine β-hydroxylase knockout
DOPAC	3,4-Dihydroxyphenylacetic acid
ED_{Max}	maximally-effective unit dose of cocaine (self-administration)
ED_{Peak}	maximally-effective dose of pre-session drug prime (reinstatement)
FI	fixed-interval
FR	fixed-ratio
HPLC	high-performance liquid chromatography
HVA	homovanillic acid
i.m.	intramuscular

i.p.	intraperitoneal
i.v.	intravenous
LC	locus coeruleus
mPFC	medial prefrontal cortex
NAc	nucleus accumbens
NE	norepinephrine
NET	norepinephrine reuptake transporter
PFC	prefrontal cortex
SEM	standard error of measurement
SERT	serotonin reuptake transporter
Veh	vehicle
VMAT	vesicular monoamine transporter
VTA	ventral tegmental area

CHAPTER I

Introduction

A. COCAINE HISTORY

Though cocaine is currently a cause of public health and criminal problems in our society, its early use was more innocuous. Drinking herbal infusions or chewing leaves from the *Erythroxylon coca* plant as a mild stimulant was documented several thousand years ago in South America. However, it was not until 1859, when Albert Niemann isolated alkaloid cocaine from the coca leaf, that cocaine abuse as it is recognized today started becoming a problem. Purified cocaine that Niemann isolated is a stronger stimulant than whole coca leaves. Cocaine was added in beverages and used as an analgesic. Sigmund Freud suggested its use for curing certain mental disorders and morphine addiction. Freud himself became addicted to cocaine, and by the end of the 19th century, an epidemic of cocaine abuse was beginning to occur. Despite increasing legislation starting in the early 20th century, cocaine abuse and dependence has been an ongoing problem.

According to the latest report by the National Survey on Drug Use and Health, over one million people in the U.S. aged 12 and older meet criteria for cocaine abuse or dependence and 24.4% of admissions to specialty substance abuse clinics are for the treatment of cocaine abuse (SAMHSA, 2011). The 2010 Drug Abuse Warning Network (DAWN) report states that cocaine use was responsible for nearly half of all emergency department visits for misuse or abuse of illicit drugs; the most of any drug of abuse (SAMHSA, 2012). The cycle of cocaine abuse and dependence typically includes recurring periods of abstinence, whether forced or voluntary. However, maintaining abstinence is a challenge that all rehabilitated cocaine users encounter. Between 40-60% of individuals who receive treatment for drug abuse in a

publicly funded treatment facility had previously been admitted in a rehabilitation program (SAMHSA, 2002). There are many factors that can cause a rehabilitated cocaine user to relapse, the strongest of those being stress and anxiety, exposure to illicit drugs, and exposure to environments related to the drug and/or drug taking.

While relapse is a problem that plagues any recovering cocaine abuser, there are some methods that have been utilized to reduce the likelihood of relapse. The National Institute on Drug Abuse (NIDA) asserts that drug abuse and dependence is a complex chronic disease that should be treated as such (NIDA et al., 1999). Because it affects both brain and behavior, successful treatment may require both behavioral therapy and pharmacological interventions. The most commonly used behavioral therapies are the 12-Step Facilitation Program and Cognitive Behavioral Therapy (CBT), each with varying reported rates of success (Rawson et al., 1991; Maude-Griffin et al., 1998). Preclinical and clinical research is currently being conducted on a myriad of pharmacological treatments with many different potentially therapeutic mechanisms of action. Some of the most recently explored strategies are vaccine therapy (Kinsey et al., 2010), agonist replacement therapy (Mooney et al., 2009; Negus et al., 2009; Rush et al., 2010), and drugs targeting glutamate receptors (Adewale et al., 2006; Peters and Kalivas, 2006; Lu et al., 2012), adrenergic receptors (Newton et al., 2012) and serotonin receptors (Cunningham et al., 2011; Manvich et al., 2012b; Pockros et al., 2012). Similar to the behavioral therapies, these pharmacological interventions have varying rates of success, both preclinically and clinically.

Cocaine abuse is an ongoing public health issue, yet despite decades of research and many clinical trials, there are no FDA approved pharmacologic

treatments for cocaine addiction. The goal of the current project was to identify a potential pharmacological treatment for reducing rates of relapse to cocaine abuse.

B. PHARMACOLOGICAL MECHANISMS

Though there are no widely accepted treatments for cocaine abuse, the neurotransmitters and neural circuitry involved in cocaine dependence have provided a framework for the discovery of such a pharmacotherapy. Cocaine blocks the reuptake of dopamine (DA), norepinephrine (NE) and serotonin (5-HT) by inhibiting their respective plasma membrane transporters (DAT, NET, and SERT), thus increasing the extracellular levels of these neurotransmitters in the brain (Reith et al., 1986; Madras et al., 1989; Ritz et al., 1990). Because of the disturbance of normal clearing and subsequent increased extracellular level of these neurotransmitters, activation at their respective receptor targets is prolonged. Since disruption of monoaminergic signaling alters several neurochemical and behavioral effects of cocaine in animals, medications that modulate these pathways are prime candidates for new therapeutics.

Cocaine binds with relatively similar affinity to these transporters, but its abuse related effects are mainly attributed to its inhibition at the DAT (Ritz et al., 1987). Animals will readily self-administer DAT-selective inhibitors (Howell and Byrd, 1991; Roberts, 1993; Howell et al., 2000; Lindsey et al., 2004), and many DAT-selective inhibitors fully substitute for cocaine (Cunningham and Callahan, 1991; Melia and Spealman, 1991; Witkin et al., 1991; Lindsey et al., 2004). Conversely, neither NET- nor SERT-selective inhibitors are readily self-administered by animals (Tessel and Woods, 1975; Woolverton, 1987; Howell and Byrd, 1995). Therefore, though cocaine disrupts the normal function of all monoamine neurotransmitters, the direct reinforcing

effects of cocaine are primarily mediated through increases in extracellular concentrations of DA.

C. Neuroanatomical Substrates Of Cocaine-Mediated Behaviors

DA has been linked to regulating motivation, emotional responses, and behavioral responses to stress (Trainor, 2011). DA is also correlated with responses to rewarding stimuli (Salamone and Correa, 2012). It is activated by rewarding stimuli, but its involvement in learning converts it to later respond to anticipation of reward and positive prediction error (Glimcher, 2011). Among the various DA systems, the mesocorticolimbic system is classically thought to modulate the abuse-related effects of cocaine (Di Chiara and Imperato, 1988; Schultz, 1997; Wise, 2004). The mesocorticolimbic system consists of DA neurons that originate in the ventral tegmental area (VTA) and project to both cortical (prefrontal cortex; PFC) and limbic (nucleus accumbens; NAc) targets (reviewed in Moore and Bloom, 1978). Each of these areas exhibits altered blood oxygen level-dependent (BOLD) responses following acute cocaine administration in humans (Kufahl et al., 2005). Most drugs of abuse, including, but not limited to psychostimulants, opiates, ethanol, and nicotine, ultimately increase extracellular DA levels in mesocorticolimbic areas (Di Chiara and Imperato, 1988). Not only do these drugs actively affect levels of DA, but there is also a relationship between DA signaling and behavioral reinforcement of both drugs of abuse and natural stimuli. In both rodents and nonhuman primates, presentation and later anticipation of a food reward activates dopamine neurons (Ljungberg et al., 1992; Schultz et al., 1993; Richardson and Gratton, 1996; Schultz, 1997). Similarly, intracranial self-stimulation (ICSS) studies, in which rats were trained to press a lever for a reinforcing electric shock in the VTA, evokes dopamine release in the NAc (Owesson-White et al., 2008). Subsecond analyses using fast-scan cyclic voltammetry

reveal that DA is released from NAc in rats immediately prior to lever pressing for cocaine and after presentation of drug associated stimuli (Phillips et al., 2003). Psychostimulants are readily self-administered when infused directly into the NAc or PFC of rats (Phillips et al., 1994; McBride et al., 1999; McKinzie et al., 1999). Lesioning the VTA (Roberts and Koob, 1982) or NAc (Suto et al., 2011) or locally infusing the DA D_1 receptor antagonist SCH23390 into the NAc (Maldonado et al., 1993) of rats disrupts the reinforcing effects of self-administered cocaine. Similarly, locally infusing DA receptor antagonists into the basolateral amygdala of rats, which also receives DA innervation from the VTA, attenuates the reinforcing effects of conditioned drug-associated stimuli (See et al., 2001; Di Ciano and Everitt, 2004). DA in the PFC is also important for cocaine-seeking. DA infused directly into the PFC increases cocaine-seeking (McFarland and Kalivas, 2001) while DA antagonists infused directly into the PFC attenuate cocaine-seeking (McFarland and Kalivas, 2001; Park et al., 2002; Sun and Rebec, 2005). Together, these studies highlight the involvement of the mesocorticolimbic DA system in the reinforcing effects of cocaine.

D. Norepinephrine And Reinforcing Behaviors

Though much research has focused on the role of DA in the reinforcing effects of cocaine, other monoamines that are affected by abused drugs also influence the subsequent behavioral effects. For instance, though not as closely associated with the behavioral and reinforcing effects of drugs of abuse as DA, NE indeed has a functional role in the behavioral effects of drugs of abuse. Noradrenergic cell bodies originate in the locus coeruleus (LC) and lateral tegmental field of the brainstem (reviewed in Moore and Bloom, 1979). The LC constitutes the largest noradrenergic nucleus in the brain and is composed solely of NE neurons. These neurons have both descending

and ascending projections. Descending projections terminate in the spinal cord, brainstem, and cerebellum; three ascending pathways terminate widely throughout the brain innervating the VTA, thalamus, neocortex, and amygdala amongst other structures (Moore and Bloom, 1979; Mejias-Aponte et al., 2009). Similar to the LC projections, both A1 and A2 brainstem nuclei project to the VTA, but additionally project to the hypothalamus, the bed nucleus of the stria terminalis (BNST), and the NAc (Lindvall and Stenevi, 1978; Moore and Bloom, 1979; Mejias-Aponte et al., 2009). These ascending projections terminate near dopaminergic cell bodies in the VTA (Jones et al., 1977; Simon et al., 1979; Liprando et al., 2004) and terminals in the NAc (Berridge et al., 1997; Delfs et al., 1998; Tong et al., 2006; Mitrano et al., 2012). Consequently, all areas within the mesocorticolimbic system are innervated by noradrenergic projections. NE also functionally affects dopaminergic output in these areas (reviewed in Weinshenker and Schroeder, 2007). NE applied to midbrain tissue (Grenhoff et al., 1995) or electrical stimulation of LC noradrenergic neurons in rats (Grenhoff et al., 1993) produces burst firing of VTA neurons. Conversely, lesioning LC neurons decreases striatal DA activity (Tassin et al., 1979) and release (Russell et al., 1989; Lategan et al., 1990, 1992). Furthermore, the α1-adrenoreceptor antagonist prazosin decreases dopaminergic excitation (Grenhoff et al., 1993).

Though modulation of NE levels affects dopaminergic neuron firing, early studies employing drug self-administration did not find a role for NE in the behavioral effects of drugs of abuse. Drug self-administration is a commonly employed and well-established technique for investigating the reinforcing properties of abused drugs. Various phases of cocaine self-administration can be studied using this technique including maintenance (a period of stable and reliable lever pressing behavior resulting in drug delivery), extinction (a period during which lever presses do not result in drug

delivery, therefore gradually reducing lever pressing behavior), and reinstatement (a session in which a stimulus reestablishes lever pressing behavior in the absence of a drug reinforcement). Early studies showed no alteration of lever pressing behavior following noradrenergic manipulation during the maintenance phase of psychostimulant self-administration.

Blockade of α1-adrenergic receptors (α1ARs) does not attenuate the reinforcing properties of either amphetamine (Risner and Jones, 1976; Yokel and Wise, 1976) or cocaine (Woolverton, 1987). Similarly, the α1AR agonist methoxamine has no effect on responding for amphetamine in self-administration studies (Risner and Jones, 1976). NE reuptake inhibitors are not self-administered, do not alter cocaine self-administration behavior, and are not substituted for cocaine in rhesus monkeys (Woolverton, 1987; Wee et al., 2006). Additionally, lesioning the ascending noradrenergic system of rats with 6-hydroxydopamine (6-OHDA) fails to affect responding for cocaine (Roberts et al., 1977). Conversely, manipulating DA transmission produces marked effects on the reinforcing properties of psychostimulants. Lever pressing is attenuated whether ascending dopaminergic systems are lesioned (Roberts et al., 1977) or DA release is pharmacologically blocked (Risner and Jones, 1976; Yokel and Wise, 1976). An intact dopaminergic system is therefore required for the reinforcing effects of psychostimulants; however, NE is not necessary to maintain psychostimulant self-administration.

Though NE does not appear to affect the primary reinforcing properties of psychostimulants measured by maintenance of self-administration, there is growing evidence that NE has influence on other abuse-related properties of these drugs (Kongyingyoes et al., 1988; Drouin et al., 2002a; Drouin et al., 2002b; Ventura et al., 2003). Treatment with the α1AR antagonist prazosin blocks enhancement of cocaine

self-administration in rats pre-exposed to cocaine (Zhang and Kosten, 2007). Prazosin also reduces the higher breakpoint for responding for cocaine under a progressive-ratio schedule of reinforcement in rats that were trained with prolonged access to cocaine (Wee et al., 2008). Furthermore, pharmacologically blocking NE synthesis in rats also lowers the breakpoint for responding for cocaine under a progressive-ratio schedule of reinforcement (Schroeder et al., 2013). This suggests that some behavioral effects of cocaine require a fully functioning noradrenergic system. More recent studies have found NE to be important in the reinstatement phase of self-administration. The reinstatement phase of self-administration is an animal model of the human relapse condition and can be precipitated by one of three different stimuli: non-contingent drug priming, presentation of a cocaine-associated cue, or a stressor. In rats, pharmacologically blocking the α1AR attenuates cocaine-primed reinstatement (Zhang and Kosten, 2005), agonists for the α2AR autoreceptor and antagonists for the βAR reduce stress-induced reinstatement (Erb et al., 2000; Leri et al., 2002), and a cocktail of α1AR and βAR antagonists suppress cue-induced reinstatement (Smith and Aston-Jones, 2011). Reinstatement can be induced by administering intracerebroventricular infusions of NE itself (Brown et al., 2009). Additionally, both α2AR antagonists, which increase NE signaling by blocking the autoreceptor, and NET inhibitors induce reinstatement of cocaine seeking in squirrel monkeys (Lee et al., 2004; Platt et al., 2007). NET inhibitor induced reinstatement can be reversed by pretreating with the α1AR antagonist prazosin, the βAR antagonist propranolol, or the α2AR agonist clonidine (Platt et al., 2007). These results indicate that increasing noradrenergic signaling is sufficient to reinstate drug-seeking behavior on its own and is necessary for stress-induced and cocaine-primed reinstatement, though via distinct adrenoreceptors.

Not surprisingly, reinstatement elicited by different stimuli not only involves different adrenoreceptors, but also involve different neuroanatomical structures and pathways. Drug-induced reinstatement is reliant on a functional connection between the NAc and the PFC. Our laboratory has recently found that infusing an α1AR antagonist into the PFC attenuates cocaine-primed reinstatement in rats (Schroeder et al., unpublished). Blocking DA receptors in the dorsal PFC attenuates cocaine-induced reinstatement; likewise, infusing DA directly into the dorsal PFC induces reinstatement (McFarland and Kalivas, 2001). Additionally, infusing DA (Cornish and Kalivas, 2000) or cocaine (Park et al., 2002) directly into the NAc can induce reinstatement. Intra-accumbal DA-induced reinstatement can be reversed by co-infusing a DA receptor antagonist (Cornish and Kalivas, 2000). In contrast, stress-induced reinstatement involves the extended amygdala and both corticotrophin releasing factor (CRF) and NE. Stressful stimuli cause release of NE from brainstem nuclei which activate CRF release from the central amygdala which, in turn, projects to the bed nucleus of the stria terminalis (BNST) (reviewed in Sinha et al., 2011). NE also projects directly to the BNST, and blocking βAR in either the BNST or central amygdala reduces stress-induced reinstatement (Leri 2002). Neurons from the BNST then project widely to many targets including dopaminergic VTA neurons. Support for this circuit is provided from studies which demonstrate that intra-VTA infusion of CRF2 receptor antagonists block footshock-induced reinstatement while intra-VTA infusion of CRF or CRF agonists induces reinstatement to levels comparable to footshock-induced reinstatement (Wang et al., 2007). Despite the different circuits involved with the various modes of reinstatement, each pathway converges with the involvement of NE and the mesocorticolimbic DA system.

E. Role Of Dopamine β-Hydroxylase In Abuse Related Behaviors

Disulfiram (Antabuse®) has been used as a pharmacotherapy for alcohol abuse for over 50 years (Moriarty, 1950; Fuller et al., 1986). Disulfiram inhibits aldehyde dehydrogenase (ALDH), an alcohol metabolizing enzyme. Inhibition of ALDH leads to an accumulation of acetaldehyde upon alcohol consumption causing the aversive "Antabuse reaction" (i.e. flushing, nausea, and vomiting) (Kitson, 1977) which serves as a psychological deterrent for reducing alcohol intake. Because of the high comorbidity of alcohol and cocaine abuse (Regier et al., 1990; Carroll et al., 1993), disulfiram was later tested in codependent abusers to determine whether it is efficacious in reducing cocaine intake in this population. Indeed, disulfiram is efficacious in reducing both alcohol intake and cocaine intake in this codependent population (Carroll et al., 1998; Carroll et al., 2000). Further studies determined that disulfiram can still reduce cocaine intake in cocaine addicts that do not meet criteria for alcohol dependence and consume very little if any alcohol (George et al., 2000; Petrakis et al., 2000). Moreover, participants that are either not alcohol dependent or abstain from alcohol throughout the duration of the study period benefit the most from the therapy (Carroll et al., 2004; Carroll et al., 2012). Additional studies have continued to find efficacy with disulfiram treatment, especially within particular subgroups. Pharmacogenetic studies have demonstrated that disulfiram is more efficacious in reducing cocaine intake in subjects who have normal dopamine β-hydroxylase (DBH) levels compared to subjects who have a variant in the gene encoding DBH which produces lower levels of DBH (Kosten et al., 2013). Genetic variants for the MTHFR gene, which promotes protein methylation, have also been associated with affecting the efficacy of disulfiram (Spellicy et al., 2012). Weight based dosing has also been shown to be vital to the efficacy of disulfiram. When distributed by milligrams of disulfiram per kilogram of body weight (similar to dosing regimens used in preclinical studies), dose was negatively correlated with number of cocaine infusions (Haile et al.,

2012). While the mechanism by which disulfiram reduces alcohol intake is known, ALDH inhibition and the subsequent acetaldehyde accumulation is not involved in the interaction between disulfiram and cocaine. Therefore, reduction of cocaine intake by disulfiram must occur through some other mechanism.

Disulfiram has many mechanisms of action and therefore inhibits a wide range of enzymes (Goldstein et al., 1964; Musacchio et al., 1966). ALDH is inhibited by disulfiram through covalent modifications of its sulfhydryl group. The primary metabolite of disulfiram, diethyldithiocarbamate, is a copper chelator that is responsible for impairing the function of any enzyme that requires copper as a co-factor (Johansson, 1989). Dopamine β-hydroxylase (DBH), a copper containing mono-oxygenase, is inhibited by diethyldithiocarbamate. DBH is an enzyme in the catecholamine biosynthetic pathway that converts DA into NE in noradrenergic and adrenergic neurons (reviewed by Weinshilboum, 1978). Disulfiram consequently inhibits NE synthesis, reducing NE levels and concomitantly increasing tissue DA levels (Karamanakos et al., 2001; Bourdelat-Parks et al., 2005). Because of the role of NE and DA in abuse related effects, it has been hypothesized that the mechanism by which disulfiram is reducing cocaine intake in clinical settings is via DBH inhibition.

Genetically modified mice lacking the DBH gene (*Dbh* –/–) support this hypothesis, as they have altered responses to cocaine. In a conditioned place preference (CPP) test examining the effect of cocaine induced behavior, *Dbh* –/– mice showed a place aversion at doses that generally produce place preference in wild-type mice (Schank et al., 2006). This place aversion could not be recreated in wild-type mice at doses that do not produce seizure activity. Additionally, *Dbh* –/– mice show increased locomotor activity and/or stereotypy in response to both cocaine and amphetamine (Weinshenker et al., 2002; Schank et al., 2006). Disulfiram does not

affect either acute cocaine-induced locomotor behavior or locomotor behavior in cocaine-sensitized *Dbh* –/– mice, however it enhances locomotor behavior in both paradigms in wild-type mice (Gaval-Cruz et al., 2012). This implies that disulfiram is facilitating cocaine sensitization through DBH inhibition.

These studies imply that *Dbh* –/– mice are hypersensitive to the effects of psychostimulants, particularly to their aversive properties. This hypersensitivity has links to a common polymorphism found in humans. A C-to-T polymorphism at the -1021 nucleotide position of the DBH gene (allele frequency of 0.22) accounts for much of the genetic variance in DBH activity (Zabetian et al., 2001). There is therefore high variability in plasma DBH activity in humans (Weinshilboum et al., 1975). Individuals heterozygous for the T allele (the low activity allele) have DBH activity levels about 50% lower than CC homozygotes, and TT homozygotes have levels about 90% lower than CC homozygotes (Zabetian et al., 2001). Interestingly, cocaine abusers with genetically low DBH activity report experiencing increased cocaine-induced paranoia (Cubells et al., 2000; Kalayasiri et al., 2007). Analogously, cocaine abusing individuals receiving disulfiram treatment report increased cocaine-induced paranoia (Hameedi et al., 1995; Mutschler et al., 2009). Furthermore, it has been demonstrated that disulfiram is efficacious specifically in subjects with a normal DBH genotype (Kosten et al., 2013). Separated by genotype, disulfiram reduces cocaine positive urine samples by about 30% in subjects who are homozygous for the C allele compared to placebo. However, there is no disulfiram effect compared to placebo in subjects who possess at least one T allele. This suggests that DBH activity and function is involved in the clinical efficacy and possibly the therapeutic mechanism of disulfiram. The combination of decreased NE levels (which, as previously stated is important in attenuating reinstatement of cocaine-seeking in animal models) and increased cocaine-induced

paranoia due to DBH inhibition may underlie the ability of disulfiram to reduce cocaine use.

Because disulfiram inhibits a wide range of enzymes, including but not limited to ALDH, DBH, and hepatic enzymes (Stripp et al., 1969; Zemaitis and Greene, 1976), a selective DBH inhibitor would be useful for determining whether disulfiram-induced changes in cocaine responses can be attributed to DBH. Nepicastat (also called "SYN-117"), is a direct, competitive inhibitor of DBH (Stanley et al., 1997; Kapoor et al., 2011). Nepicastat does not chelate copper, acts directly on DBH, does not affect a panel of various receptors and enzymes and is a more potent inhibitor of DBH than disulfiram (disulfiram IC_{50}=1μM, nepicastat IC_{50}=9nM) (Stanley et al., 1997; Kapoor et al., 2011). As such, not only is nepicastat a more selective inhibitor of DBH than disulfiram, it also does not have the aversive side effect profile that characterizes disulfiram (Wilson, 1962; Heath et al., 1965; Ewing et al., 1977; Ewing et al., 1978; Major et al., 1979). It has recently been demonstrated that nepicastat suppresses some subjective effects of cocaine such as maximum high, craving, and "drug effect" (Cunningham et al., 2010), indicating that it is a promising candidate pharmacotherapy for treating cocaine abuse. A multisite phase II clinical trial is scheduled to begin April, 2013, to test the efficacy of nepicastat in cocaine addicted subjects (ClinicalTrials.gov, 2012).

F. Advantages Of Nonhuman Primate Models of Pharmacological Effects

The impact of pharmacologically manipulating NE levels on cocaine mediated effects has mainly been studied in rodent models. There are many advantages for using rodents in research involving drugs of abuse. Amongst these are the ability to utilize genetically manipulated mouse models (i.e. DBH -/- mice) and the ease of use

as a model organism for screening the effects of novel compounds. However, there are important concerns to note that may impact the generalization of findings in rodents to human cocaine users.

Firstly, there are marked neuroanatomical differences between rodents and humans in brain regions that are pertinent to the abuse-related effects of cocaine. Nonhuman primates are more similar to humans in regards to many of these differences. For example, the distribution of DA D1-like and D2-like receptors differs between rodents and primates. The D1:D2 receptor ratio is higher in rats than nonhuman primates and humans (reviewed in Weerts et al., 2007). There are also reported differences in distribution of α1, α2 and β adrenergic receptors (Weerts et al., 2007). Additionally, NET distribution in nonhuman primates is similar to humans in various brain regions, and both differ from NET distribution in rats (Weerts et al., 2007). Furthermore, there are species differences in regional interconnectivity, in particular, in DA rich areas of the PFC and striatum. Dopaminergic afferents and efferent synaptic connections in these areas in humans are more homologous to nonhuman primates than to rats (Haber et al., 2000; Uylings et al., 2003; Seamans et al., 2008; Haber and Knutson, 2010). Not only are nonhuman primates more similar to humans with respect to the neuroanatomy of their monoamine neurotransmitter systems, but nonhuman primates may also be a more valid animal model than rodents for studying the pharmacological effects of compounds acting on these systems (Weerts et al., 2007). For instance, NET in rats has differing affinity for substrates and inhibitors, including cocaine, than human NET (Paczkowski et al., 1999). These differences suggest that the impact of novel pharmacotherapies on cocaine effects may be better modeled in nonhuman primates.

Another advantage for using nonhuman primates for evaluating potential pharmacotherapies is the similarity to drug pharmacokinetics in humans.

Pharmacokinetics pertains to rates of drug uptake, clearance, and metabolism. Rodents typically have higher metabolic rates than nonhuman primates and humans. Therefore, pharmacokinetic parameters measured in nonhuman primates are thought to be better predictors of drug pharmacokinetics found in humans (Ward and Smith, 2004b, a; Jolivette and Ward, 2005; Ward et al., 2005). There are even reported differences between rodents and both nonhuman primates and humans in psychostimulant metabolism (Weerts et al., 2007). Drugs can therefore be metabolized into different active and inactive metabolites, which can result in varying drug-induced effects. These effects may be stronger, weaker, or absent in rats compared to nonhuman primates and humans. In this regard, the similarity in pharmacokinetics between nonhuman primates and humans is advantageous when investigating the pharmacotherapeutic potential of experimental compounds.

An additional consideration when conducting studies on the behavioral effects of drugs of abuse is methodological differences. Because nonhuman primates have a longer lifespan than rodents, long-term within-subject experimental designs are more feasible in nonhuman primates. This in turn reduces the number of subjects required for statistical power. The within-subject design is also more feasible in nonhuman primates because of the ability to maintain intravenous catheter patency. Self-administration studies in rats are typically limited to several weeks or months due to difficulties in maintaining catheter patency. Conversely, patency in a single catheter preparation in nonhuman primates can be maintained for several years. Furthermore, the duration of drug-taking can be extended in nonhuman primates with multiple, consecutive catheter preparations if a catheter becomes occluded. This more closely models human drug abuse which often spans several years. Additionally, any important neuroadaptations that occur in response to chronic drug use will likely also be incorporated in animal models designed with extended drug exposure. Because of

the shorter duration for catheter patency, it is nearly impossible to create an analogous model in rodents.

Though the use of rodents for investigating novel pharmacotherapies for drug abuse produces valuable information, the aforementioned factors indicate a necessity for also using nonhuman primate subjects. The present study took advantage of the unique value of each animal model for investigating potential pharmacotherapeutic interventions.

G. Summary And Experimental Rationales

Preclinical cocaine self-administration studies have demonstrated that reducing brain levels of NE results in an attenuation of reinstatement of cocaine-seeking. In clinical settings, disulfiram has shown promise as a cocaine abuse pharmacotherapy. A probable mechanism by which these results are occurring is through inhibition of DBH and reduction of brain NE levels. Additionally, the increase in tissue DA may function as a DA replacement therapy further aiding in the efficacy of disulfiram. Pharmacological DBH inhibition may be a promising target for substance abuse medication development. Further investigation, using animal models, needs to be carried out to confirm this mechanism. While disulfiram has several biological targets and subsequently various adverse side effects, the selective DBH inhibitor nepicastat does not present the same adverse side effect profile. Hence, nepicastat is an ideal candidate for a pharmacotherapeutic intervention that targets DBH. Therefore, the aims of this research project were as follows:

1. To explore the effects of DBH inhibition on cocaine-induced reinstatement of cocaine-seeking behavior in rats

2. To explore the effects of DBH inhibition on cocaine-seeking behavior in squirrel monkeys

3. To determine the effect of DBH inhibition on catecholamine neurochemistry in the ventral striatum (NAc) following cocaine administration in squirrel monkeys

CHAPTER II

Pharmacological DBH Inhibition: Effect on Cocaine Reinforcement and Reinstatement in Rats

A. INTRODUCTION

Disulfiram (Antabuse) has been used for more than 50 years in the treatment of alcoholism (Fuller et al., 1986). Disulfiram inhibits aldehyde dehydrogenase (ALDH), which results in the accumulation of acetaldehyde upon ethanol ingestion. This toxic metabolite produces aversive symptoms, such as flushing, nausea, and vomiting, and a desire to avoid this aversive reaction encourages abstinence. Because 50-90% of patients who abuse cocaine also abuse alcohol (Weiss et al., 1988; Grant and Harford, 1990; Closser and Kosten, 1992; Khalsa et al., 1992), the belief was that discouraging alcohol consumption in cocaine- and alcohol-dependent individuals might lower cocaine use. Indeed, disulfiram was found to reduce alcohol and cocaine intake in this patient population (Carroll et al., 1993; Carroll et al., 1998; Carroll et al., 2000). Surprisingly, further studies went on to reveal that disulfiram is at least as effective at treating cocaine addicts who do not consume alcohol, and may even be more effective (George et al., 2000; Petrakis et al., 2000; Carroll et al., 2004). Therefore, an aldehyde dehydrogenase-independent mechanism must be responsible for the ability of disulfiram to promote cocaine abstinence (Weinshenker and Schroeder, 2007; Gaval-Cruz and Weinshenker, 2009).

Cocaine increases extracellular levels of dopamine (DA), norepinephrine (NE), and serotonin (5-HT) in the brain by blocking plasma membrane monoamine transporters. Thus, pathways critical for the production or transmission of these neurotransmitters are a reasonable place to look for targets underlying the efficacy of disulfiram in the treatment of cocaine dependence. Because the primary metabolite of

disulfiram, diethyldithiocarbamate (DDC), is a copper chelator (Hald and Jacobsen, 1948; Johnston, 1953), disulfiram impairs the function of many copper-containing enzymes, including carboxylesterase, cholinesterase, and dopamine β-hydroxylase (DBH). Of particular interest, the inhibition of DBH by disulfiram reduces production of NE, with a concomitant increase in tissue levels of DA in rodents (Goldstein, 1966; Musacchio et al., 1966; Bourdelat-Parks et al., 2005). Disulfiram also decreases NE and its metabolites in the urine, blood, and CSF of humans (Takahashi and Gjessing, 1972; Major et al., 1979; Rogers et al., 1979; Hoeldtke and Stetson, 1980; Rosen and Lobo, 1987; Paradisi et al., 1991). We have shown that disulfiram has no effect on catecholamine levels in DBH knockout (*Dbh -/-*) mice, which lack NE, indicating that disulfiram's effects on NE and DA are mediated solely by DBH inhibition (Bourdelat-Parks et al., 2005). Disulfiram also inhibits cocaine metabolizing enzymes and increases peak plasma cocaine levels under some conditions in humans (McCance-Katz et al., 1998b, a; Baker et al., 2007) but not rodents (Gaval-Cruz et al., 2008).

The efficacy of disulfiram in treating cocaine dependence has been attributed to several different mechanisms, including a decrease in cocaine reward, an increase in cocaine aversion, and as a "DA replacement therapy" that elevates DA levels and restores normal reward function in hypodopaminergic addicts (Weinshenker and Schroeder, 2007; Sofuoglu et al., 2008; Gaval-Cruz and Weinshenker, 2009); however, the data have been ambiguous. Different human laboratory studies report that genetic or pharmacological DBH inhibition increases cocaine-induced paranoia and decreases, increases, or has no effect on psychostimulant-induced euphoria (Hameedi et al., 1995; McCance-Katz et al., 1998b, a; Cubells et al., 2000; Petrakis et al., 2000; Baker et al., 2007; Kalayasiri et al., 2007; Sofuoglu et al., 2008). In rodents, disulfiram decreases the

locomotor-activating effects of acute cocaine administration, but facilitates cocaine sensitization (Maj et al., 1968; Haile et al., 2003).

The available human and animal data give us a hazy picture of how disulfiram discourages cocaine use. The influence of disulfiram on the reinforcing properties of cocaine have yet to be investigated in an animal model, and while DBH inhibition has been suggested to underlie disulfiram's efficacy, this hypothesis has not been tested directly. In an effort to resolve these issues, we assessed the effects of disulfiram in operant rat paradigms of drug taking (cocaine self-administration) and relapse (cocaine-primed reinstatement) at doses that inhibit DBH in the brain. To determine whether the effects of disulfiram were mediated by inhibition of DBH, we employed the selective DBH inhibitor, nepicastat. Nepicastat is a direct, competitive inhibitor of DBH with greater potency than disulfiram (IC_{50} = 9 nM for nepicastat versus $IC_{50} \cong 1$ μM for disulfiram); (Green, 1964; Goldstein, 1966; Stanley et al., 1997), as well as better selectivity (does not chelate copper, no significant interaction with a panel of other enzymes and receptors tested, including aldehyde dehydrogenase and tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis) (Stanley et al., 1997; K. Walker, Roche Biosciences, personal communication).

B. METHODS

1. Subjects

Male Sprague-Dawley rats (175-200 g) were purchased from Charles River (Wilmington, MA, USA). All subjects were maintained in a temperature-controlled environment on a 12-h reverse light/dark cycle with the lights on from 7 pm to 7 am with ad libitum access to food and water. Rats were acclimated to the vivarium for 1 week prior to catheter

implantation surgery. All self-administration sessions occurred during the dark cycle and were performed using standard methods with minor modifications (McFarland and Kalivas, 2001; Fuchs et al., 2006). All animals were treated in accordance with NIH policy, and experiments were approved by the Emory IACUC committee.

2. Drugs

In initial pilot experiments, we tested the effects of disulfiram (10, 25, 50, 75, 100, or 200 mg/kg, i.p.) and nepicastat (50 or 100 mg/kg, i.p.) on brain catecholamine levels and operant responding for food. Disulfiram was obtained from Sigma-Aldrich (St. Louis, MO), sonicated in sterile saline, and injected as a suspension. Nepicastat was obtained from Synosia Therapeutics (South San Francisco, CA), sonicated in sterile saline containing 1.5% DMSO and 1.5% Cremaphor EL (Sigma), and injected as a suspension. We chose the 100 mg/kg dose of disulfiram based on 3 criteria. First, 100 mg/kg was the maximum dose that significantly inhibited DBH but did not impair the ability of rats to perform operant responses. Second, the 100 mg/kg dose has been shown by others to alter other behavioral effects of cocaine in rats, such as locomotor activity and sensitization (e.g. Haile et al., 2003). Third, the 100 mg/kg dose inhibits aldehyde dehydrogenase in rats and is in the range typically used for alcohol studies (e.g. Deitrich and Erwin, 1971; Yourick and Faiman, 1991; Karamanakos et al., 2001). Fourth, the 100 mg/kg dose is therapeutically relevant. The typical therapeutic dose for the cocaine studies performed in humans is 250-500 mg/day (e.g. Carroll et al., 1998; McCance-Katz et al., 1998), which translates to ~ 3-7 mg/kg for a 70 kg human, or ~ 10-fold lower than we used in our study. Because of their higher metabolic rate, rodents require much larger doses of psychoactive drugs to produce behavioral and neurochemical effects compared to humans, and the 3-7 mg/kg dose has been shown to inhibit DBH in humans with a magnitude similar to the 100 mg/kg dose in rats (e.g. compare Vesell et

al., 1971; Major et al., 1979; Rogers et al., 1979; Paradisi et al., 1991 human studies to our current rat study). Thus, use of the 100 mg/kg dose in rats is a close functional match to therapeutic doses in humans. We chose the 10 mg/kg dose of disulfiram for an additional experiment because it was the maximum dose in our pilot studies that did not significantly reduce brain NE levels. The 50 mg/kg dose of nepicastat was chosen to match the level of DBH inhibition observed with the 100 mg/kg dose of disulfiram.

3. Quantification of Catecholamine Levels

Rats were injected with disulfiram (10 or 100 mg/kg, i.p.), nepicastat (50 mg/kg, i.p.), or vehicle (saline for disulfiram, 1.5% DMSO + 1.5% Cremaphor EL in saline for nepicastat; 1 ml/kg, i.p.). Two hours later, rats were euthanized by CO_2, brains were removed, and the prefrontal cortex was dissected on ice and frozen. The prefrontal cortex was chosen because it contains comparable amounts of NE and DA, and thus can be used to accurately assess DBH inhibition. NE and DA levels were determined using HPLC followed by coulometric detection. DA and NE concentrations were normalized to wet tissue weight for each sample.

Analytical samples from saline- and disulfiram-treated rats were prepared by adding 10 volumes of ice-cold mobile phase [0.1 mM NaHSO4, monohydrate 0.1 mM EDTA, 0.2 mM octane sulfonic acid, 6.5% acetonitrile (pH 3.1)], and sonicated until completely homogenized. Samples were centrifuged at 13.2 rpm x 1000 for 30 min at 4°C, and the supernatant removed from the tubes. The supernatant was centrifuged again at 13.2 rpm x 1000 for 30 min at 4°C using a 22-micron filter column. The resulting eluant was injected using an ESA 542 Autosampler (ESA Biosciences Inc., Chelmsford, MA) onto a Synergi Max-RP 4u (150 x 4.6mm) with Security Guard precolumn filter with Max-RP cartridges (Phenomenex, Inc., Torrance, CA) at a constant rate of 1 ml/min

maintained by ESA 584 pumps. An ESA CoulArray 5600A detector with a potential set at -150 mV, 200 mV was used to visualize the peaks. The retention time and height of NE and DA peaks were compared with reference standard solutions (Sigma). Peak heights were quantified by CoulArray software (ESA Biosciences Inc.).

Analytical samples of vehicle and nepicastat-treated rats were prepared by adding 70 µL of ice-cold 0.1 N perchloric acid and 0.04% sodium metabisulfite to the tissue, and then sonicating until completely homogenized. Samples were centrifuged at 15 rpm x 1000 for 10 min at 4°C. This supernatant was injected at a constant flow rate of 1 mL/min onto an Ultrasphere ODS 250 × 4.6 mm column, 5 µm (Beckman Coulter, Fullerton, CA, USA) with mobile phase (0.1 mM EDTA; 0.35mM sodium octyl sulfate; 0.6% phosphoric acid; 5% acetonitrile (pH 2.7)). A coulometric electrochemical array detector (Agilent Technologies; guard cell set at 600 mV and analytical cell at 300 mV) was used to visualize the peaks. The retention time, height, and area of NE and DA peaks were compared with reference standard solutions (Sigma) and quantified by ChemStation chromatography software (Agilent Technologies).

4. Food Training

Rats were trained to lever-press for food in standard rat operant chambers (Med Associates, St. Albans, VT) prior to drug exposure to facilitate acquisition of drug self-administration, as described (Fuchs et al., 2004). Each chamber was equipped with a house light, two levers (active and inactive), and stimulus lights above both levers. Fan motors provided ventilation and masked noise for each chamber. A microcomputer with Logic ‘1’ interface and MED-PC software (MED Associates) controlled schedule contingencies and recorded data. Animals had access to a water bottle and received 45-mg food pellets following active lever presses on a fixed ratio 1 (FR1) schedule, meaning

the rat received a reinforcer following each active lever press. The food training sessions lasted for 8 h, or until the animal met criteria, defined as at least 70% selection of the active lever and at least 100 food pellets obtained. Most rats met criteria on the first day of food training, but a few rats required 2-3 days.

5. Surgery

Following food training, rats were anesthetized with isoflurane and implanted with indwelling jugular catheters using standard methods. Briefly, catheters were inserted into the jugular vein and anchored with suture material and tissue adhesive. The catheter was then threaded subcutaneously through the skin between the shoulder blades, and the catheter was anchored. Catheters were flushed daily with 0.05 mL gentamicin (4 mg/mL) and 0.1 mL heparin solution (30 U/mL in sterile saline). Catheter patency was verified periodically by infusing 0.08-0.12 ml of methohexital sodium (10 mg/ml, IV; Eli Lilly and Co., Indianapolis, Ind., USA), which produces a rapid loss of muscle tone only when administered intravenously.

6. Cocaine self-administration and reinstatement

a. Cocaine self-administration

Daily self-administration sessions were run for 2 h on a FR1 schedule. At the start of each session, both active and inactive levers were extended, and rats received a non-contingent infusion of cocaine (0.5 mg/kg). During training, each press of the active lever resulted in a cocaine infusion (0.5 mg/kg in a volume of 167 μl/kg) accompanied by a discrete flashing light above the lever. Following a 20-s timeout period (during which time active lever presses did not result in drug infusion), the stimulus light was extinguished, and responses were again reinforced. Responses on the inactive lever

had no programmed consequences. To prevent overdose, the session was terminated early if the number of cocaine infusions exceeded 40.

Once rats reached a stable level of responding (number of drug infusions varied by <20% of the mean, and preference for the active lever was at least 75% for 3 consecutive days, with a minimum of 5 total days of cocaine self-administration), the effects of disulfiram were assessed. Rats received an injection of saline (2 ml/kg, i.p.) or disulfiram (100 mg/kg, i.p.) 2 h prior to the self-administration session. The rats were then allowed 1-2 days of self-administration sessions with no pretreatment. The following day, rats received the opposite pretreatment (saline or disulfiram) 2 h prior to the self-administration session in a counterbalanced fashion.

b. Extinction

Following the completion of the maintenance phase of cocaine self-administration, lever pressing was extinguished in daily 2-h sessions during which presses on the previously active lever no longer resulted in delivery of cocaine or presentation of cocaine-paired cues. Behavior was considered extinguished when active lever presses over 3 consecutive days was <25% of the average number of active lever presses during the last 3 days of maintenance.

c. Cocaine-Primed Reinstatement

The day after extinction criteria were met, rats were pretreated with saline (2 ml/kg, i.p.) or disulfiram (10 or 100 mg/kg, i.p.). Two hours later, they were given a noncontingent priming injection of cocaine (10 mg/kg, i.p.) and placed in the operant chambers under extinction conditions (i.e., presses on the “active” lever had no programmed consequences) for 2 h. Rats then underwent a second round of extinction, as described

above. When extinction criteria were met, rats were again tested for cocaine-primed reinstatement, but received the opposite pretreatment (saline or disulfiram) in a counterbalanced fashion (order was randomized). Some of the rats used for the reinstatement tests were the same ones that received disulfiram at the end of the maintenance phase of cocaine self-administration, while others were from a separate group that did not receive any pretreatments during maintenance. We found no differences in reinstatement, and these groups were combined. To determine whether the effects of disulfiram on reinstatement were mediated by DBH inhibition, separate groups of rats went through cocaine self-administration and extinction, then were pretreated with vehicle (1.5% DMSO, 1.5% Cremaphor EL in saline, 1 ml/kg, i.p.) or nepicastat (50 mg/kg, i.p.) prior to counterbalanced reinstatement sessions, as described for disulfiram.

7. Food Self-Administration and reinstatement

a. Food self-administration

Separate groups of rats were used for the food self-administration and reinstatement experiments. Rats were maintained on a restricted diet of 16 g of normal rat chow per day, given in the evening at least 1 h after self-administration sessions had ended. Parameters of food self-administration were identical to the cocaine self-administration experiments, except that rats received a food pellet instead of a cocaine infusion for each active lever press, and sessions lasted 1 h and were terminated if the reinforcers obtained exceeded 60.

b. Food-Primed Reinstatement

Food-primed reinstatement of food seeking was performed using a modified version of published protocols (Sun and Rebec, 2005; Peters and Kalivas, 2006). Once

maintenance criteria for operant food self-administration were met (maintenance criteria and extinction criteria were identical to those used for cocaine-primed reinstatement), rats were pretreated with vehicle (1.5% DMSO, 1.5% Cremaphor EL in saline, 1 ml/kg, i.p.) or nepicastat (50 mg/kg, i.p.). 2 h later, they were placed in the operant chambers and the reinstatement session was started. Three food pellets were delivered non-contingently in the first ten seconds of the session and the levers were presented to the subjects. As during extinction, responses on either of the levers had no programmed consequence. Throughout the 60 min food reinstatement session, a food pellet was delivered every 3 min non-contingently, and responses upon the formerly active and inactive levers were recorded. Rats then underwent a second round of maintenance and extinction training for operant food self-administration, as described above, then were tested for food-primed reinstatement following the opposite pretreatment (vehicle or nepicastat) in a counterbalanced fashion (order was randomized).

8. Data Analysis

Catecholamine level data were analyzed by Student's t-test, and self-administration data were analyzed by ANOVA followed by Bonferroni post hoc tests using Prism 4.0 for Macintosh.

C. RESULTS

1. Disulfiram Inhibits DBH and Decreases Brain NE Levels

DBH is the enzyme in the catecholamine biosynthetic pathway that converts DA to NE in noradrenergic neurons. Thus, inhibition of DBH has the unique effect of simultaneously decreasing NE production and increasing DA (*Fig. 1*). To confirm previous reports that systemic disulfiram administration inhibits DBH in the rat brain,

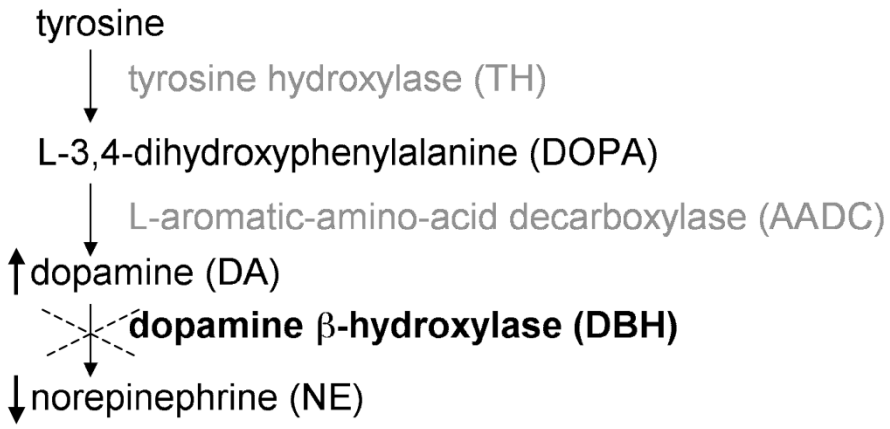

Figure 1: Catecholamine biosynthetic pathway. Because DBH converts DA to NE in noradrenergic neurons, inhibition of DBH is unique in its ability to decrease NE while increasing DA.

we measured NE, DA, and the NE/DA ratio in the frontal cortex following administration of saline or disulfiram (100 mg/kg, i.p.). We chose the frontal cortex because it contains NE and DA in similar concentrations, thereby allowing the detection of both decreases and increases in these neurotransmitters. As expected, disulfiram was a bona fide DBH inhibitor, as it decreased NE, increased DA, and decreased the NE/DA ratio (*Fig. 2*). Inhibition of other catecholamine biosynthetic enzymes would have had different patterns, such as decreases in both NE and DA following tyrosine hydroxylase (TH) inhibition.

2. Disulfiram Has No Effect on Self-Administration of Food or Cocaine

To ensure that we were using a dose of disulfiram that did not impair the ability of rats to perform an operant task, we assessed responding for food pellets following saline or disulfiram (100 mg/kg, i.p.) administration. Disulfiram had no effect on food responding; all rats obtained the maximum number of reinforcers possible during the session (61), regardless of pretreatment (n = 4 per group). To determine whether disulfiram altered the reinforcing or aversive effects of cocaine, we assessed maintenance levels of responding for cocaine infusions (0.5 mg/kg/infusion) following saline or disulfiram (100 mg/kg, i.p.). Disulfiram had no effect on cocaine self-administration (*Fig. 3*). Repeated measures ANOVA revealed no significant effects for active lever presses ($F_{23,2} = 0.77$, $p = 0.48$) or reinforcers obtained ($F_{23,2} = 0.97$, $p = 0.4$). Inactive lever presses were negligible (0-2 presses per animal) and did not differ between groups.

3. Disulfiram Blocks Cocaine-Primed Reinstatement of Cocaine Seeking

We next tested the effects of disulfiram on drug-primed reinstatement of cocaine seeking. Following the attainment of stable self-administration and extinction, rats

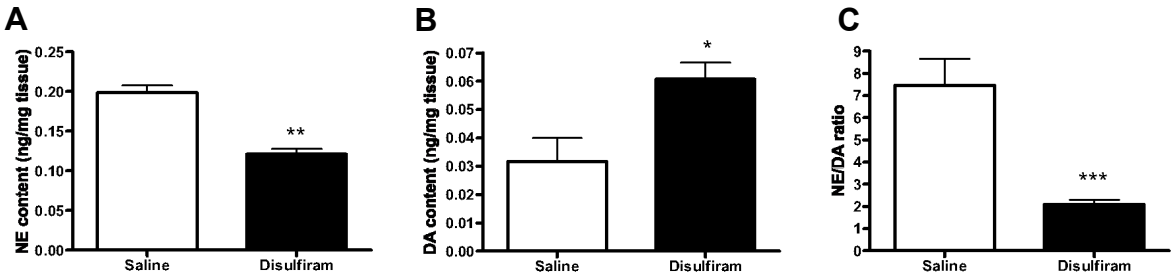

Figure 2: Effect of disulfiram on catecholamine levels in the rat prefrontal cortex. Shown is the mean ± SEM for (A) NE levels, (B) DA levels, and (C) the NE/DA ratio in the prefrontal cortex of rats after treatment with saline or disulfiram (single injection of 100 mg/kg, i.p., catecholamines measured 2 hours after disulfiram administration by HPLC followed by electrochemical detection; N = 6 per group). *$p<0.05$, **$p<0.01$, ***$p<0.001$ compared with vehicle.

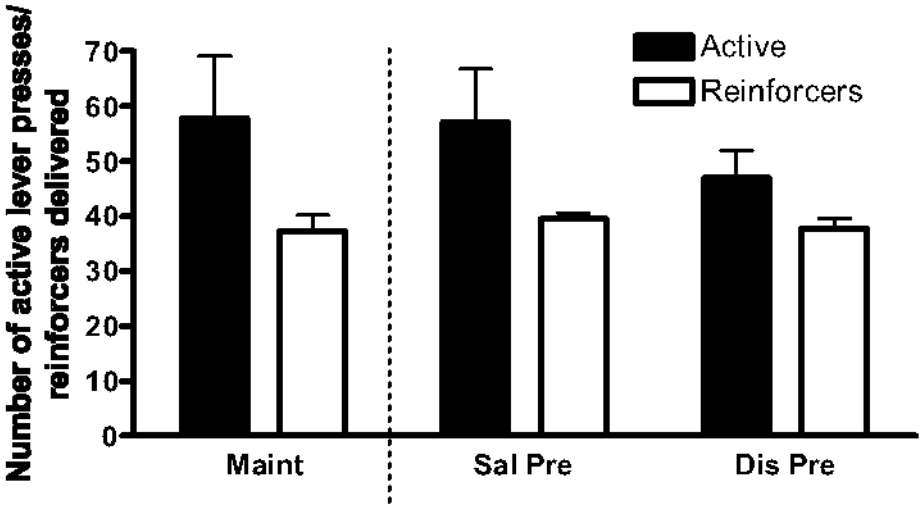

Figure 3: Effect of a 2-hr pretreatment of disulfiram (100mg/kg, i.p.; "Dis Pre") on maintenance of cocaine self-administration in rats (n=8 per group). Shown are mean ± SEM of active lever responses and number of reinforcers obtained over a 2-hour session. Maintenance ("Maint") values reflect an average number of responses and reinforcers obtained over the last 3 days of maintenance. Occasional active lever pressing during the 20-second timeout periods result in more active lever presses than reinforcers received.

were treated with saline or disulfiram (100 mg/kg, i.p.) prior to a noncontingent priming injection of cocaine (10 mg/kg, i.p.). Rats that were pretreated with saline showed a robust reinstatement of responding on the previously active lever following cocaine prime. In contrast, disulfiram pretreatment completely blocked cocaine-primed reinstatement (*Fig. 4*). ANOVA revealed a significant effect of treatment phase ($F_{4,51}$ = 8.17, $p < 0.0001$), and Bonferroni post hoc analysis showed a significant difference between extinction responding and cocaine-primed reinstatement following saline pretreatment ($t = 3.62$, $p < 0.05$), but not between extinction responding and disulfiram pretreatment ($t = 0.22$, $p > 0.05$). In addition, there was a significant difference between reinstatement responding with saline pretreatment and disulfiram pretreatment ($t = 2.81$, $p < 0.05$). There was no effect of pretreatment on inactive lever responding.

We next tested the ability of a lower dose of disulfiram (10 mg/kg, i.p.) to attenuate cocaine-primed reinstatement. This dose of disulfiram, which we found in pilot studies to be the highest one that does not significantly reduce NE levels in the PFC (vehicle = 0.32 ± 0.04 ng/mg tissue, disulfiram = 0.29 ± 0.08, $p > 0.05$, n = 4 per group), did not impair cocaine-primed reinstatement (*Fig. 4*). Bonferroni post hoc analysis showed a significant difference between extinction responding and cocaine-primed reinstatement following low dose disulfiram pretreatment ($t = 2.69$, $p < 0.05$, but not between saline and low dose disulfiram pretreatment ($t = 0.18$, $p > 0.05$).

4. Nepicastat Blocks Cocaine-Primed Reinstatement of Cocaine Seeking

The previous experiments indicated that a dose high enough to inhibit DBH is required for the efficacy of disulfiram in blocking cocaine-primed reinstatement. However, because DBH has many other targets, it was unclear whether DBH

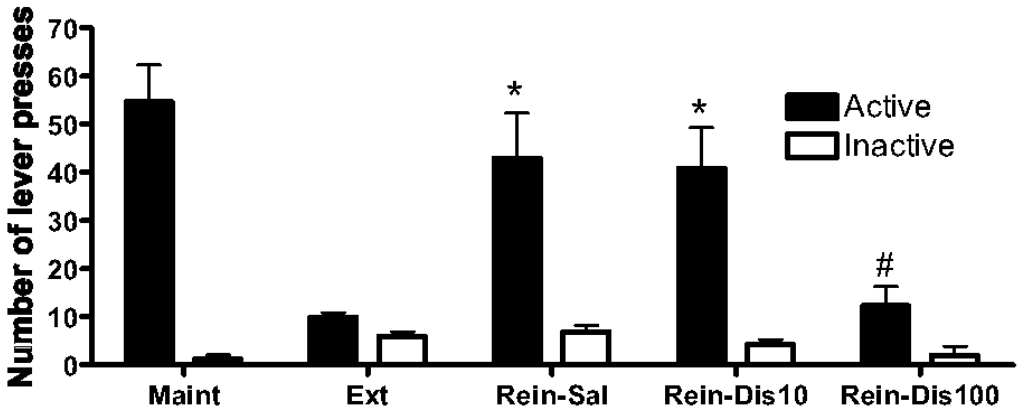

Figure 4: Effect of a 2-hr pretreatment of disulfiram (0, 10, 100 mg/kg, i.p.) on reinstatement primed with cocaine (10mg/kg, i.p.) in rats ("Rein-Sal" n=13; "Rein-Dis10" n=6; "Rein-Dis100" n=7). Shown are active and inactive lever responses. Maintenance values ("Maint") reflect an average of the last 3 days of maintenance sessions, and extinction values ("Ext") reflect an average of the last 3 days of extinction. *$p<0.05$ compared with active lever responses during extinction, #$p<0.05$ compared with active lever responses during cocaine-induced reinstatement tests with saline pretreatment.

inhibition alone was sufficient to block reinstatement. Thus, we repeated the self-administration experiments with the selective DBH inhibitor, nepicastat, at a dose (50 mg/kg, i.p.) that inhibited DBH to a similar extent as the effective dose of disulfiram (100 mg/kg, i.p.) (*Fig. 5*), and found that nepicastat pretreatment mimicked the effects of disulfiram in several ways. First, nepicastat had no effect on the maintenance phase of cocaine self-administration (*Fig. 6*). Repeated measures ANOVA revealed a non-significant trend for active lever presses ($F_{26,2}$ = 3.36, p = 0.06) and no effect on reinforcers obtained ($F_{26,2}$ = 0.38, p = 0.69). Inactive lever presses were negligible and did not differ between groups. Second, nepicastat blocked cocaine-primed reinstatement (*Fig. 7*). Repeated measures ANOVA revealed a significant effect of treatment phase ($F_{3,23}$ = 18.14, p < 0.0001), and Bonferroni post hoc analysis showed a significant difference between extinction responding and cocaine-primed reinstatement following saline pretreatment (t = 5.17, p < 0.001) and between vehicle pretreatment and nepicastat pretreatment (t = 4.67, p < 0.01), but not between extinction responding and cocaine-primed reinstatement following nepicastat pretreatment (t = 0.5, p > 0.05). Pretreatment had no effect on inactive lever responding. Third, nepicastat (50 mg/kg, i.p.) had no effect on food responding; all rats obtained the maximum number of reinforcers possible during the session (61), regardless of pretreatment (n = 8 per group).

Because the neural and molecular pathways underlying reinstatement of cocaine and food seeking are partially overlapping (Nair et al., 2009), we tested whether the attenuation of reinstatement by DBH inhibition was specific to cocaine, and found that nepicastat did not significantly reduce food-primed reinstatement of food seeking (*Fig. 8*). Repeated measures ANOVA revealed a significant effect of treatment phase ($F_{3,27}$ = 29.49, p < 0.0001), and Bonferroni post hoc analysis showed a significant

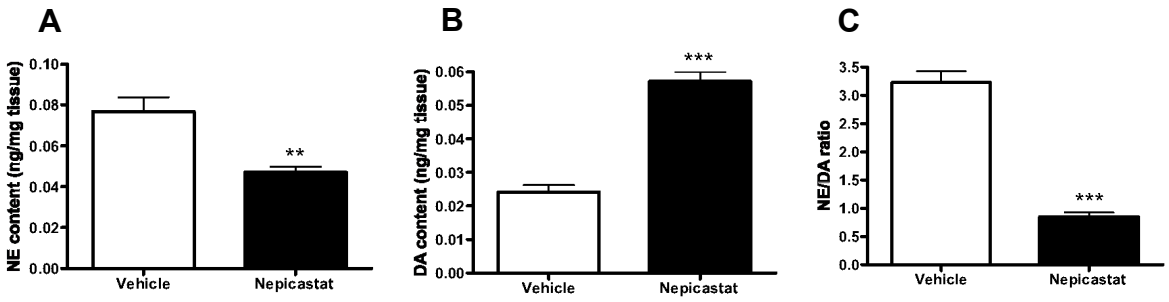

Figure 5: Effect of nepicastat on catecholamine levels in the rat prefrontal cortex. Shown is the mean ± SEM for (A) NE levels, (B) DA levels, and (C) the NE/DA ratio in the prefrontal cortex of rats after treatment with vehicle or nepicastat (single injection of 50 mg/kg, i.p., catecholamines measured 2 hours after nepicastat administration by HPLC followed by electrochemical detection; N = 8 per group). **p<0.01, ***p<0.001 compared with vehicle.

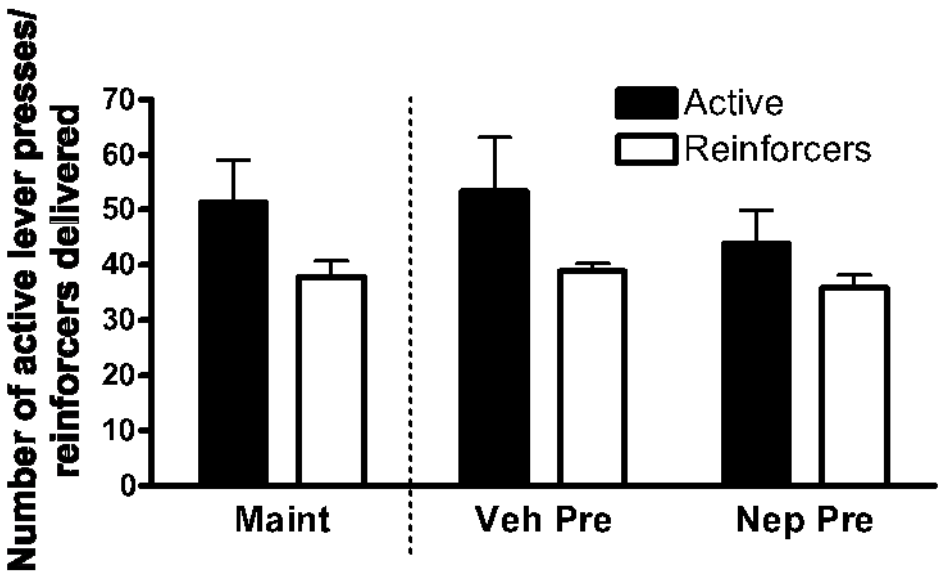

Figure 6: Effect of a 2-hr pretreatment of nepicastat (50mg/kg, i.p; "Nep Pre") on maintenance of cocaine self-administration. Shown are mean ± SEM active lever responses and number of reinforcers obtained over a 2-hour session. Maintenance values ("Maint") reflect an average number of responses and reinforcers obtained over the last 3 days of maintenance. Occasional active lever pressing during the 20-second timeout periods result in more active lever presses than reinforcers received. n=6 per group.

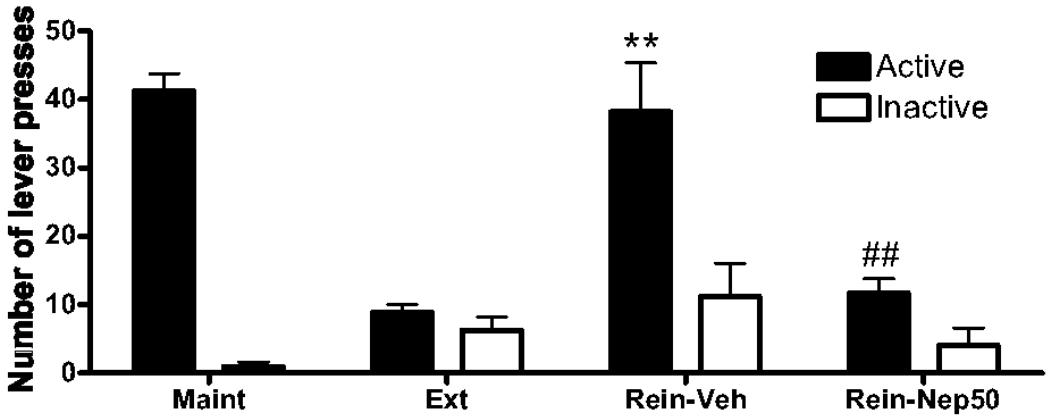

Figure 7: Effect of a 2-hr nepicastat pretreatment (50mg/kg, i.p.) on reinstatement primed with 10mg/kg, i.p. cocaine. Shown are the mean ± SEM active and inactive lever responses. Maintenance values ("Maint") reflect an average of the last 3 days of maintenance sessions, and extinction ("Ext") values reflect an average of the last 3 days of extinction. **p<0.01 compared with active lever responses during extinction, ##p<0.01 compared with active lever responses during cocaine-induced reinstatement tests with saline pretreatment (n=6 per group).

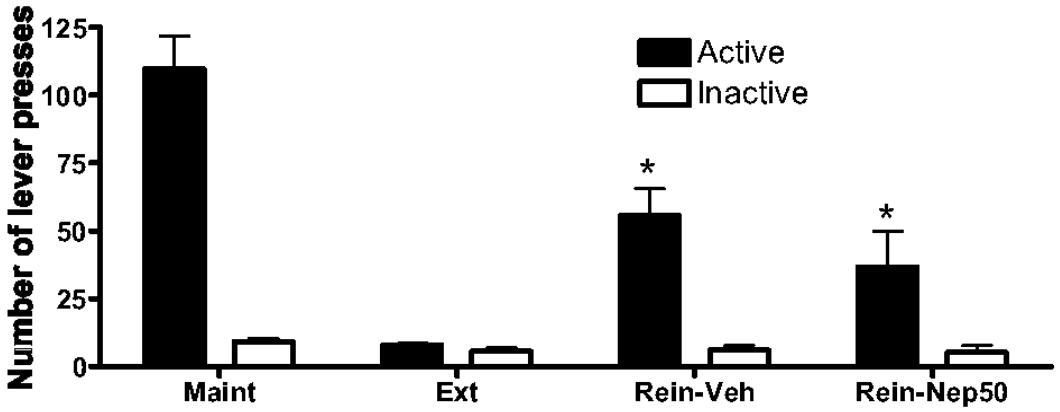

Figure 8: Effect of a 2-hr pretreatment of nepicastat on food-primed reinstatement of food seeking. Shown are mean ± SEM active and inactive lever responses. Maintenance values reflect an average of the last 3 days of maintenance sessions, and extinction values reflect an average of the last 3 days of extinction. *$p<0.05$ compared with active lever responses during extinction (n=7 per group).

difference between extinction responding and cocaine-primed reinstatement following vehicle or nepicastat pretreatment (vehicle $t = 4.27$, $p < 0.05$; nepicastat $t = 2.57$, $p < 0.05$), but not between cocaine-primed reinstatement following vehicle and nepicastat pretreatment ($t = 1.70$, $p > 0.05$). These results indicate that the blockade of cocaine-primed reinstatement by nepicastat cannot be attributed to an inability to perform the operant task and that DBH inhibition does not impair reinstatement of responding for a natural reward.

D. DISCUSSION

Disulfiram has shown promise as a treatment for cocaine dependence in several clinical trials (Carroll et al., 1993; Carroll et al., 1998; Carroll et al., 2000; George et al., 2000; Petrakis et al., 2000; Carroll et al., 2004; Grassi et al., 2007; Pettinati et al., 2008; Carroll et al., 2012). Because concurrent alcohol use is not necessary for disulfiram to have beneficial effects on cocaine addiction, an ALDH-independent mechanism is likely. Furthermore, whatever the underlying molecular mechanism, why disulfiram treatment reduces cocaine use remains unclear; several human laboratory studies have produced conflicting results over how DBH inhibition influences the rewarding and aversive effects of cocaine. The purpose of our study was therefore two-fold. First, to gain insight into which aspects of addiction were being altered in the clinic, we determined which “phase” of cocaine self-administration (i.e., maintenance vs. reinstatement) was affected by disulfiram in rats. Second, to test the hypothesis that disulfiram was acting via DBH inhibition to reduce cocaine intake in clinical studies, we used a lower dose of disulfiram that does not inhibit DBH and the selective DBH inhibitor, nepicastat.

Treatments that alter the reinforcing effects of cocaine, such as dopaminergic manipulations, typically change cocaine self-administration behavior (Koob et al., 1994).

Given the history of NE manipulations and cocaine self-administration, it is not surprising that disulfiram had no effect on maintenance responding for cocaine. NE transporter (NET) inhibitors themselves do not support self-administration, and neither NET inhibitors nor adrenergic receptor antagonists alter cocaine self-administration (Yokel and Wise, 1976; Roberts et al., 1977; Woolverton, 1987; Howell and Byrd, 1991; Skjoldager et al., 1993; Tella, 1995; Wee et al., 2006; Weinshenker and Schroeder, 2007; Gaval-Cruz and Weinshenker, 2009).

Drug addiction is a chronic relapsing disorder (Hunt et al., 1971; Leshner, 1997), as patients in treatment often slip back into drug taking after periods of sobriety. Several types of stimuli can trigger drug craving and lead to relapse, including re-exposure to the drug, stress, and drug-associated cues; these stimuli also trigger reinstatement in the rat model. The reliability, species generality, as well as face and construct validity of the reinstatement model are high, because it recapitulates many of the features of human addiction (Panlilio and Goldberg, 2007). In contrast to the lack of data to support an influence on the maintenance phase of psychostimulant self-administration, the role of NE in the reinstatement of drug seeking is clear (Erb et al., 2000; Weinshenker and Schroeder, 2007; Gaval-Cruz and Weinshenker, 2009). Central infusion of NE itself, or the facilitation of NE transmission with reuptake inhibitors or inhibitory autoreceptor antagonists, induces reinstatement in rats and non-human primates (Lee et al., 2004; Platt et al., 2007; Brown et al., 2009). Conversely, blockade of β-adrenergic receptors (βARs) prevents stress-induced reinstatement, whereas blockade of α1ARs prevents drug-primed reinstatement (Leri et al., 2002; Zhang and Kosten, 2005). Because we examined cocaine-primed reinstatement, it is likely that reinstatement was blunted following disulfiram or nepicastat pretreatment due to reduced NE production and a failure to engage α1ARs. The ability of DBH inhibition to block cocaine-primed

reinstatement provides further support for the critical role of NE in this paradigm, and we propose that the clinical efficacy of disulfiram, via DBH inhibition and reduction of NE, reduces the risk for relapse. Most disulfiram clinical trials to date have not been designed to examine cocaine relapse specifically. It will be important to build measures into future trials that can distinguish between abstinence due to altered subjective drug effects vs. healthier responses to environmental triggers.

The evidence available suggests that blockade of cocaine-primed reinstatement by disulfiram involves the impairment of neurotransmission in the nucleus accumbens (NAc). DA and glutamate release in the NAc are both essential for cocaine-primed reinstatement (Schmidt et al., 2005; Kalivas et al., 2009). Noradrenergic neurons project to the mesocorticolimbic DA system, and NE promotes DA transmission, primarily via activation of α1ARs. For example, depletion of NE, or attenuation of α1AR signaling via genetic, pharmacological, or neurotoxic means impairs psychostimulant-induced DA overflow in the NAc (Darracq et al., 1998; Drouin et al., 2002b; Ventura et al., 2003). It is important to note that while DBH inhibition increases tissue levels of DA, it decreases DA release because NE-mediated excitation of DA neurons is reduced (Schank et al., 2006; Weinshenker and Schroeder, 2007; Weinshenker et al., 2008). Thus the failure of a cocaine prime to provoke DA release in the NAc may underlie the efficacy of disulfiram in this paradigm. While proof of a direct role for NE in regulating cocaine-induced glutamate release in the NAc is lacking, we have recently found that α1-adrenergic receptors are enriched in presumptive glutamatergic terminals throughout the mesocorticolimbic system (Rommelfanger et al., 2009), and we predict that a loss of noradrenergic tone may also attenuate the glutamate release essential for cocaine-primed reinstatement.

Although the blockade of cocaine-primed reinstatement by disulfiram could involve several targets, our results strongly suggest that it is mediated primarily by DBH inhibition, NE reduction, and a decrease in α1AR signaling, as the effects of disulfiram require a dose that significantly inhibits DBH and are mimicked by the selective DBH inhibitor, nepicastat (present study), and the α1AR antagonist, prazosin (Zhang and Kosten, 2005). What remains unclear is why a reduction of NE/α1AR signaling hampers drug-primed reinstatement, but not the maintenance phase of cocaine self-administration. Earlier findings revealed that blockade of α1ARs does not affect "conventional" operant responding for cocaine, but does attenuate the escalation of cocaine self-administration elicited by long-access "binge" paradigms or prior drug sensitization (Zhang and Kosten, 2007; Wee et al., 2008). Combined, these results suggest that while NE does not play a critical role in the primary reinforcing effects of cocaine, as measured by standard operant self-administration, it does have significant effects under conditions that escalate or reinstate drug-seeking behavior. Furthermore, medications that impair NE production, such as disulfiram or nepicastat, may short circuit the ability of environmental triggers to promote relapse, and therefore make promising pharmacotherapies for the treatment of dependence on cocaine and other stimulants.

CHAPTER III

Pharmacological DBH Inhibition: Effect on Cocaine Reinstatement in Squirrel Monkeys

A. INTRODUCTION

Disulfiram has shown promise in clinical trials as a pharmacotherapy for cocaine dependence. Due to its inhibition of aldehyde dehydrogenase (ALDH) and subsequent accumulation of acetaldehyde following ethanol ingestion, disulfiram was originally used as an anti-alcoholism medication (Moriarty, 1950; Fuller et al., 1986). It was later found to reduce both alcohol and cocaine intake in a patient population that abused both substances (Carroll et al., 1998; Carroll et al., 2000). Further trials found that disulfiram is equally, if not more, effective at reducing cocaine intake in subjects who are not consuming alcohol (Carroll et al., 2004; Carroll et al., 2012). Because ALDH is not involved in cocaine metabolism, it is likely that the ability of disulfiram to reduce cocaine intake does not rely on an ALDH-dependent mechanism.

The clinical efficacy of disulfiram may be more closely linked to pathways involved with dopamine (DA), norepinephrine (NE), and serotonin (5-HT). Because cocaine blocks plasma membrane monoamine transporters and increases extracellular levels of DA, NE and 5-HT, pathways and molecules involved in the production or transmission of these neurotransmitters are ideal candidates for determining the efficacy of disulfiram. Disulfiram inhibits a wide range of enzymes including ALDH, which underlies the efficacy of disulfiram in alcohol addiction. The major metabolite of disulfiram, diethyldithiocarbamate, is also a copper chelator and therefore interferes with the action of any enzyme that is dependent on copper (Johansson, 1989). One of these copper-containing enzymes that is inhibited by disulfiram is dopamine β-hydroxylase (DBH), the enzyme that converts DA to NE (Goldstein et al., 1964; Musacchio et al.,

1966). When disulfiram inhibits DBH, production of NE is decreased, consequently reducing tissue NE levels in rats and concomitantly increasing DA levels (Goldstein et al., 1964; Musacchio et al., 1966; Bourdelat-Parks et al., 2005; Schroeder et al., 2010). Though disulfiram has many targets, its effects on catecholamine levels and cocaine-induced behaviors are due solely to DBH inhibition. The evidence for this is as follows. First, disulfiram decreases NE and increases DA in wild-type animals but has no effect on catecholamine levels in DBH knockout (*Dbh* –/–) mice (Bourdelat-Parks et al., 2005). Second, both naïve *Dbh* –/– mice and disulfiram-treated control mice are hypersensitive to the locomotor effects of cocaine, and disulfiram has no further effect in the knockouts. Finally, the selective DBH inhibitor nepicastat can mimic the ability of disulfiram to facilitate cocaine sensitization in control mice but is without effect in *Dbh -/-* mice (Gaval-Cruz et al., 2012).

Manipulating NE levels has been shown to affect the reinstatement phase of drug self-administration in animal models. Specifically, attenuating NE production and/or transmission can attenuate drug-induced (Zhang and Kosten, 2005), stress-induced (Erb et al., 2000; Leri et al., 2002), and cue-induced reinstatement (Smith and Aston-Jones, 2011; Schroeder et al., 2013) in rats. Reinstatement to cocaine-seeking can also be induced by increasing extracellular NE levels, such as by administering NE reuptake transporter (NET) inhibitors (Platt et al., 2007) or α2-adrenergic receptor (α2AR) antagonists (Lee et al., 2004) in squirrel monkeys. Because NE plays an important role in reinstatement in animal models, it is believed that DBH inhibition and the subsequent reduction in NE levels mediates the therapeutic effects of disulfiram. In addition to altering catecholamine levels in rats, disulfiram also blocks cocaine-induced reinstatement (Schroeder et al., 2010), corroborating with clinical results. Furthermore, nepicastat, a selective DBH inhibitor that lacks disulfiram's target promiscuity and

adverse side effects also decreases NE and increases DA brain tissue levels in rats, consistent with a DBH inhibitor (Schroeder et al., 2010). Nepicastat also blocks cocaine-, footshock-, and cue-induced reinstatement in rats (Schroeder et al., 2010; Schroeder et al., 2013), supporting the therapeutic use of DBH inhibitors.

Currently, the effects of DBH inhibition on cocaine-induced behavioral changes in nonhuman primates have not been assessed. There are important considerations and advantages to studying the effects of these treatments in nonhuman primates in order to translate to a human population. Firstly, there are notable neuroanatomical differences between rodents and primates in brain regions crucial to the abuse-related effects of cocaine and other drugs of abuse (Frankle et al., 2006; Smith et al., 2006; Haber and Knutson, 2010). For instance, the distribution of NET in the nucleus accumbens (NAc) and basolateral amygdala differs between rodents and nonhuman primates (Smith et al., 2006). Some of these NET binding patterns in nonhuman primates correlate closely with patterns found in humans. Also, the pharmacokinetics of pharmacological treatments tends to be different between rodents and primates. Rodents typically have higher rates of drug uptake, clearance, and metabolism than nonhuman primates or humans. Often, pharmacokinetic parameters measured in nonhuman primates are better predictors of drug pharmacokinetics than will be found in humans (Ward and Smith, 2004b, a; Jolivette and Ward, 2005; Ward et al., 2005). Additionally, because of longer life-span and ability to maintain intravenous catheter preparations longer than rodents, it is more feasible to perform within-subject designed studies in nonhuman primates. Finally, there are few studies that elucidate the role of NE in cocaine-seeking behavior in nonhuman primates. Given the importance of establishing nonhuman primate models for the translation of pharmacotherapies to a human population, more experiments exploring the effects of NE in cocaine-seeking behavior in nonhuman primates are warranted. The

goal of the present study was to determine whether DBH inhibition also reduces drug seeking in nonhuman primates (squirrel monkeys) as previously found in rats. Squirrel monkeys trained to self-administer cocaine under a second-order schedule were pretreated with disulfiram or nepicastat prior to cocaine-primed reinstatement sessions. We hypothesized that DBH inhibition would attenuate cocaine-primed reinstatement in squirrel monkeys, corroborating with both rodent and human clinical literature.

B. METHODS

1. Subjects

Eight male squirrel monkeys (*Saimiri sciureus*) weighing between 850g – 1100g were used as subjects for the following experiments. Animals were individually housed, had ad libitum access to water, and were fed twice daily (LabDiet 5045 High Protein Monkey Chow, PMI Nutrition International, Brentwood, MO; fresh fruit/vegetables; cereal). Monkeys were provided daily enrichment with access to foraging devices, toys, climbing devices, swings and nature sounds. Animals previously served in behavioral studies that involved administration of compounds acting on monoaminergic systems (Kimmel et al., 2007; Bauzo et al., 2009; Fantegrossi et al., 2009; Kimmel et al., 2009; Bauzo et al., 2012; Manvich et al., 2012a; Manvich et al., 2012b). All studies were conducted in strict accordance with the National Institutes of Health's "Guide for Care and Use of Laboratory Animals", the American Association for Accreditation of Laboratory Animal Care (AAALAC), and were approved by the Institutional Animal Care and Use Committee of Emory University.

2. Apparatus

Animals were comfortably seated in a commercially-available plexiglass chair within a ventilated, sound-attenuating chamber (Med Associates Inc., St. Albans, VT)

during behavioral sessions. The chair was equipped with an operant panel consisting of a series of red and white lights, a lever, and a white noise amplifier which remained activated for the duration of all behavioral sessions to lessen the influence of ambient noise. Med-PC IV software (Med Associates Inc., St. Albans, VT) was interfaced with each chamber to allow for automated output control and lever-press recording.

3. Surgeries

Animals in self-administration and reinstatement experiments were prepared with a chronic indwelling venous catheter under aseptic conditions. Animals were initially anesthetized with Telazol (tiletamine HCl and zolazepam HCl, 2.0mg, i.m.) and ketamine HCl (20mg, i.m.). Anesthesia was maintained throughout the procedure with inhaled isoflurane (0.5-1.5%). A polyvinyl chloride catheter (diameter: 0.025" inner, 0.035" outer) was inserted into either the left or right femoral vein or left or right external jugular vein and allowed to rest near the right atrium. The distal end of the catheter was routed subcutaneously and exited at the interscapular region of the subject's back. The end of the catheter was locked using a 25-guage stainless-steel obturator. Animals were fitted with a custom-made nylon mesh jacket (Lomir Biomedical Inc., Malone, NY) to protect the outer portion of the catheter. Animals were allowed to recover for 5-7 days before resuming operant-behavioral sessions. To maintain catheter patency, catheters were flushed several days per week with 0.2 ml saline and when not in use, filled with heparinized saline (100units/mL). If a catheter became occluded or damaged during the course of the study, it was promptly removed and replaced with a new catheter that was implanted into the same vessel when possible or into another vessel. For all surgical procedures, preoperative antibiotics (ceftriaxone) and postoperative analgesics (meloxicam) were administered by veterinary staff who closely monitored the animals.

4. Cocaine self-administration and reinstatement

a. Second order self-administration

Daily sessions were conducted 5-6 days per week and lasted approximately 60 minutes. Subjects were allowed to self-administer cocaine during these sessions under a second-order operant schedule of reinforcement (Schindler et al., 2002). Briefly, sessions began with the illumination of a pair of red lights. A fixed-ratio 20 (FR20) operant schedule was embedded within a 600-sec fixed-interval (FI600). Every 20th lever press resulted in a brief termination of the red lights and a 2-sec illumination of a white stimulus light, followed immediately by re-illumination of the red lights. Responding during the 2-sec white light was recorded but did not contribute to the subsequent ratio requirement. Once the 600-sec FI elapsed, the schedule progressed into a 200-sec limited hold. A FR requirement completed during the limited hold resulted in an intravenous bolus infusion of cocaine (veh, 0.01-0.3 mg/kg/infusion in 0.5 ml; 25 ml/min flow rate). Simultaneously, the red lights were extinguished and the white stimulus light was illuminated for 15-sec. The schedule promptly moved to a 60-sec time out in which all lights were extinguished and responses were recorded but had no programmed consequences. If the animal did not complete an FR during the limited hold, the red lights were extinguished and the schedule moved directly into the timeout. Following the timeout, the schedule was repeated for a total of five FI components per session. Response rates were calculated for each individual component and then averaged across the session. Responding was considered stable when response rates for each session varied less than 20% across 3 consecutive days. Once responding was stable, the unit dose of cocaine was altered and behavior was allowed to stabilize until the maximally-effective unit dose of cocaine (ED_{max}, i.e. the unit dose of cocaine that maintained highest rates of responding) was identified for each individual subject. The

ED_{max} for most subjects was 0.1 mg/kg/infusion, but ranged from 0.03-0.3 mg/kg/infusion across all subjects. The ED_{max} dose for each subject was used as the maintenance dose between reinstatement sessions.

b. Cocaine-primed reinstatement

Once response rates were stable, subjects progressed to the extinction phase during which saline infusions were substituted for cocaine and completed FRs within the 600-sec fixed-interval or the limited hold were recorded but did not produce conditioned reinforcement (i.e. the white stimulus light was withheld). Under extinction conditions, response rates for all subjects rapidly decreased across sessions. Extinction criteria were met when the overall response rate within a single session reached ≤ 20% of the mean response rate of the three previous maintenance sessions. Reinstatement tests occurred on the day immediately following successful extinction of responding. Five minutes prior to the onset of the session, animals were administered a non-contingent, intravenous bolus infusion ("prime") of cocaine (veh, 0.03 – 1.0 mg/kg). The white stimulus light was reintroduced, consistent with maintenance sessions, but saline was still substituted for cocaine infusions throughout the duration of the reinstatement session. Therefore, all responding during a reinstatement test was dependent upon the dose of the non-contingent prime and the reintroduction of the conditioned reinforcer, but not by cocaine reinforcement. For each subject, the dose of cocaine prime that induced maximal rates of responding was determined and deemed the ED_{Peak}. The ED_{Peak} for each individual subject was typically one-half log-step above the ED_{Max} unit dose for maintenance cocaine self-administration sessions. Reinstatement sessions were preceded by a drug pretreatment of either disulfiram [(veh, 10mg/kg, i.m.) given acutely 2-hr prior to the cocaine prime] or nepicastat [(veh, 10mg/kg, 30mg/kg, i.m.) given acutely 30-min – 24-hr prior to the cocaine prime or subchronically for 5 consecutive

days prior to the session]. Within each drug-interaction experiment, reinstatement tests for each drug dose were separated by the reestablishment of maintenance cocaine self-administration and subsequent extinction. The dose order of drug combinations for reinstatement tests was randomized and counterbalanced within subjects.

c. Yohimbine-Primed Reinstatement

Yohimbine-primed reinstatement was used as a positive control to investigate the effectiveness of pharmacological DBH inhibition in squirrel monkeys in a norepinephrine dependent paradigm. When administered systemically, yohimbine, an α2AR antagonist, increases extracellular norepinephrine levels (Palij and Stamford, 1993; Forray et al., 1995; Forray et al., 1997; Khoshbouei et al., 2002). Additionally, yohimbine has previously been shown to induce reinstatement in squirrel monkeys (Lee et al., 2004). Reinstatement sessions proceeded as previously described with the exception that an ED_{Peak} dose of yohimbine was determined and, on reinstatement test days, subjects were given a non-contingent injection of yohimbine (0.1-03.mg/kg, i.m.) instead of cocaine 5-min prior to the onset of the session. For drug interaction studies, a nepicastat pretreatment (veh, 10mg/kg, 30mg/kg, i.m.) was given 30-min or 120-min prior to the yohimbine prime.

d. Nepicastat-Primed Reinstatement

To test the ability of nepicastat to induce reinstatement, an injection of nepicastat was given as a prime prior to the onset of reinstatement sessions. Reinstatement sessions proceeded as previously described with the exception that a nepicastat prime (veh, 10mg/kg, 30mg/kg, i.m.) was administered either 30-min or 120-min prior to the onset of sessions instead of a cocaine prime.

5. Drugs

Cocaine HCl (National Institute on Drug Abuse, Research Technology Branch, Research Triangle Park, NC) was dissolved in 0.9% sterile saline. Yohimbine HCl (Sigma-Aldrich, St. Louis, MO) was sonicated and dissolved in 0.9% sterile saline. Nepicastat (Synosia Therapeutics, South San Francisco, CA) was sonicated and dissolved at a concentration of 30 mg/ml in a 20:20:60 mixture of 95% ethanol, Tween 80 (Sigma-Aldrich, St. Louis, MO), and 0.9% sterile saline for the low dose. The high dose of nepicastat was sonicated and dissolved at a concentration of 80 mg/ml in a 25:25:50 mixture of 95% ethanol, Tween 80 (Sigma-Aldrich, St. Louis, MO), and 0.9% sterile saline. Disulfiram (Sigma-Aldrich, St Louis, MO) was sonicated in sterile water and injected as a suspension. All drug solutions were passed through a 0.2 μm-pore polysulfone filter prior to use. Doses were calculated from the salt weights. Unless otherwise specified, all drugs were administered via the intramuscular route into the thigh muscle.

6. Data analysis

For reinstatement experiments, response rates across sessions were normalized to the percent of average responding maintained during the last three maintenance sessions of cocaine self-administration. Data were analyzed using repeated-measures ANOVAs with post hoc Bonferroni tests, or paired t-tests, as specified. Data were graphically plotted and analyzed using GraphPad v. 5.01 (GraphPad Software Inc., La Jolla, CA). For all statistical analyses, significance was accepted at the 95% level of confidence ($\alpha = 0.05$).

C. RESULTS

1. Cocaine-Primed Reinstatement

   a. Disulfiram pretreatment

   The effects of a 2-hr pretreatment of disulfiram are shown in Figure 9. The mean response rate (± SEM) during maintenance ED_{Max} cocaine self-administration sessions was 1.32 ± 0.21 responses/sec. When given a pretreatment with disulfiram vehicle, cocaine induced responding during reinstatement to levels nearly 100% of the response rate maintained during maintenance of cocaine self-administration. Following pretreatment with disulfiram, cocaine-induced reinstatement was near the level of responding during maintenance of cocaine self-administration (95%). Two-way repeated-measures ANOVA indicated a main effect of cocaine dose ($F_{(1,3)}$ = 15.57, p = 0.029), but no significant main effect of disulfiram pretreatment ($F_{(1,3)}$ = 0.01, p = 0.939) or interaction ($F_{(1,3)}$ = 0.27, p = 0.641).

   b. Nepicastat pretreatment

   To remain consistent with behavioral tests performed in rats, the effects of a 2-hr pretreatment with nepicastat were evaluated in squirrel monkeys (*Fig. 10*). The mean response rate (± SEM) during maintenance ED_{Max} cocaine self-administration sessions was 1.56 ± 0.10 responses/sec. During reinstatement sessions, a noncontingent cocaine prime was given immediately before the start of the session. The ED_{Peak} priming dose of cocaine increased responding to between 40-90% of levels maintained during cocaine self-administration. Nepicastat (10mg/kg, i.m.) given 2 hours before the start of the reinstatement session did not affect responding during reinstatement (paired t(2)=3.8, p=0.068) (*Fig. 10a*). Similarly, a 2-hr pretreatment of a higher dose of nepicastat (30mg/kg,

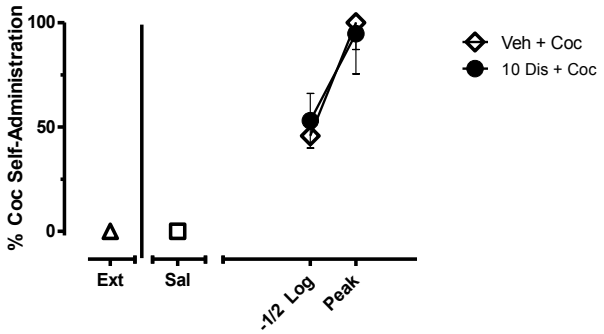

Figure 9: Effects of a 2-hr pretreatment with 10mg/kg, i.m. disulfiram on cocaine-induced reinstatement in squirrel monkeys (n=2). Data (mean ± SEM) are expressed as the percent of responding maintained during cocaine self-administration sessions. Priming with ED_{Peak} cocaine reinstated responding to levels near that maintained by cocaine self-administration while priming with a cocaine dose a half log step less than the ED_{Peak} reinstated responding levels to nearly 50% of those maintained by cocaine self-administration. Disulfiram did not alter the reinstatement effect of either priming dose of cocaine.

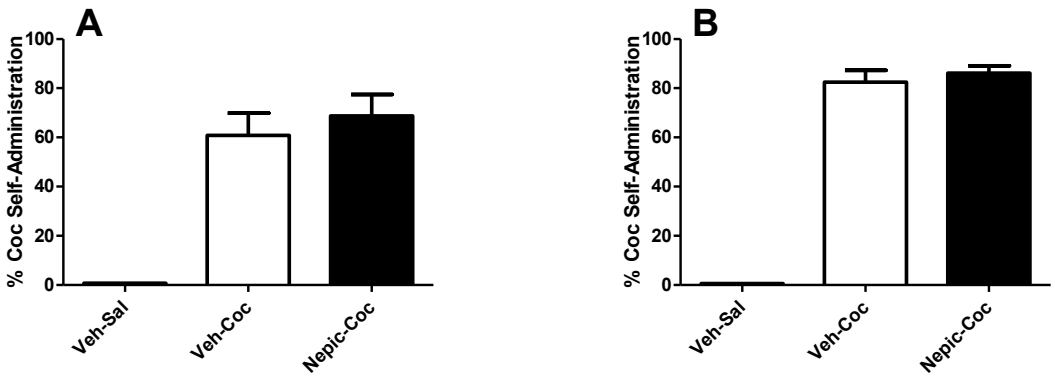

Figure 10: Effects of a 2-hr pretreatment of with (**A**) a low dose (10mg/kg, i.m.) and (**B**) a high dose (30mg/kg, i.m.) of nepicastat on cocaine-induced reinstatement in squirrel monkeys (n=3). Data (mean ± SEM) are expressed as the percent of responding maintained during cocaine self-administration sessions. Priming with ED_{Peak} cocaine reinstated responding to levels near that maintained by cocaine self-administration; nepicastat did not alter the reinstatement effect of cocaine.

i.m.) did not affect responding during reinstatement (paired t(2)=0.75, p=0.534) (*Fig. 10b*).

Because nonhuman primates typically have a slower rate of metabolism than rodents, longer pretreatment times were tested (*Fig. 11*). The mean response rate (± SEM) during maintenance ED_{Max} cocaine self-administration sessions was 1.38 ± 0.08 responses/sec. A pretreatment with a high dose of nepicastat (30 mg/kg, i.m.) 4 hours prior to the noncontingent cocaine prime did not affect responding (paired t(2)=0.34, p=0.769) (*Fig. 11a*). A 5-day subchronic regimen was also conducted prior to the reinstatement session. Animals were treated daily with nepicastat (30mg/kg, i.m.) at the same time for 5 days in their homecage, with no intermediate behavioral sessions. The last pretreatment was administered on the 5^{th} day, two-hours prior to the start of the reinstatement session. The subchronic treatment did not affect reinstatement responding (paired t(2)=0.94, p=0.447) (*Fig. 11b*).

To determine whether nepicastat has an effect on cocaine-induced reinstatement when a lower priming dose of cocaine was administered, animals were primed with both their ED_{Peak} cocaine prime as well as a priming dose that was one-half log-step lower than their ED_{Peak} (*Fig. 12*). A shorter, 30-min, pretreatment time was examined with two different priming doses of cocaine (*Fig. 12a*). When given a vehicle pretreatment, the maximally effective priming dose of cocaine (ED_{Peak}) induced responding to 85% of maintenance levels. A nepicastat (10mg/kg, i.m.) pretreatment did not affect responding at either priming dose of cocaine. Two-way repeated-measures ANOVA indicated significant main effects for cocaine dose ($F_{(1,4)}$ = 8.29, p = 0.045) but not a significant main effect for nepicastat

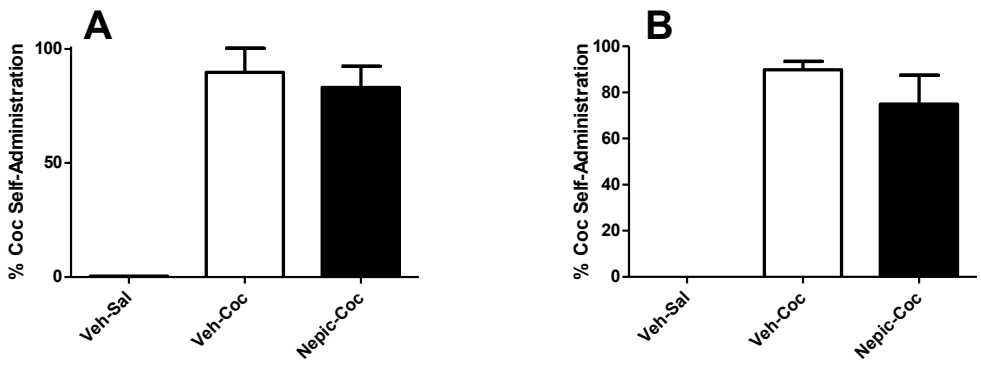

Figure 11: Effects of pretreatment with nepicastat on cocaine-induced reinstatement in squirrel monkeys (n=3). Data (mean ± SEM) are expressed as the percent of responding maintained during cocaine self-administration sessions. Priming with ED_{Peak} cocaine reinstated responding to levels near that maintained by cocaine self-administration. (**A**) Reinstatement following an acute 4-hr pretreatment with a low dose of nepicastat (10mg/kg, i.m.) did not alter the reinstatement effect of cocaine. (**B**) Subchronic 5 day pretreatment with 30mg/kg, i.m. nepicastat did not alter the reinstatement effect of cocaine (compared to an acute 2-hr pretreatment vehicle condition).

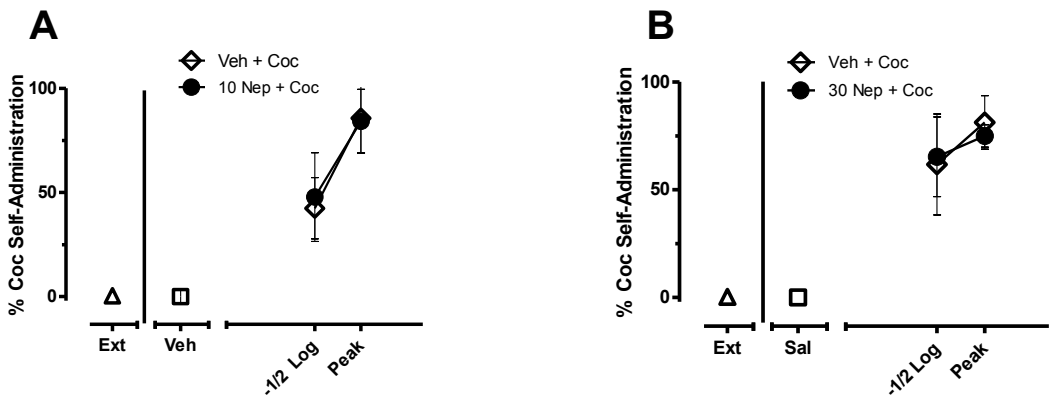

Figure 12: Effects of nepicastat pretreatment on cocaine-induced reinstatement in squirrel monkeys (n=3). Data (mean ± SEM) are expressed as the percent of responding maintained during cocaine self-administration sessions. (**A**) Priming with a maximally effective ED_{Peak} cocaine dose after a 30-min vehicle pretreatment reinstated responding to levels near that maintained by cocaine self-administration while priming with a cocaine dose a half log lower than the ED_{Peak} reinstated responding levels to nearly 50% of those maintained by cocaine self-administration. Nepicastat (10mg/kg, i.m.) did not alter the reinstatement effect of either priming dose of cocaine. (**B**) Priming with ED_{Peak} cocaine dose after a 24-hr vehicle pretreatment reinstated responding to levels near that maintained by cocaine self-administration while priming with a cocaine dose a half log step less than the ED_{Peak} reinstated responding levels to nearly 75% of those maintained by cocaine self-administration. Nepicastat (30mg/kg, i.m.) did not alter the reinstatement effect of either priming dose of cocaine.

pretreatment ($F_{(1,4)}$ = 0.69, p = 0.453) or interaction ($F_{(1,4)}$ = 2.20, p = 0.212). Similarly, a 24-hr pretreatment time was examined with two priming doses of cocaine (*Fig. 12b*). After a vehicle pretreatment, the maximally effective priming dose of cocaine (ED_{Peak}) induced responding to around 80% of levels maintained during maintenance of cocaine self-administration. Pretreating with nepicastat (30mg/kg, i.m.) did not affect reinstatement responding Two-way repeated-measures ANOVA did not indicate a significant main effect for cocaine dose ($F_{(1,4)}$ = 5.50, p = 0.079), nepicastat pretreatment ($F_{(1,4)}$ = 0.01, p = 0.914) or interaction ($F_{(1,4)}$ = 0.18, p = 0.697).

2. Yohimbine-Primed Reinstatement

Yohimbine-primed reinstatement was used as a positive control to test the effectiveness of pharmacological DBH inhibition in a NE dependent paradigm. While a yohimbine prime did not induce a full reinstatement effect, subjects did increase lever pressing from extinction levels; average response rate was near 50% of responding during maintenance (*Fig. 13*). A nepicastat pretreatment (10mg/kg, i.m.) given 30-min prior to the yohimbine prime increased response rates to ~50% of rates maintained during maintenance of self-administration (t(2)=18.18, p=0.003).

3. Nepicastat-Primed Reinstatement

Because nepicastat enhanced yohimbine-induced reinstatement, it was necessary to test whether a nepicastat prime alone is sufficient to induce reinstatement to cocaine-seeking. Priming injections of nepicastat (0, 10, 30mg/kg, i.m.) were given 30-min or 120-min before the start of reinstatement sessions (*Fig 14*). When given

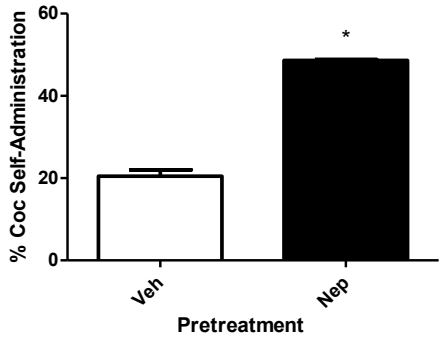

Figure 13: Effects of a 2-hr pretreatment with 10mg/kg, i.m. nepicastat on yohimbine-induced reinstatement (0.3mg/kg, i.m.) in squirrel monkeys (n=2). Data (mean ± SEM) are expressed as the percent of responding maintained during cocaine self-administration sessions. Yohimbine had a modest effect on responding during reinstatement, while a nepicastat pretreatment in combination with yohimbine enhanced responding to nearly 50% of levels maintained by cocaine self-administration. *$p<0.005$, compared to vehicle control.

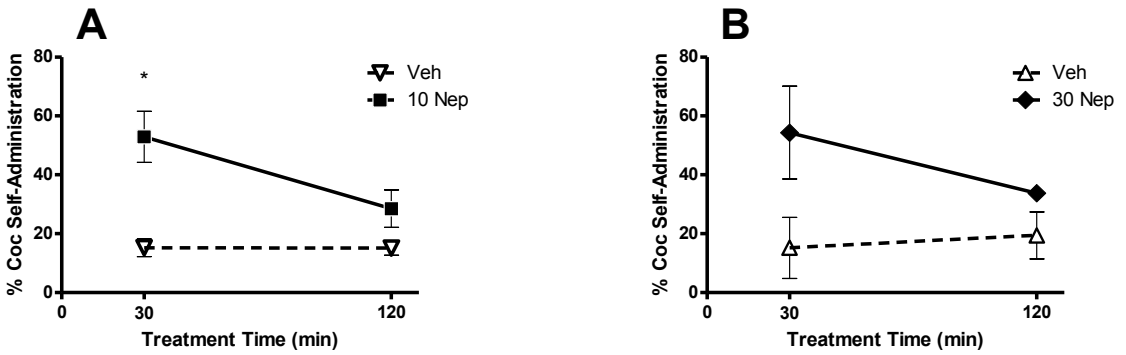

Figure 14: Reinstatement effects of nepicastat administered alone. (**A**) 10mg/kg, i.m. or (**B**) 30mg/kg, i.m. nepicastat was administered at two time points (30-min and 120-min) before the start of a reinstatement session. Data (mean ± SEM) are expressed as the percent of responding maintained during cocaine self-administration sessions. Both doses of nepicastat increased response rates when the prime was given 30-min before the start of the session. *$p<0.01$, compared to vehicle control at same pretreatment time.

30-min prior to the start of the session, both priming of doses of nepicastat resulted in an increase in responding to greater than 50% of response rates maintained during maintenance of cocaine self-administration. When the prime was given 120-min prior to the session, neither priming dose of nepicastat significantly affected response rates, although there were trends towards an increase in responding. Two-way repeated-measures ANOVA for the lower dose of nepicastat (10mg/kg) indicated a main effect of nepicastat treatment ($F_{(1,7)}$ = 19.65, p = 0.003), but no significant main effect of time ($F_{(1,7)}$ = 4.51, p = 0.071) or interaction ($F_{(1,7)}$ = 4.44, p = 0.073). Post hoc analyses indicated nepicastat treatment was significantly different than vehicle treatment ($p<0.01$). Two-way repeated-measures ANOVA for the higher dose of nepicastat (30mg/kg) did not indicate a main effect of nepicastat treatment ($F_{(1,5)}$ = 4.24, p = 0.095), time ($F_{(1,5)}$ = 0.39, p = 0.558) or interaction ($F_{(1,5)}$ = 0.91, p = 0.385).

D. DISCUSSION

The aim of the present study was to assess the effects of pharmacological DBH inhibition on cocaine-induced reinstatement in squirrel monkeys. Though these effects have been evaluated in rodents, the results here demonstrate for first time the consequences of DBH inhibition on cocaine-mediated behavior in nonhuman primates. In squirrel monkeys, pharmacological DBH inhibition via nepicastat or disulfiram was ineffective in influencing cocaine-induced reinstatement. Additionally, nepicastat, in the absence of cocaine, was sufficient to partially reinstatement cocaine-seeking behavior.

We have previously shown that in rats, pharmacological DBH inhibition attenuated cocaine-induced reinstatement (see Chapter II), which supported clinical findings with DBH inhibitors. To extend the generality of the studies in rats to nonhuman

primates, drug effects on cocaine-induced reinstatement were evaluated in squirrel monkeys. A pretreatment time of 2 hours was kept consistent when disulfiram was administered, however, disulfiram did not affect cocaine-induced reinstatement in squirrel monkeys. Because of its many biological targets, disulfiram may have nonspecific effects that influence the behavioral response in squirrel monkeys. Despite the lack of an effect with a disulfiram treatment, nepicastat was also examined in the squirrel monkeys. Because it exhibited similarly favorable results as disulfiram in the rats, yet is a more promising pharmacotherapy due to its low adverse side effect profile, it was necessary to examine the effects of nepicastat in squirrel monkeys. The pretreatment time of two hours was initially kept consistent with previous studies in rodents. Neither a low nor high dose of nepicastat affected reinstatement following a 2-hour pretreatment time. Therefore, pretreatment time and dose were parametrically manipulated to further evaluate the effectiveness of nepicastat at attenuating cocaine-induced reinstatement in squirrel monkeys. Both longer (4 hr) and shorter (30 min) pretreatment times were tested with the lower dose of nepicastat. A subchronic pretreatment was also evaluated, as it would more closely approximate dosing regimens used in a clinical setting. Twenty-four hours following the subchronic treatment, a trend seemed to appear in two monkeys in which responding was uncharacteristically low. This prompted a trial with a 24-hour pretreatment with the same dose. However, none of these parametric changes affected responding during reinstatement. Hence, although DBH inhibition attenuated cocaine-induced reinstatement in rats, these results were not replicated in squirrel monkeys.

There are several potential explanations for the discrepancy observed in rodent and squirrel monkey studies. There are published studies documenting drug treatments that affect rodents and nonhuman primates disparately. For example, there is an

extensive literature on the role of group II metabotropic glutamate receptors (mGluR2/3) on cocaine mediated behavior (reviewed in Kalivas and Volkow, 2011). The majority of these studies focus on rodent behavior. Those studies concluded that mGluR2/3 agonists administered either systemically or locally in the NAc or ventral tegmental area (VTA) inhibit cocaine-induced reinstatement in rats (Peters and Kalivas, 2006; Lu et al., 2012). However, there are conflicting results on the effect of pharmacologically stimulating mGluR2/3 in squirrel monkeys. Bauzo and colleagues (2009) found that the mGluR2/3 agonist LY379268 had no significant effect on cocaine-induced reinstatement. Conversely, in a separate study, LY379268 was found to reduce cocaine-induced reinstatement in squirrel monkeys (Adewale et al., 2006). There are also conflicting results on the effects of enhancing the cystine-glutamate antiporter with N-acetyl-$_L$-cysteine (NAC). Cocaine- and cue-induced reinstatement were attenuated with NAC administration in rats (Kau et al., 2008; Kupchik et al., 2012), while NAC had no effect on reinstatement of previously extinguished cocaine self-administration in squirrel monkeys (Bauzo et al., 2012). Though there are speculative hypotheses to address these discrepancies, there are no definitive explanations that resolve the inconsistencies.

One possible explanation for the results observed in the present study is that there may be inherent species differences in responses of the noradrenergic system in these behavioral paradigms. The DBH dependent mechanism for disulfiram and nepicastat relies on the noradrenergic system. For rodents and nonhuman primates to exhibit similar behavior in response to these pharmacological treatments, their noradrenergic responses in a cocaine-induced reinstatement paradigm should also function similarly. Unfortunately, there have been very few studies that investigate the influence of the noradrenergic system on cocaine self-administration in nonhuman primates (Woolverton, 1987; Macey et al., 2003; Beveridge et al., 2005; Wee et al.,

2006; Negus et al., 2007), and even fewer that specifically study its influence on reinstatement (Lee et al., 2004; Platt et al., 2007; Valdez et al., 2007). Additionally, there are examples of divergent effects of noradrenergic treatments on cocaine-induced behavioral responses between rodent and nonhuman primate. For example, the effects of a pretreatment with the α1AR antagonist prazosin differed between rats and squirrel monkeys. Zhang and Kosten (2005) found that prazosin dose dependently attenuated cocaine-induced reinstatement in rats; however, prazosin had no effect on cocaine-induced reinstatement in squirrel monkeys (Platt et al., 2007). Furthermore, while the α2AR antagonist yohimbine was able to induce reinstatement in both rats and squirrel monkeys, the effect of a pretreatment with the α2AR agonist clonidine differed between the species. In rats, clonidine had no effect on yohimbine-induced reinstatement (Brown et al., 2009) while clonidine dose-dependently attenuated yohimbine-induced reinstatement in squirrel monkeys (Lee et al., 2004). An additional discrepancy between rats and squirrel monkeys in the effects of noradrenergic manipulations involves NET inhibition on cocaine-seeking. Systemic administration of the NET inhibitor, nisoxetine, did not reinstate cocaine-seeking in rats (Schmidt and Pierce, 2006); however, nisoxetine did reinstate cocaine-seeking in squirrel monkeys (Platt et al., 2007). The present study produces another example of noradrenergic manipulation that does not coincide between species in regards to cocaine-mediated behavior. The reasons underlying the divergent effects of DBH inhibition on cocaine-induced reinstatement are unclear; however, there are a few noteworthy differences that can explain the discrepancies.

There is a notable methodological difference between the reinstatement paradigms used in the rats and squirrel monkeys to test the effects of DBH inhibition. In the study by Schroeder and colleagues (2010), the cue lights that functioned as the

conditioned reinforcer for the rats were removed during extinction sessions and remained absent during reinstatement tests. Conversely, in the present study, the conditioned reinforcer cue lights were removed during extinction sessions but restored during the reinstatement sessions. Thus the squirrel monkeys were effectively experiencing a combined cocaine- and cue-induced reinstatement session. Previously, studies have shown that in squirrel monkeys, reinstatement precipitated by this combination of stimuli results in the most robust reinstatement effect (Spealman et al., 1999). Though we have used this reinstatement paradigm for previous studies in our laboratory (Bauzo et al., 2009, 2012; Manvich et al., 2012a; Manvich et al., 2012b), it is possible that the combined reinstatement, especially following the DBH inhibition, influenced the results in the squirrel monkeys disparately from in the rats.

Both pharmacological and genetic DBH inhibition increase tissue DA levels because these manipulations prevent noradrenergic neurons from converting DA to NE (Bourdelat-Parks et al., 2005; Schroeder et al., 2010). This, however, does not necessarily translate into an increase in basal extracellular DA levels (Schank et al., 2006). Under normal circumstances in rats, noradrenergic neurons that originate in the locus coeruleus (LC) and project to the PFC co-release DA and NE. Stimulating the LC increases extracellular levels of both NA and DA in the PFC (Devoto et al., 2005a, b); however, lesioning the VTA has no effect on extracellular levels of DA in the PFC (Devoto et al., 2008). Additionally, the DA reuptake transporter (DAT) inhibitor, GBR 12909, locally perfused into the medial PFC has no effect on extracellular DA or NA levels, while the NET inhibitor desipramine increases extracellular levels of both neurotransmitters (Devoto et al., 2004). This suggests that the sources of DA in the PFC are likely from noradrenergic neurons projecting from the LC in addition to dopaminergic neurons projecting from the VTA. Because DBH inhibition causes an excess of DA in

normally noradrenergic neurons and DA can be released from noradrenergic neurons terminating in the PFC, it is conceivable that the excess DA is being released in the PFC of the squirrel monkeys. Furthermore, DA is packaged into vesicles via the vesicular monoamine transporter (VMAT2) where it is then converted into NE by DBH (Lagercrantz, 1976; Eiden and Weihe, 2011). If DBH is inhibited, the vesicular contents released from noradrenergic neurons would be the packaged DA that was not converted into NE. Because DA in the PFC is involved in reinstatement (McFarland and Kalivas, 2001; Park et al., 2002; Sun and Rebec, 2005), this release of DA in the PFC can potentially facilitate reinstatement. This effect occurs in the squirrel monkeys and not in the rats because the conditioned reinforcer (the drug cue) in the combined reinstatement paradigm may drive the release of the excess DA in the PFC of the squirrel monkeys.

Following the cocaine-induced reinstatement sessions, it was unclear whether DBH inhibition did not affect cocaine-induced reinstatement in the squirrel monkeys because the noradrenergic system is not as essential in the squirrel monkeys as it is in the rats or because disulfiram and nepicastat function differently in the squirrel monkeys. To test whether nepicastat would block reinstatement in a paradigm that was NE-dependent, yohimbine-induced reinstatement was used as a positive control. Yohimbine increases extracellular NE levels and induces reinstatement to cocaine-seeking. In the present study, yohimbine induced a modest reinstatement, but nepicastat unexpectedly enhanced that effect. DA release from noradrenergic neurons may also explain why the yohimbine-primed reinstatement experiments did not function as a positive control for the effects of a nepicastat pretreatment. DA co-release from noradrenergic neurons is thought to be mediated by the α2AR (Devoto et al., 2001; Devoto et al., 2004). The α2AR antagonist RS 79948 increases extracellular levels of both DA and NE in the in the medial PFC (Devoto et al., 2004). Conversely, the α2AR agonist clonidine reverses

the DA increasing effects DBH inhibition in the medial PFC of rats (Devoto et al., 2012; Devoto et al., 2013). Yohimbine also functions as an α2AR antagonist and has previously been found to increase extracellular DA in the medial PFC (Tanda et al., 1996). In the present study, a yohimbine prime may have released the excess DA that accumulated in the PFC following the nepicastat pretreatment, therefore enhancing the effect of yohimbine-induced reinstatement.

Another possible explanation for the behavioral differences between the rats and the squirrel monkeys may be due to differences in drug metabolism and pharmacokinetics. It is well known that drug clearance can differ between species, which may underlie the difference exhibited in these behavioral paradigms. For example, DA metabolism differs greatly between rodents and primates. Homovanillic acid (HVA) and 3,4-Dihydroxyphenylacetic acid (DOPAC) are both DA metabolites, but their relative striatal concentration is species dependent. In primates, HVA is found in higher concentration than DOPAC in the striatum (Bacopoulos et al., 1978; Wilk and Stanley, 1978; Rollema et al., 1989), while DOPAC is found in higher concentration in rat striatum (Wilk et al., 1975). Additionally, the proportion of the two forms of monoamine oxidase (MAO), the enzyme that deaminates DA, differs between rodents and primates. MAO type B (MAO-B) is present in greater concentration in primates while the concentration of MAO-A is higher in rodents (Garrick and Murphy, 1980). The metabolic profile of nepicastat is not well characterized and it is unknown if it is comparable for rodents and primates. Because both the metabolizing enzymes as well as the corresponding active drug metabolites can differ within species, it is possible that nepicastat and disulfiram are metabolized differently in rats and squirrel monkeys. Difference in drug clearance and pharmacokinetics can potentially explain the dissimilarities in behavioral responses to DBH inhibition.

Based solely on the inability of nepicastat to affect cocaine-induced reinstatement, its ability to cross the blood-brain barrier became questionable. While there is evidence that nepicastat crosses the blood brain barrier in beagles and rats (Stanley et al., 1997; Schroeder et al., 2010), it has not been indisputably determined whether it crosses the blood-brain barrier in nonhuman primates. Because nepicastat has been found to cross the blood-brain barrier in some species, it is likely that it crosses the blood-brain barrier in squirrel monkeys as well. While there were no behavioral effects of nepicastat in a cocaine-induced reinstatement paradigm, nepicastat did increase responding in yohimbine-induced and nepicastat-induced reinstatement. This suggests that cocaine-mediated behavioral responses to nepicastat in squirrel monkeys are paradigm-dependent, and it is possible that nepicastat is crossing the blood-brain barrier to influence these responses. However, definitive evidence that nepicastat crosses the blood brain barrier in squirrel monkeys cannot be determined until nepicastat levels are measured in brain tissue or cerebrospinal fluid following nepicastat administration. Though it is possible that nepicastat is crossing the blood-brain barrier, it is unknown whether there are any differences in the rate and extent of drug delivery between rats and squirrel monkeys. Along with the potential disparity in drug metabolism, differences in drug distribution in the brain may underlie the discrepancies observed in behavioral responses to nepicastat in the rats and squirrel monkeys.

In summary, the present study demonstrated that, unlike in rats, DBH inhibition in squirrel monkeys did not affect cocaine-induced reinstatement. The selective DBH inhibitor, nepicastat, was sufficient to induce reinstatement to cocaine-seeking when administered alone. The behavioral effects of DBH inhibition in squirrel monkeys complicate the extrapolation of these treatments to human conditions. However, the promising effects observed in rat and human clinical studies warrant further testing to

conclude whether DBH inhibition will be a potential pharmacotherapy for cocaine relapse prevention.

CHAPTER IV

Pharmacological DBH Inhibition: Effect on Striatal Neurochemistry in Squirrel Monkeys

A. INTRODUCTION

Disulfiram (Antabuse) has been used as an alcohol abuse pharmacotherapy for more than 50 years (Moriarty, 1950; Fuller et al., 1986). One of the many biological targets of disulfiram is aldehyde dehydrogenase (ALDH), an enzyme involved in the alcohol metabolic pathway. When alcohol is consumed after disulfiram is taken, ALDH is inhibited and subsequently levels of acetaldehyde increase. This increase in acetaldehyde causes a reaction called the 'Antabuse effect' (extreme nausea, flushing, vomiting). This aversive reaction is thought to deter alcohol consumption, thereby underlying disulfiram's efficacy in alcohol abuse. More recently, disulfiram has been implicated as a potential pharmacotherapy for cocaine abuse. Disulfiram is able to reduce both cocaine and alcohol intake in a population that abuses both substances (Carroll et al., 1998; Carroll et al., 2000). Further studies found that disulfiram is equally, if not more, effective at reducing cocaine intake in subjects who are not consuming alcohol (Carroll et al., 2004; Carroll et al., 2012). Because ALDH is not involved in cocaine metabolism, and there is no subsequent acetaldehyde buildup or Antabuse reaction, it is likely that a mechanism other than ALDH inhibition underlies the ability of disulfiram to reduce cocaine intake.

The efficacy of disulfiram to reduce cocaine intake has been attributed to its ability to inhibit dopamine β-hydroxylase (DBH). DBH is a copper containing mono-oxygenase enzyme that converts dopamine (DA) into norepinephrine (NE). The major metabolite of disulfiram, diethyldithiocarbamate, is a copper chelator that affects the activity of any enzyme that utilizes copper, including DBH. Disulfiram indeed has been

shown to reduce tissue NE levels and increase tissue DA levels in rat (Musacchio et al., 1966; Goldstein and Nakajima, 1967; Karamanakos et al., 2001; Schroeder et al., 2010) and mouse (Bourdelat-Parks et al., 2005) brain. Both DA and NE have a significant role in cocaine abuse pharmacology. Cocaine blocks plasma membrane monoamine transporters which subsequently increases extracellular levels of DA, NE and serotonin. Dopaminergic systems are primarily responsible for the reinforcing effects of cocaine and reinstatement to cocaine-seeking, an animal model of relapse (Risner and Jones, 1976; Yokel and Wise, 1976; Roberts et al., 1977; Spealman et al., 1999; Shaham et al., 2003). The dopaminergic system most closely linked with the abuse-related effects of cocaine is the mesocorticolimbic system. This system consists of dopaminergic neurons localized in the ventral tegmental area (VTA) that send several axonal projections terminating in the nucleus accumbens (NAc), amygdala, and prefrontal cortex (PFC) (Moore and Bloom, 1978; Haber and McFarland, 1999). It has been demonstrated that lesioning the VTA or NAc within this system reduces the reinforcing effects of cocaine in rodents (Roberts et al., 1977; Roberts et al., 1980; Roberts and Koob, 1982). Though there is ample evidence for the role of DA in cocaine related effects, the role for NE has only more recently become elucidated. Though NE does not have a significant role in maintaining the reinforcing effects of cocaine (Roberts et al., 1977; Woolverton, 1987; Wee et al., 2006), it is associated with influencing reinstatement of cocaine-seeking. Pharmacologically reducing NE levels attenuates reinstatement (Erb et al., 2000; Leri et al., 2002; Zhang and Kosten, 2005; Schroeder et al., 2010; Smith and Aston-Jones, 2011) while increasing NE induces reinstatement (Lee et al., 2004; Erb, 2010). Because of the contribution of these neurotransmitters to the abuse-related effects of cocaine, they are ideal targets for potential pharmacotherapies. It is therefore likely that the clinical efficacy of disulfiram is due to its inhibition of DBH and subsequent alteration of DA and NE levels.

Consistent with an intervention that lowers brain NE levels, disulfiram blocks cocaine-induced reinstatement of cocaine-seeking in rats (Schroeder et al., 2010). A specific DBH inhibitor, nepicastat, also blocks cocaine-induced reinstatement (Schroeder et al., 2010), as well as attenuates stress- and cue- induced reinstatement (Schroeder et al., 2013) in rats. It is important to note that reinstatement was attenuated in these experiments despite the increase in tissue DA. Because NE facilitates DA neuron firing and DA release that is imperative for psychostimulant-induced responses, DBH inhibition subsequently reduces firing of noradrenergic neurons onto mesolimbic DA neurons (Gaval-Cruz and Weinshenker, 2009). Therefore, even though brain tissue levels of DA increase with DBH inhibition, there is not necessarily a correlative increase in extracellular DA levels. Consistent with hypothesis, mice treated with the DBH inhibitor fusaric acid (Weinshenker et al., 2008) and mice genetically lacking DBH (Schank et al., 2006) have decreases in amphetamine- and methamphetamine-induced extracellular DA release in the striatum. Contrary to these results, however, Devoto and colleagues found that neither disulfiram (Devoto et al., 2012) nor nepicastat (Devoto et al., 2013) have an effect on cocaine-induced DA overflow in the nucleus accumbens, yet both markedly increase cocaine-induced DA overflow in the medial prefrontal cortex (mPFC) of rats. This suggests that perhaps the increase in cortical DA following DBH inhibition functions as a DA replacement therapy and is effective in reversing a hypodopaminergic state.

There are currently no studies that assess the effects on DBH inhibition on catecholamine levels in nonhuman primates. Based on differences in their behavioral responses to DBH inhibition, it is possible that the effects on catecholamine neurochemistry differ between the species. Though both disulfiram and nepicastat block reinstatement to cocaine-seeking in rats, these treatments have contrasting effects in a

nonhuman primate model (see Chapter III). In squirrel monkeys, neither disulfiram nor nepicastat affect responding during cocaine-induced reinstatement. Moreover, nepicastat was sufficient to induce reinstatement to cocaine-seeking. It is not yet understood why there is a difference between the species in cocaine-induced behavior following DBH inhibition, but one plausible explanation is due to differences in the effects of DBH inhibition on catecholamine synthesis and/or release. The goal of the current study was to use *in vivo* microdialysis in awake squirrel monkeys to assess whether loss of noradrenergic drive onto midbrain DA neurons, via DBH inhibition, affects basal and cocaine-induced DA output in the striatum.

B. METHODS

1. Subjects

Four male squirrel monkeys (*Saimiri sciureus*) weighing between 930g - 1050g were used as subjects for the following experiments. Animals were individually housed, had ad libitum access to water, and were fed twice daily (LabDiet 5045 High Protein Monkey Chow, PMI Nutrition International, Brentwood, MO; fresh fruit/vegetables; cereal). Monkeys were provided daily enrichment with access to foraging devices, toys, climbing devices, swings and nature sounds. Animals previously served in behavioral studies that involved administration of compounds acting on monoaminergic systems (Kimmel et al., 2007; Bauzo et al., 2009; Fantegrossi et al., 2009; Kimmel et al., 2009; Bauzo et al., 2012; Manvich et al., 2012a; Manvich et al., 2012b). Animals also previously served in *in vivo* microdialysis studies that targeted the caudate nucleus (within the same dorsal-ventral plane as the nucleus accumbens) (Manvich et al., 2012a; Manvich et al., 2012b). All studies were conducted in strict accordance with the National Institutes of Health's "Guide for Care and Use of Laboratory Animals", the American

Association for Accreditation of Laboratory Animal Care (AAALAC), and were approved by the Institutional Animal Care and Use Committee of Emory University.

2. Apparatus

Animals were comfortably seated in a commercially-available plexiglass chair within a ventilated, sound-attenuating chamber (Med Associates Inc., St. Albans, VT) supplemented with an adjustable Lexan barrier that was situated slightly above the level of the animal's shoulders to prevent disturbance to microdialysis probes and connective tubing. A motor-drive syringe pump (Model 11Plus Dual-Syringe, Harvard Apparatus, Holliston, MA) was mounted on top of the operant chamber for automated delivery of microinfused solutions.

3. Surgeries

Animals in *in vivo* microdialysis studies were implanted with bilateral guide cannulae (CMA/11; CMA/Microdialysis, Acton, MA) using stereotaxic techniques under aseptic conditions as described previously (Czoty et al., 2000). Animals were initially anesthetized with Telazol (tiletamine HCl and zolazepam HCl, 2.0mg, i.m.) and ketamine HCl (20mg, i.m.). Anesthesia was maintained throughout the procedure with inhaled isoflurane (0.5-1.5%). Guide cannulae targeted the nucleus accumbens using the following coordinates from the earbar: anterior/posterior + 15.0, medial/lateral +/- 3.0. When subjects were not actively participating in microdialysis experiments, stainless-steel stylets were situated within the cannulae to maintain the integrity and sterility of the tissue site. For all surgical procedures, preoperative and postoperative antibiotics (ceftriaxone) and postoperative analgesics (meloxicam) were administered by veterinary staff who closely monitored the animals. Subjects were allowed one month of recovery before microdialysis experiments commenced.

4. *In Vivo* Microdialysis

The microdialysis protocols used in the present study were similar to those described previously (Czoty et al., 2000; Kimmel et al., 2005; Kimmel et al., 2007; Bauzo et al., 2009; Manvich et al., 2012a; Manvich et al., 2012b). CMA/11 dialysis probes (CMA Microdialysis, North Chelmsford, MA) with a shaft length of 20mm and active dialysis membrane measuring 2 x 0.24 mm were used for all studies. The probe inlet was connected via FEP Teflon tubing (CMA Microdialysis, North Chelmsford, MA; 1.2μL/100mm) to a microinfusion syringe (Hamilton Co., Reno, NV) mounted on a motor-driven syringe pump (Harvard Apparatus, Holliston, MA). FEP Teflon tubing was connected to the probe outlet and was directed outside of the experimental chamber where dialysate samples were collected in microcentrifuge tubes.

Prior to the start of an experiment, probes were flushed with artificial cerebrospinal fluid (aCSF: 1.0 mM Na_2HPO_4, 150 mM NaCl, 3 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$, and 0.15 mM ascorbic acid). After the probes were flushed for 30 min, the stylets were removed and probes were inserted into the guide cannulae. For the duration of an experiment, aCSF was perfused through the probe at a flow rate of 0.2 μl/min. Once probes were inserted into the guide cannula a 60-min equilibration sample was collected. Following the equilibrium period, three baseline samples were collected at 10-min intervals for determination of basal DA concentrations. Following baseline sample collection, microdialysis proceeded with the following drug administration conditions: disulfiram (10 mg/kg), disulfiram (10 mg/kg) administered 30-min prior to cocaine (1.0 mg/kg), nepicastat (veh, 10 mg/kg), and nepicastat (veh, 10 mg/kg) administered 30-min prior to cocaine (1.0 mg/kg). The disulfiram + cocaine combination was compared to the vehicle + cocaine combination used in the nepicastat experiments.

Following drug administration, additional 10-min samples were collected for a total session duration of 4-5 hours. The interval between pretreatments and cocaine administration and the doses of all drugs were chosen based on results from previous behavioral studies in which nepicastat induced reinstatement when given 30-min before the start of a reinstatement session. All samples were refrigerated or frozen until immediately prior to analysis. Probes were tested in vitro both prior to and immediately after each session to determine probe viability and percent-recovery. To confirm integrity of the site, following experimental sample collection, the KCl concentration within the perfused aCSF was increased to 100 mM and a final 10-min sample was collected. A robust increase in extracellular DA levels confirmed site viability. We have previously shown repeated microdialysis accesses without a resultant loss of site viability (Czoty et al., 2000). Each experimental session was conducted in a single brain hemisphere. For each subject, all drug combinations within a given experiment were acquired from the same ipsilateral hemisphere. Accesses at each brain site were separated by at least two weeks. The order of drug dose combinations was randomized within subjects.

Levels of DA were quantified within each sample using high-performance liquid chromatography with electrochemical detection as described previously (Czoty et al., 2000; Kimmel et al., 2005; Kimmel et al., 2007; Bauzo et al., 2009; Manvich et al., 2012a; Manvich et al., 2012b). The HPLC system consisted of a small-bore (3 mm i.d. x 100 mm) column (MD-150 Analytical, 3 mm i.d. x15 cm; ESA, Chelmsford, MA) with a commercially-available mobile phase (ESA, Chelmsford, MA). Experimental samples (20 µl) collected into microcentrifuge vials were loaded into a refrigerated CMA/200 autosampler. Before each sample was analyzed, 3 µl of ascorbate oxidase was mixed into the sample and 10 µl of the mixture was injected into the HPLC system via an ESA 582 solvent delivery pump at a flow rate of 0.6 ml/min. Electrochemical analyses were

performed using an ESA dual-channel analytical cell (model 5040) and guard cell (model 5020) and an ESA Coulochem II detector. Potentials were set as follows: channel 1, -150 mV (oxidation); channel 2, +275 mV (reduction); guard cell, 350 mV. EZChrome Elite v. 3.1 software (Scientific Software, Pleasanton, CA) was used to generate chromatograms for each sample analyzed. A set of DA standards containing experimenter-prepared concentrations of DA (0.5-25 nM) were analyzed in duplicate before and after each set of experimental samples. Area under the curve (AUC) was calculated for each standard. A standard plot (AUC x DA concentration) was generated from which the estimated DA concentration for each experimental sample could be extrapolated.

5. Drugs

Cocaine HCl (National Institute on Drug Abuse, Research Technology Branch, Research Triangle Park, NC) was dissolved in 0.9% sterile saline. Nepicastat (Synosia Therapeutics, South San Francisco, CA) was sonicated and dissolved at a concentration of 30 mg/ml in a 20:20:60 mixture of 95% ethanol, Tween 80 (Sigma-Aldrich, St. Louis, MO), and 0.9% sterile saline. Disulfiram (Sigma-Aldrich, St Louis, MO) was sonicated in sterile water and injected as a suspension. Doses were calculated from the salt weights. All drugs were administered via the intramuscular route into the thigh muscle.

6. Data Analysis

Because the effects of cocaine typically returned to near-baseline levels within 60-min post cocaine administration, only samples collected within the first 60-min following a cocaine challenge were analyzed. For each subject, DA levels within each test session were normalized as the percent of the mean of three baseline values

acquired prior to drug administration. Data were analyzed using a two-way repeated-measures ANOVA. Bonferroni post hoc tests then determined at each timepoint whether DA levels were affected by nepicastat compared to vehicle treatment. Data were graphically plotted and analyzed using GraphPad v. 5.01 (GraphPad Software Inc., La Jolla, CA). For all statistical analyses, significance was accepted at the 95% level of confidence ($\alpha = 0.05$).

C. RESULTS

1. *in vivo* Microdialysis

a. Basal DA levels

The effects of pharmacological DBH inhibition on basal DA levels in the NAc were evaluated in awake squirrel monkeys. Over the 2-hr timecourse, disulfiram treatment steadily lowered DA levels compared to vehicle treatment (*Fig. 15*). Two-way repeated-measures ANOVA indicated a significant main effect of time ($F_{(18,72)} = 3.22$, $p = 0.0002$), and interaction ($F_{(18,72)} = 4.35$, $p < 0.0001$), but not disulfiram dose ($F_{(1,72)} = 3.35$, $p = 0.1412$). Subsequent post hoc analyses indicated that although there was no main effect of treatment, there was a significant difference in DA concentration nearly 80-min following drug administration ($p<0.05$).

Mean ± SEM basal DA levels uncorrected for probe recovery before nepicastat administration were 3.30 ± 1.09 nM (*Fig. 16*). Over the 2-hr timecourse, nepicastat treatment did not significantly alter DA levels compared to vehicle treatment. Two-way repeated-measures ANOVA indicated a significant main effect of time ($F_{(13,78)} = 2.59$, $p = 0.0048$), but not nepicastat dose ($F_{(1,78)} = 0.01$, $p = 0.940$) or interaction ($F_{(13,78)} = 0.77$, $p = 0.690$).

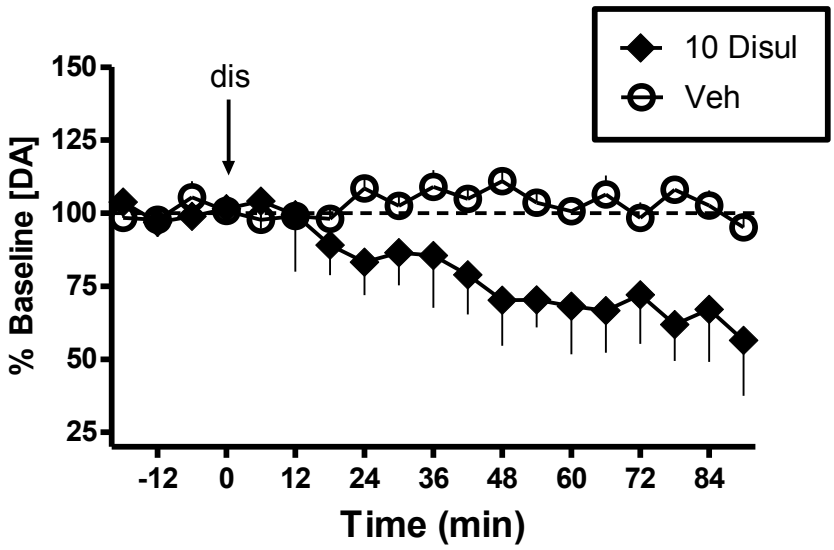

Figure 15: Shown are the effects of 10 mg/kg disulfiram on extracellular DA levels in the NAc of squirrel monkeys. Data points (mean ± S.E.M.) are expressed as the percentage of baseline DA levels before drug administration (n=3). (Data collected by Heather Kimmel, Ph.D.)

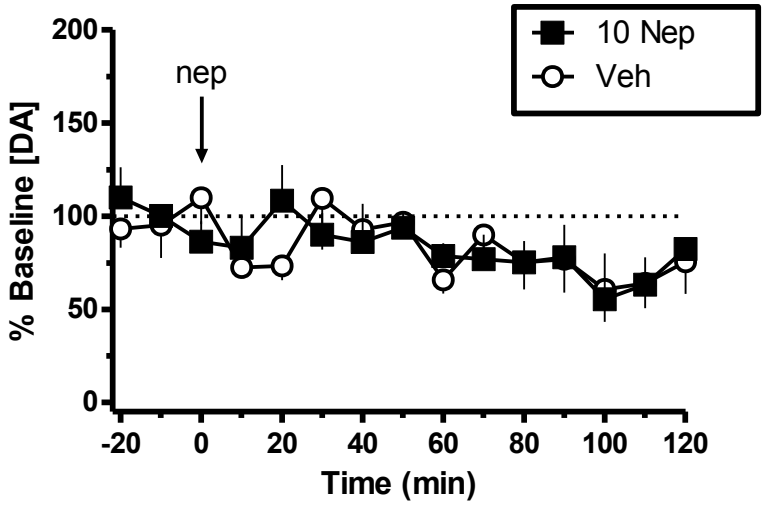

Figure 16: Shown are the effects of 10 mg/kg nepicastat on extracellular DA levels in the NAc of squirrel monkeys. Data points (mean ± S.E.M.) are expressed as the percentage of baseline DA levels before drug administration (n=4).

b. Cocaine induced DA overflow

The effects of DBH inhibition on cocaine-induced DA overflow in the NAc were evaluated in awake squirrel monkeys. Following basal sample collection, a disulfiram pretreatment (10mg/kg, i.m.) was administered. 30-min after the pretreatment, a cocaine (1.0mg/kg, i.m.) challenge was administered. There was no peak increase in DA concentration following the combined administration of disulfiram and cocaine (*Fig 17*). Two-way repeated-measures ANOVA indicated a significant main effect of disulfiram dose ($F_{(1,20)}$ = 63.40, p = 0.0013), but not time ($F_{(5,20)}$ = 1.80, p < 0.1594), or interaction ($F_{(5,20)}$ = 2.29, p = 0.0844). Subsequent post hoc analyses indicated that although nepicastat did not affect DA levels during the interval preceding cocaine administration, the peak increase of DA levels following cocaine administration (20-min) was significantly lowered when a nepicastat pretreatment was given as compared to the increase following cocaine and vehicle pretreatment (p<0.01).

In a separate experiment, a nepicastat pretreatment (0, 10mg/kg, i.m.) was administered 30-min before cocaine (1.0mg/kg, i.m.) administration. Cocaine administered following a vehicle pretreatment increased extracellular DA in the NAc to ~240% of basal DA levels within 20-min after cocaine administration, which returned to near-baseline levels within 60-min post drug injection. Pretreatment

with nepicastat attenuated the effects of cocaine on extracellular DA levels (*Fig. 18*). Two-way repeated-measures ANOVA indicated a significant main effect of time ($F_{(8,48)}$ = 4.51, p = 0.0004), nepicastat dose ($F_{(1,48)}$ = 6.08, p = 0.0487) and interaction ($F_{(8,48)}$ = 2.56, p = 0.0208). Subsequent post hoc analyses indicated that although nepicastat did not affect DA levels during the interval preceding cocaine administration, the peak increase of DA levels following cocaine administration (20-

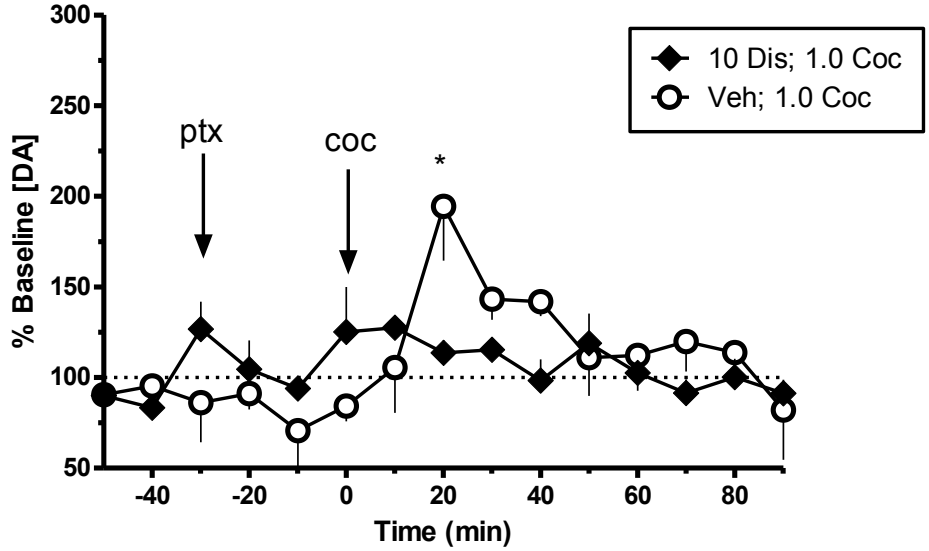

Figure 17: Shown is the effect of cocaine (1.0 mg/kg) on extracellular levels of DA in the NAc after pretreatment with 10 mg/kg disulfiram (filled diamonds) in squirrel monkeys. Data points (mean ± S.E.M.) are expressed as the percentage of baseline DA levels before drug administration (n=3). *p<0.01, compared to vehicle control.

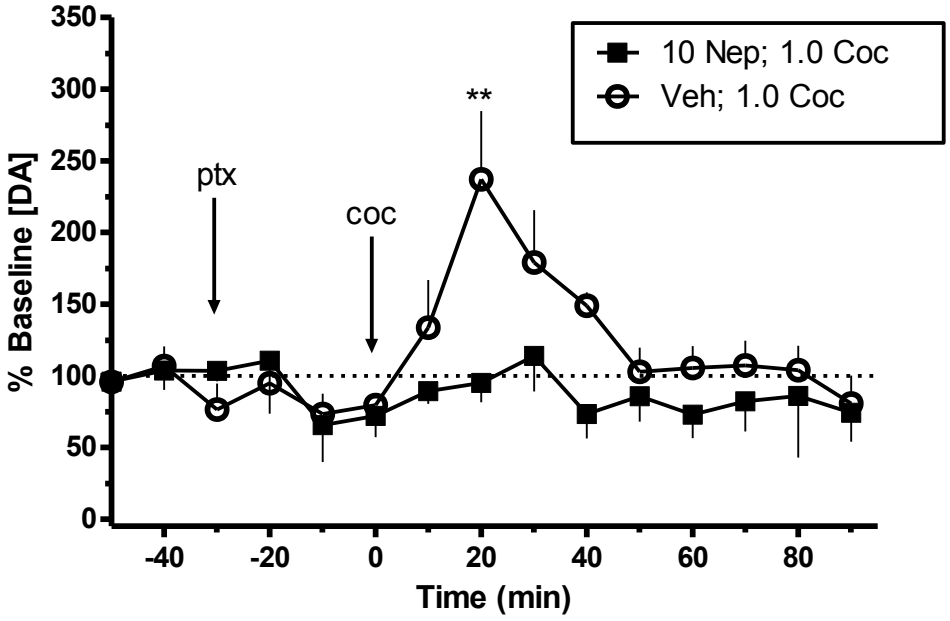

Figure 18: Shown are the effects of cocaine (1.0 mg/kg) on extracellular levels of DA in the nucleus accumbens after pretreatment with 10 mg/kg nepicastat (filled squares) or its vehicle (open squares) in squirrel monkeys. Data points (mean ± S.E.M.) are expressed as the percentage of baseline DA levels before drug administration (n=4). **p<0.001, compared to vehicle control

min) was significantly lowered when a nepicastat pretreatment was given as compared to the increase following cocaine and vehicle pretreatment ($p<0.001$).

D. Discussion

The aim of the present study was to assess the effects of pharmacological DBH inhibition on catecholamine neurochemistry in squirrel monkeys. These results demonstrate the first time that effects of DBH inhibition on cocaine-mediated DA overflow have been investigated in nonhuman primates. Though these effects have been evaluated in rodents, the effects of pharmacological DBH inhibition on cocaine-seeking behavior differ between rodents and squirrel monkeys. Pharmacological DBH inhibition via disulfiram or nepicastat blocked reinstatement in rats while, in squirrel monkeys, pharmacological DBH inhibition had no effect on cocaine-induced reinstatement. The current study attempted to examine whether differences in catecholamine neurochemistry were responsible for the differences in behavioral responses. In squirrel monkeys, nepicastat did not affect basal DA levels in the NAc but did attenuate the cocaine-mediated increase in DA release in the NAc. Similarly, cocaine administration following a disulfiram pretreatment did not generate a peak increase in DA concentration in the NAc.

Several studies have shown that changes in noradrenergic signaling can modulate DA transmission within the mesocorticolimbic DA system, primarily via α1ARs. For instance, administering the α1AR antagonist prazosin decreases midbrain DA cell firing (Grenhoff et al., 1993; Grenhoff and Svensson, 1993). Furthermore, prazosin administration reduces basal (Sommermeyer et al., 1995) and psychostimulant-induced (Darracq et al., 1998; Mitrano et al., 2012) DA release in the NAc. DBH inhibition lowers NE levels by blocking the conversion of DA into NE, consequently increasing tissue DA

levels (Karamanakos et al., 2001; Bourdelat-Parks et al., 2005; Schroeder et al., 2010). However, the effects of DBH inhibition on psychostimulant-induced extracellular DA levels in the NAc of rodents have shown conflicting reports with either a decrease found in mice (Schank et al., 2006; Weinshenker et al., 2008) or no change detected in rats (Devoto et al., 2012; Devoto et al., 2013). Decreases in extracellular DA in the NAc were attributed to reduced noradrenergic firing on midbrain DA neurons. In direct opposition, it was postulated that striatal DA release is not tonically controlled by NE, which explains the lack of effect on extracellular DA. To determine whether lowered NE levels via DBH inhibition has an effect on striatal DA release in nonhuman primates, dialysate samples were analyzed from the NAc of squirrel monkeys. Because NE innervation in the NAc is low, and DBH inhibition would further lower any basal NE levels, only DA was analyzed.

We have previously shown that nepicastat alone is sufficient to induce reinstatement to cocaine-seeking in squirrel monkeys (see Chapter III). The lowest dose of nepicastat that induced reinstatement (10mg/kg, i.m.) was used to test the effects of DBH inhibition on catecholamine neurochemistry in squirrel monkeys. Reinstatement sessions that began 30-min following nepicastat administration resulted in the most robust reinstatement effect; therefore, a 30-min pretreatment of nepicastat was used to test the interaction with cocaine. The cocaine dose was chosen because prior studies demonstrated that 1.0mg/kg cocaine induces robust and long-lasting increases in DA levels in the NAc of awake squirrel monkeys (Manvich et al., 2012a; Manvich et al., 2012b). These results indicate that although systemic administration of nepicastat did not affect basal DA levels, it significantly attenuated cocaine-induced increases in DA levels in the NAc of squirrel monkeys.

These results align with studies in mice that have pharmacologically or genetically lowered DBH levels. Psychostimulant-induced DA overflow is attenuated in

the NAc of mice treated with fusaric acid and DBH knock-out mice (Schank et al., 2006; Weinshenker et al., 2008). This supports the notion that midbrain DA neurons are tonically regulated by noradrenergic neurons, and DBH inhibition results in a loss of that tone. However, these results are in contrast to those found in rats in which neither disulfiram nor nepicastat administration has an effect on cocaine-induced DA overflow in the NAc (Devoto et al., 2012; Devoto et al., 2013). The reason for these discrepancies are unknown, but may be related to differences in drug preparation, dosing, route of drug administration or species.

In addition to dialysate sampling in the NAc, Devoto and colleagues also analyzed both NE and DA in the mPFC (Devoto et al., 2012; Devoto et al., 2013). While DBH inhibition in both regions decreases extracellular NE, the effect on DA release is strikingly different in the two brain regions. Disulfiram modestly increases basal DA release in the NAc while nepicastat has no effect on basal DA levels. However, in the mPFC, both disulfiram and nepicastat cause a robust increase in basal DA release. Both treatments also significantly increase cocaine-induced DA overflow in the mPFC. This DA is believed to be released from noradrenergic terminals in the mPFC. Under normal circumstances, some DA release in the PFC is attributed to co-release from noradrenergic neurons projecting from the LC (Devoto et al., 2005a, b). This DA co-release from noradrenergic neurons is mediated by the α2AR (Devoto et al., 2001; Devoto et al., 2004). Antagonists of the α2AR increase extracellular DA (Tanda et al., 1996; Millan et al., 2000; Devoto et al., 2004) while α2AR agonists decrease DA in the PFC. Following DBH inhibition, there is an excess of intracellular DA in the noradrenergic terminals in the mPFC. This excess elicits increased basal and psychostimulant-induced DA overflow. It is unknown whether a similar increase in DA release would occur in the PFC of squirrel monkeys. In the present study, we were

unable to develop a paradigm to target the PFC of squirrel monkeys for *in vivo* microdialysis. We were also unable to confidently measure NE levels in the dialysate samples. These technical limitations restricted the present study to analysis of DA in the NAc, but it is critical for future experiments to assess the effects of catecholamine neurochemistry following DBH inhibition in the PFC of squirrel monkeys.

A potential explanation for the lack of effect of DBH inhibition on cocaine-induced reinstatement, as well as nepicastat's ability to induce reinstatement in squirrel monkeys (see Chapter III), is that DA can be co-released from noradrenergic neurons in the PFC of squirrel monkeys. If DA release in the PFC of squirrel monkeys is increased following DBH inhibition, it would corroborate with the reinstatement results. However, the ability of DBH inhibition to attenuate the DA releasing effects of cocaine in the NAc of squirrel monkeys seemingly complicates this interpretation. It must be noted that the monkeys used in the microdialysis experiments were not actively self-administering or seeking cocaine. Others have previously demonstrated that cocaine-induced DA overflow can be markedly different depending on whether drug administration was contingent or noncontingent (Hemby et al., 1997; Kimmel et al., 2005). The magnitude of cocaine-induced increases in extracellular DA concentrations in the NAc is greater in animals responding for cocaine than yoked control subjects receiving simultaneous noncontingent infusions of cocaine (Hemby et al., 1997). Nepicastat may therefore differentially alter the DA increasing effects of cocaine if given in the context of a drug-associated environment.

It has been demonstrated that DA transmission in the shell of the NAc is both necessary and sufficient for cocaine-induced reinstatement in rats. Locally infusing D1-like or D2 receptor antagonists into the NAc shell attenuates cocaine-induced reinstatement (Anderson et al., 2003; Anderson et al., 2006), while local infusions of D1-

like or D2 agonists reinstate cocaine-seeking behavior (Schmidt et al., 2006). However, to date, the involvement of accumbal DA in reinstatement of cocaine seeking in nonhuman primates has not been investigated. It is possible that the impact of accumbal DA differs between rodents and nonhuman primates. Thus, while DBH inhibition attenuated the DA increasing effects of cocaine in the NAc of squirrel monkeys, it may not have been sufficient to induce a corresponding attenuation in cocaine-elicited behavior.

In summary, these studies are the first to demonstrate the effect of DBH inhibition on striatal catecholamine neurochemistry in nonhuman primates. Nepicastat administration had no effect on basal extracellular accumbal DA concentration, but attenuated cocaine-induced DA overflow in the NAc. This is in contrast to the effects of DBH inhibition previously observed in squirrel monkeys in which DBH inhibition had no effect on cocaine-induced reinstatement. The species differences in neurochemistry in response to nepicastat administration provide a possible explanation for the disparate responses to nepicastat pretreatment in cocaine-induced reinstatement.

CHAPTER V

General Discussion

By blocking monoamine transporters, cocaine increases extracellular levels of norepinephrine (NE), dopamine (DA), and serotonin (5-HT). The abuse-related effects of cocaine are due to its disruption of the normal function of these neurotransmitters, making their respective systems ideal targets for cocaine abuse pharmacotherapy. Many studies have investigated the influence of targeting the dopaminergic system in order to manipulate cocaine-mediated effects. However, fewer studies have focused on the effects of the noradrenergic system as a target for cocaine abuse pharmacotherapy. The majority of these studies have been preclinical studies in animals (reviewed in Weinshenker and Schroeder, 2007), while few studies have investigated noradrenergic targets in human clinical trials (Kosten et al., 2005; McDowell et al., 2005; Szerman et al., 2005; Jobes et al., 2011). Preclinical studies have demonstrated that inhibiting noradrenergic signaling can attenuate reinstatement responding in animal models. Because reinstatement is an animal model of relapse, the noradrenergic system provides a potential target for preventing relapse in a human clinical setting. One mechanism for decreasing NE levels is through inhibition of the enzyme dopamine β-hydroxylase (DBH), which is essential for the synthesis of NE.

The aim for the current project was to assess the effect of DBH inhibitors on cocaine-mediated behavior and neurochemistry in animal models. The nonselective DBH inhibitor, disulfiram, has shown promise in clinical studies as a cocaine abuse pharmacotherapy. However, because of its non-selectivity and wide array of biological targets, it is unclear whether the therapeutic mechanism of action for disulfiram in regards to cocaine abuse is through its actions on DBH. Disulfiram was originally used as a treatment for alcohol abuse; its efficacy for alcohol abuse is attributed to its

inhibition of aldehyde dehydrogenase (ALDH), an enzyme in the ethanol metabolic pathway. When ALDH is inhibited, levels of acetaldehyde increase causing an aversive "Antabuse reaction." ALDH, however, is not involved in the cocaine metabolic pathway, yet previous studies have demonstrated that disulfiram may be more effective in reducing cocaine intake in subjects that are not also abusing alcohol. This suggests that disulfiram may be working through mechanisms other than ALDH inhibition to reduce cocaine intake. It has been demonstrated, however, that ALDH is involved in DA metabolism (Maring et al., 1985). DA is metabolized into 3,4-Dihydroxyphenylacetaldehyde (DOPAL), which is then reduced to 3,4-dihydroxyphenylethanol (DOPET) by aldehyde/aldose reductase or oxidized to 3,4-dihydroxyphenylacetic acid (DOPAC) by ALDH. The metabolism of DA by ALDH may therefore play a role in the efficacy of disulfiram in cocaine abuse pharmacotherapy (Yao et al., 2010). Through a feedback loop involving tetrahydropapaveroline (THP), phosphorylated protein kinase A (PKA) and protein kinase C (PKC), selective ALDH2 inhibitors reduce intra- and extracellular DA concentration in cell cultures. ALDH2 inhibition also attenuates cocaine- and cue-induced reinstatement in rats. These results align with reported studies in rats in which disulfiram blocks cocaine-induced reinstatement. Unfortunately, these studies did not investigate how ALDH inhibition affects DA levels in an awake animal. Neither did these studies evaluate whether disulfiram, as a non-selective ALDH inhibitor, decreases DA levels comparable to the selective ALDH2 inhibitor.

A more widely accepted hypothesis to explain the efficacy of disulfiram is through its ability to inhibit DBH. Because DBH activity regulates relative DA and NE levels in noradrenergic neurons, and these neurotransmitters are heavily involved in the reinforcing and abuse-related effects of cocaine, it is a likely pharmacotherapeutic target.

Furthermore, the effects of disulfiram on cocaine sensitization are abolished in mice genetically lacking DBH (Dbh -/-) suggesting that disulfiram's effects are mediated via DBH inhibition (Gaval-Cruz et al., 2012). However, it is difficult to definitively attribute disulfiram's efficacy to DBH inhibition due to its ability to inhibit a variety of enzymes. The selective DBH inhibitor nepicastat, however, lacks disulfiram's target promiscuity. Hence, its therapeutic effects can definitively be linked to DBH inhibition. Throughout every set of experiments in the current project, responses following nepicastat treatment were comparable to those observed after a disulfiram treatment. This supports the hypothesis that disulfiram's therapeutic mechanism of action for cocaine-mediated responses is through DBH inhibition.

Although the responses between nepicastat and disulfiram did not differ, the effects of DBH inhibition between rodent and nonhuman primate animal models did differ. In rats, pharmacological DBH inhibition attenuated three different modes of reinstatement (drug-, cue- and stress-induced), while there was no effect in squirrel monkeys during cocaine-primed reinstatement. Additionally, nepicastat was sufficient to induce reinstatement in squirrel monkeys when administered alone. It is not known why the discrepancy between the species exists, but there is precedence for treatments that have disparate effects in rodents and nonhuman primates. For example, treatments affecting the glutamatergic system have shown promising results as a potential pharmacotherapy in rodents (Peters and Kalivas, 2006; Kau et al., 2008; Kupchik et al., 2012; Lu et al., 2012), while results have not been as conclusively promising in nonhuman primates (Adewale et al., 2006; Bauzo et al., 2009, 2012). Furthermore, even some treatments targeting the noradrenergic system have shown conflicting results. The α1-adrenergic receptor (α1AR) antagonist prazosin attenuates cocaine-induced reinstatement in rats (Zhang and Kosten, 2005), but has no effect in squirrel monkeys

(Platt et al., 2007). Though these discrepancies exist, there currently are no clear explanations for the divergences. There are, however, a number of possible explanations for the current project.

One possible explanation is related to the neuroanatomical differences in rodents and nonhuman primates. The aforementioned examples of divergent treatment outcomes manipulate different neurotransmitter systems, so differences in the neurotransmitter systems would affect these outcomes. There are documented examples of differences in neuroanatomical structures and connectivity between rodents and nonhuman primates (Frankle et al., 2006; Smith et al., 2006; Haber and Knutson, 2010). For example, there are species differences in noradrenergic receptors and norepinephrine reuptake transporter (NET) distribution (reviewed in Weerts et al., 2007). Since reduction of NE and decreased activation of noradrenergic targets in the midbrain, striatum, and cortex provides the framework behind the ability of disulfiram and nepicastat to attenuate reinstatement, inherent differences in the noradrenergic system would affect the influence of DBH on cocaine responses.

Another possible explanation accounts for the methodological differences in the behavioral paradigms. The squirrel monkey behavioral paradigm utilizes a combined cocaine- and cue-induced reinstatement while a strictly cocaine-induced reinstatement is used with the rats. The extra cue stimulus may activate the release of the excess DA that has accumulated in normally noradrenergic neurons following DBH inhibition, possibly increasing DA concentration in the prefrontal cortex (PFC). This unanticipated DA release may be responsible for facilitating nepicastat-induced reinstatement and for not effecting combined cocaine- and cue-induced reinstatement in squirrel monkeys.

Although the behavioral responses to DBH inhibition were different in squirrel monkeys than in rats, there were responses in squirrel monkeys that suggested that nepicastat was crossing the blood brain barrier and exerting its effects. For example, nepicastat induced reinstatement when administered alone and both disulfiram and nepicastat attenuated cocaine-induced DA overflow in the nucleus accumbens (NAc). While it is believed that nepicastat crosses the blood brain barrier in squirrel monkeys as it does in rats, there may still be metabolic differences that account for the differences in responses. Drugs can be metabolized in varying ways in different species creating diverging active and inactive metabolites. The concentrations of these metabolites also vary which can cause divergent reactions at their respective targets. The primary metabolite of disulfiram, diethyldithiocarbamate, is a copper chelator and is responsible for inhibiting DBH. However, the rates that disulfiram is metabolized into diethyldithiocarbamate and that diethyldithiocarbamate is metabolized into its metabolite in rats and squirrel monkeys are unknown. Nepicastat itself binds to the DBH active site thereby inhibiting the enzyme (Kapoor et al., 2011), and its major N-acetyl metabolite, RS-4783 1-007, also inhibits DBH *in vitro* (though it is a weaker inhibitor) (Hegde and Friday, 1998). The rate of metabolism from nepicastat to RS-4783 1-007 may differ between species which would also create varying efficacy of DBH inhibition. Differing rates of metabolism and DBH inhibition may be responsible for some of the observed differences between rats and squirrel monkeys.

These potential differences highlight the importance of choosing the appropriate experimental model and paradigm to investigate potential cocaine abuse pharmacotherapies. Experiments using animal models are designed such that no more than the fewest number of animals possible are used and the most appropriate animal model is used. It is currently unclear whether rodents or squirrel monkeys are the most

ideal animal model to study the effect of DBH inhibition on cocaine-mediated behavior and neurochemistry, as well as any related studies that may emerge from these results. Rodents are often used to assess the effects of a novel treatment *in vivo*. Nonhuman primates typically more closely approximate the effects of treatments in humans than rodents and are used to test the effects of a treatment that generates promising results in rodents. Human trials usually commence following positive results in nonhuman primates. In this instance, that particular sequence of events did not occur in that order. Because both disulfiram and nepicastat failed to effect cocaine-induced reinstatement in squirrel monkeys and nepicastat induced reinstatement on its own, DBH inhibition may not have been recommended for use in human cocaine abusers. However, the effects of DBH inhibition in a human cocaine-abusing population are already being assessed. This presents a rare instance in which rodents and humans share similar effects of a treatment, which differs from nonhuman primates. Human trials have shown varying rates for disulfiram's efficacy in reducing cocaine intake (Carroll et al., 1998; Carroll et al., 2000; Carroll et al., 2004; Baker et al., 2007; Carroll et al., 2012; Spellicy et al., 2012). However, because disulfiram is not a selective DBH inhibitor, its efficacy may be limited by its actions at other biological targets. Nepicastat trials in humans will therefore portray a more accurate assessment on the effect of DBH inhibition on cocaine-abuse in humans. Cunningham and colleagues (2010) have already demonstrated that nepicastat reduces some of the subjective effects of cocaine. A multi-center, phase II clinical trial is currently underway to assess the effects of nepicastat on cocaine abstinence in humans (ClinicalTrials.gov, 2012).

Despite the ongoing clinical trial, there are still relevant experimental questions that can be answered in animal models in order to optimize future translational treatments. Additional experiments should be performed to address whether the

combined cocaine- and cue-induced reinstatement paradigm that was used in the current study affects results in such a way that is not conducive to translation. One approach to address this is to locally infuse dopamine receptor antagonists or α2AR agonists into the PFC. It has been demonstrated that some DA in the PFC is released from noradrenergic neurons (Devoto et al., 2005a, b) and transmission is facilitated through α2AR antagonists (Tanda et al., 1996; Devoto et al., 2001; Devoto et al., 2004). Following DBH inhibition, the paired presence of the additional drug-associated cue may be sufficient to release the excess DA that accumulates in noradrenergic neurons projecting to the PFC. Blocking dopaminergic transmission in the PFC will therefore assess whether DA release in the PFC contributes to the results observed in squirrel monkeys. Another potential experiment is to conduct cocaine-induced reinstatement in the absence of drug associated cues. As aforementioned, the additional presence of the drug-associated cue may influence the release of excess DA. Perhaps pharmacologically inhibiting DBH in squirrel monkeys will be effective in attenuating cocaine-induced reinstatement without the drug-associated cues. These experiments can elucidate more ideal parameters for conducting reinstatement in squirrel monkeys in order to translate the results to humans.

In addition to assessing the behavioral effect of DA in the PFC following DBH inhibition, the effect on extracellular DA should be directly measured using *in vivo* microdialysis. Unfortunately, technical limitations in the current project prevented conducting *in vivo* microdialysis sampling from the PFC of squirrel monkeys. In rats, both disulfiram and nepicastat increase basal and cocaine-induced DA overflow in the medial PFC (Devoto et al., 2012; Devoto et al., 2013). The effects of DBH inhibition on both basal and cocaine-induced DA overflow in the PFC of squirrel monkeys are unknown. Data from *in vivo* microdialysis in the PFC of squirrel monkeys would serve two

purposes: firstly, it would provide further comparison of the effects of DBH inhibition in rats and squirrel monkeys, which can contribute to determining which animal model is better suited to use for translation of DBH inhibitors as a treatment in humans. Secondly, the effects of DBH inhibition on catecholamine neurochemistry in the PFC of squirrel monkeys will be helpful for understanding the behavioral effects. DA release in the PFC is a potential cause for the behavioral results that were observed in the current project and *in vivo* microdialysis in the PFC could validate that hypothesis.

The evidence that some of the DA in the PFC is released from noradrenergic neurons comes from studies in rats. If DBH inhibition increases basal DA release in the PFC of squirrel monkeys, evidence would be provided that DA is being released from noradrenergic terminals. Further studies can assess this by locally perfusing an α2AR agonist such as clonidine into the PFC which could determine whether stimulating the noradrenergic autoreceptor reverses the effects of DBH inhibition on extracellular DA levels in the PFC. However, this would be an indirect measure, as there are α2ARs that are found on non-noradrenergic neurons. A more direct method to study the inactivation of noradrenergic terminals in the PFC would be through the use of optogenetics. Optogenetics is a relatively new technique for transiently controlling the activation and inactivation of subpopulations of neurons in an intact brain. The technique has mostly been utilized in rodents, but has more recently been applied to the nonhuman primate cerebral cortex, striatum, and thalamus (Han et al., 2009; Diester et al., 2011; Galvan et al., 2012). Extracellular DA levels in the PFC can be measured after transiently inactivating noradrenergic neurons that project to the PFC. Optogenetics can also be used to assess the effects of inactivation of noradrenergic neurons following pharmacological DBH inhibition. Lesioning studies in mice have previously demonstrated that permanently inactivating noradrenergic transmission in the PFC has no effect on

basal or amphetamine-induced DA release in that structure (Ventura et al., 2003). However, optogenetics presents the advantage of examining reversible inactivation with better temporal resolution. Furthermore, there may be differences in responses between rodents and nonhuman primates, and this could address potential differences. Determining whether DA in the PFC of squirrel monkeys is released from noradrenergic neurons will be valuable for future studies that involve manipulating DA and NE levels in the mesocorticolimbic and related neurotransmitter systems.

Though it was not feasible to measure DA in the PFC of squirrel monkeys in the current study, extracellular DA was measured in the NAc using *in vivo* microdialysis. Despite not affecting cocaine-induced reinstatement, pharmacologically inhibiting DBH attenuated the DA increasing effects of cocaine in the NAc. Moreover, nepicastat did not affect basal DA even though it induced reinstatement when administered alone. Perhaps accumbal DA does not have a crucial role for reinstatement in squirrel monkeys. There is strong evidence that accumbal DA is imperative for the reinforcing effects in both rodents and nonhuman primates (McGregor and Roberts, 1993; McKinzie et al., 1999; Porrino et al., 2004). In rats, DA in the NAc is important for reinstatement. An intra-accumbal infusion of DA induces reinstatement which can be reversed with co-infusion of a DA receptor antagonist (Cornish and Kalivas, 2000). However, the role of DA in the NAc during reinstatement is not well defined in nonhuman primates. The discrepancies in the current study are an example of the undefined role of striatal DA in reinstatement in nonhuman primates. Additional experiments from our laboratory allude to the complicated relationship between accumbal DA and reinstatement in nonhuman primates. The 5-HT2C receptor agonist, Ro 60-0175, blocks both the DA releasing effects of cocaine in the NAc and cocaine-induced reinstatement in squirrel monkeys (Manvich et al., 2012b). Moreover, in squirrel monkeys, the 5-HT2C antagonist, SB

242084, enhances both cocaine-induced reinstatement and cocaine-induced DA overflow in the NAc, but has no effect on cocaine-induced DA overflow in the caudate nucleus, suggesting separate roles of the ventral and dorsal striatum (Manvich et al., 2012a). In contrast, in rhesus macaques, the 5-HT2A receptor antagonist, M100,907, blocks cocaine-induced reinstatement and cocaine-induced DA overflow in the caudate nucleus, but has no effect on cocaine-induced DA overflow in the NAc (Murnane et al., submitted). While accumbens DA is necessary for self-administration, it may not be sufficient to influence reinstatement. To address whether DA in the NAc is important for reinstatement in squirrel monkeys, DA signaling can be locally blocked prior to reinstatement. A DA receptor antagonist can be infused into the NAc before either a cocaine- or cue-induced reinstatement session to evaluate the influence of accumbens DA in relation to the different modes of reinstatement. Another approach would be to measure extracellular DA in the NAc using *in vivo* microdialysis while monkeys are actively responding for reinstatement. Though technically difficult to undertake, concurrent *in vivo* microdialysis and self-administration has previously been performed in our laboratory (Kimmel et al., 2005). Those experiments, however, did not measure the relationship between DA in the NAc and reinstatement. *In vivo* microdialysis concurrent with cocaine- or cue-induced reinstatement can be employed to determine whether DA is altered in the NAc in these behavioral paradigms.

In humans, a C to T polymorphism at the -1021 nucleotide position of the DBH gene results in naturally occurring genetic variance in the DBH activity (Zabetian et al., 2001). Individuals with genetically lower DBH activity tend to experience increased cocaine-induced paranoia and do not benefit as much from the pharmacotherapeutic effects of disulfiram treatment (Cubells et al., 2000; Kalayasiri et al., 2007; Kosten et al., 2013). Similar to humans, there are differences in plasma DBH activity between different

strains of rats and amongst different primate species (Lamprecht et al., 1974; Stolk et al., 1979; Dunnette and Weinshilboum, 1983). However, it is not known whether there is a similar polymorphism in rats or squirrel monkeys that produces varying levels of DBH activity. If there is a SNP that produces varying DBH activity, the allele frequency may differ greatly from the frequency found in humans. It is possible that the squirrel monkeys in the current study have comparably lower DBH activity than either the rats used in similar studies or humans. One study found that there was not much variation in DBH activity amongst a sample of ten squirrel monkeys, but the average enzyme activity was lower than that of humans (Dunnette and Weinshilboum, 1983). Lower plasma DBH activity could explain why DBH inhibition did not affect cocaine-induced reinstatement in squirrel monkeys. Determining if plasma DBH activity varies as widely in rats and squirrel monkeys as it does in humans would give insight into relative DBH activity between the species. Further, sequencing their respective DBH genes and performing quantitative trait analyses with genotype/phenotype correlations would provide evidence of common polymorphisms that produce variation in plasma DBH activity. Comparison of DBH activity from rats, squirrel monkeys, and humans would also influence determining the more ideal animal model for translation of treatments involving DBH inhibition.

In summary, the overall purpose of the experiments in the present study was to assess the impact of disulfiram and nepicastat on the behavioral and neurochemical effect of cocaine in animal models. These studies also represent the first time that the effects of DBH inhibition have been examined in nonhuman primates. The overall effects remain inconclusive since contradictory results were generated in rats and squirrel monkeys. Despite the contradictory effects of DBH inhibition on cocaine-mediated behavior and neurochemistry in animal models, both disulfiram and nepicastat have shown promising effects in humans. Though disulfiram has many biological targets,

evidence gathered over the past two decades suggests that the therapeutic effects of disulfiram are due to its action on DBH. However, because of its many biological targets, disulfiram produces several undesirable side effects in humans, including the “Antabuse reaction” caused when alcohol is consumed, hepatotoxicity, and in some cases, psychotic symptoms. These side effects result in poor compliance in an alcohol abusing population and would likely result in similar poor compliance in a cocaine abusing population, therefore limiting the clinical utility of disulfiram. Nepicastat, however, does not have the same adverse side effect profile as disulfiram. Furthermore, the effects of the selective DBH inhibitor, nepicastat, on cocaine-mediated behavior and subjective responses are comparable to that of the nonselective DBH inhibitor, disulfiram, rendering nepicastat a more suitable treatment in a clinical setting than disulfiram.

CHAPTER VI

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