EVALUATION OF AGONIST PHARMACOTHERAPY FOR COCAINE DEPENDENCE: EFFECTS OF CONTINUOUS D-AMPHE TAMINE TREATMENT ON COCAINE SELF-ADMINISTRATION IN RATS

BY

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A Dissertation Submitted to the Graduate Faculty of WAKE FOREST UNIVERSITY GRADUATE SCHOOL OF ARTS AND SCIENCES
In Partial Fulfillment of the Requirement For the Degree of

DOCTOR OF PHILOSOPHY
In the Neuroscience Program
Wake Forest University School of Medicine

MAY 2009
Winston-Salem, North Carolina

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ACKNOWLEDGEMENTS

First and foremost, this dissertation would not have been possible without my advisor, Dr. David Roberts. I thank Dave for taking me under his wing when I was on the verge of admitting defeat. Somehow a “one-month trial period” in his lab turned into four years and I am grateful to Dave for seeing my potential and helping me to believe in myself as a scientist. Over the years Dave has also become a valuable mentor in life and I appreciate the wisdom he imparted to me through his many stories and metaphors (many of which included the phrase, “reinvent the wheel”).

I would also like to thank Dr. Terry Blumenthal, Dr. David Friedman, Dr. Anthony Liguori and Dr. Michelle Nicolle for their guidance and advice as members of my committee. I appreciate the considerable amount of time and energy that they graciously devoted to my success in graduate school.

In addition, several other professors at Wake Forest University have been invaluable mentors to me throughout my graduate school career. I thank Dr. James Smith for introducing me to drug abuse research when I was an undergraduate student. I will always cherish the summer I spent in his laboratory as well as his helpful advice about pursuing a Ph.D. I also thank Dr. Ronald Oppenheim, for accepting me into the Neuroscience Program and supporting me for the past six years. I would also like to acknowledge Dr. Allyn Howlett and Dr. Dwayne Godwin for giving me several opportunities to develop my teaching skills both inside and outside of the classroom.

It has been a pleasure to work with the other members of the Roberts lab. I thank Leanne, Xiao Yu, Chris, and Aubrae for welcoming me to the lab and teaching me the skills I needed to complete my research. I also thank Erik, Carson and Caroline for their assistance, candid advice, and tolerance for my love of holiday music.
I am especially grateful to Jody Dedo for her unending support. Jody’s selfless devotion to the students in the Neuroscience program is unsurpassed and I truly cannot imagine making it through graduate school without her.

Many thanks to my Academic Computing friends for graciously replacing my hard drive three times and reassuring me that I do not hold the record for the most encounters with the “blue screen of death”.

Finally, I will be forever indebted to my family and friends who were more integral to this process than they will ever realize. I am incredibly grateful to my parents for empowering me throughout my life and giving me the freedom to make my own decisions. I sincerely appreciate them for dropping everything and crossing the Mason-Dixon Line to be here when I needed their support. I also appreciate my brother, Tim, for always being a model student because I would not have had the strength to make it through graduate school had I not spent the first 18 years of my life working hard to emerge from his academic shadow. I have grown to truly appreciate Tim’s friendship (as well as his willingness to use his success in “Corporate America” to support our parents after they retire). I would also like to thank Dani, Alli, Amy, Sarah, Heather, Carla, Kim, Bethany, Amelia, Nathalie and Ms. Pat for pulling me back into reality on several occasions by reminding me that this dissertation is not a testament to my value in life.
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<td>serotonin</td>
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<td>ADHD</td>
<td>attention deficit hyperactivity disorder</td>
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<td>ANOVA</td>
<td>analysis of variance</td>
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<td>DA</td>
<td>dopamine</td>
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<td>DAT</td>
<td>dopamine transporter</td>
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<td>DBH</td>
<td>dopamine-beta-hydroxylase</td>
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<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual of Mental Disorders (4th edition)</td>
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<td>DT</td>
<td>discrete trials</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>FR</td>
<td>fixed ratio</td>
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<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
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<td>HVA</td>
<td>homovanillic acid</td>
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<tr>
<td>ICD-10</td>
<td>International Statistical Classification of Diseases and Health Related Problems</td>
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<tr>
<td>IP</td>
<td>intraperitoneal</td>
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<tr>
<td>IV</td>
<td>intravenous</td>
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<tr>
<td>LAAM</td>
<td>levo-alpha-acetylmethadol</td>
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<td>NAc</td>
<td>nucleus accumbens</td>
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<td>NE</td>
<td>norepinephrine</td>
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<tr>
<td>NET</td>
<td>norepinephrine transporter</td>
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<tr>
<td>NIDA</td>
<td>National Institute on Drug Abuse</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>NRT</td>
<td>nicotine replacement therapy</td>
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<tr>
<td>PET</td>
<td>positron emission tomography</td>
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<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
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<td>PR</td>
<td>progressive ratio</td>
</tr>
<tr>
<td>SC</td>
<td>subcutaneous</td>
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<tr>
<td>SEM</td>
<td>standard error of the mean</td>
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<td>SERT</td>
<td>serotonin transporter</td>
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<td>SPECT</td>
<td>single photon emission computed tomography</td>
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<tr>
<td>VMAT</td>
<td>vesicular monoamine transporter</td>
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ABSTRACT

KERI ANN CHIODO

EVALUATION OF AGONIST PHARMACOTHERAPY FOR COCAINE DEPENDENCE:
EFFECTS OF CONTINUOUS D-AMPHETAMINE TREATMENT ON
COCAINE SELF-ADMINISTRATION IN RATS

Dissertation under the direction of
David C.S. Roberts, Ph.D., Professor of Physiology and Pharmacology

Cocaine dependence is a chronic disease which causes an immeasurable amount of detriment at both individual and societal levels. Although several promising pharmacological treatments are under development, the process of bringing an investigational drug to market requires substantial investments of both time and money. In view of this, recent research efforts have also been focused on investigating the utility of medications that are currently available for the treatment of other disorders. d-Amphetamine, an FDA-approved medication for the treatment of attention deficit hyperactivity disorder and narcolepsy, has shown promise as an agonist/replacement pharmacotherapy for cocaine dependence in both clinical and preclinical studies. Thus far, studies have shown that the route of administration and treatment regimen greatly influence the therapeutic effectiveness of d-amphetamine in that continuous, but not acute, administration of d-amphetamine decreases the reinforcing effects of cocaine.
The studies presented in Chapters II and III extend the preclinical research by investigating the effects of \textit{d}-amphetamine treatment via subcutaneous osmotic mini-pump on cocaine self-administration on a progressive ratio (PR) schedule of reinforcement in rats. In Chapter II, we show that continuous \textit{d}-amphetamine infusion causes a decrease in breakpoints (i.e., the number of cocaine injections self-administered in a PR session) that can last for up to two weeks after the treatment period. These experiments demonstrate that the duration of the \textit{d}-amphetamine treatment period and the unit dose of self-administered cocaine determine the extent to which \textit{d}-amphetamine attenuates the reinforcing effects of cocaine. Additionally, we show that cocaine self-administration had to occur during the \textit{d}-amphetamine treatment period in order for post-treatment breakpoints to be decreased. In Chapter III, we demonstrate that continuous \textit{d}-amphetamine treatment produces a substantial downward shift in the PR dose-response curve for cocaine which is influenced by the level of cocaine exposure during the \textit{d}-amphetamine period.

Collectively, these studies suggest that continuous \textit{d}-amphetamine treatment selectively attenuates the reinforcing efficacy of cocaine. Future study is needed to determine whether this effect is due to a gradual learning process and/or a pharmacological interaction between the two drugs.
CHAPTER I

MEDICATION STRATEGIES FOR THE TREATMENT OF COCAINE DEPENDENCE

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The following review is in preparation for submission.
CHAPTER OUTLINE

1. Introduction

2. Defining the goals of drug dependence treatment

3. Agonist medications to alleviate withdrawal effects
   3.1. Physical dependence associated with opiate and nicotine abuse
   3.2. Replacement therapy to alleviate withdrawal symptoms in opiate and nicotine dependence
       3.2.1. First-line replacement medications
       3.2.2. Partial agonist medications
   3.3. Cocaine dependence is qualitatively different than opiate and nicotine dependence
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4. Medications for “neurochemical normalization” after extended cocaine abuse
   4.1. Dysregulation of the dopamine system
   4.2. Dopamine agonist medications
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       4.2.2. Partial dopamine agonists
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   4.3. Dysregulation of the serotonin system
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5. The use of medications to prevent or block the reinforcing effects of cocaine

6. The treatment of “drug dependence” instead of “cocaine dependence”
   6.1. Cocaine abusers typically use other drugs as well
   6.2. Craving is a universal feature of drug dependence
   6.3. Medications which target drug craving

7. Conclusions
1. **INTRODUCTION**

Many pharmacological agents have been investigated as potential treatments for cocaine dependence over the past few decades, but none have yet received FDA-approval for this purpose (Perti 2007; Vocci and Ling 2005). Multiple strategies have been applied to the search for the ideal medication for cocaine dependence. Researchers and clinicians have investigated drugs to serve as agonist replacement medications, to block the euphoric effects of cocaine, and combat drug craving in general. This review will discuss the overall goals of treatment and summarize several medication strategies in terms of their ability to help cocaine-dependent individuals meet these goals.

2. **DEFINING THE GOALS OF DRUG DEPENDENCE TREATMENT**

As with other drug addictions, long-term abstinence is the primary goal of treating cocaine dependence, as it is thought that long-term abstinence would yield the greatest benefit to both the individual and society. Although acute abstinence is attainable when cocaine addicts do not have access to the drug due to hospitalization or incarceration, this rarely leads to permanent abstinence. The majority of cocaine addicts relapse once they are re-exposed to a familiar environment laden with conditioned cues, regardless of their treatment progress or intentions to stay sober (O'Brien 2005). It is unrealistic to use abstinence as the only measure of a successful treatment since very few drug-dependent individuals are able to remain sober for the rest of their lives.
Instead of this all-or-nothing approach, a more realistic way to measure the utility of a treatment is in terms of harm reduction.

Cocaine addiction is associated with multiple negative consequences for the individual including medical (e.g., movement disorders, seizure, stroke, and other cardiovascular problems), psychological (e.g., anxiety, paranoia, psychosis, and HIV-associated dementia), and social (e.g., damage to interpersonal relationships, job/income loss) problems (see Benowitz 1991; Bolla et al. 1998; Buttner et al. 2003; Ferris et al. 2008; Peterson et al. 1991 for review). Additionally, our society pays a high price for cocaine addiction both economically (i.e., due to costs of imprisonment, hospital visits, etc.) and in terms of use-related crime and violence, propagation of infectious diseases (e.g., AIDS, hepatitis, tuberculosis), and developmental deficits due to neonatal cocaine exposure (Schempf and Strobino 2008; Soares et al. 2003). By reducing these harms, the quality of life for both the cocaine user and members of society can be improved.

Harm reduction can be achieved by either decreasing the amount of cocaine that an individual takes or by changing his pattern of use. Cocaine users tend to develop a non-daily pattern of intake wherein they take several doses of cocaine in binges lasting from 8-24 hours (Gawin 1989). Considering that cocaine is involved in almost 150,000 emergency department visits annually (National Institute on Drug Abuse 2001), a treatment may have beneficial effects if it helps the user eliminate cocaine binges in favor of
shorter, less intense periods of cocaine administration. This could potentially
decrease the occurrence of cocaine-induced medical emergencies (e.g.,
seizures, hyperthermia, arrhythmias; Glauser and Queen 2007) and crimes
associated with obtaining cocaine (Smart 1996).

Similarly, by changing the route of administration, an intravenous (IV)
cocaine user could decrease the spread of health problems including HIV and
hepatitis (Ball et al. 1988; Des Jarlais et al. 1993; Hagan et al. 2005). In terms
of treatment, slower routes of administration would be beneficial for
decreasing illicit cocaine use because its reinforcing effects are correlated with
the speed of drug onset (Nelson et al. 2006). Even daily prescriptions for oral
cocaine could theoretically reduce IV drug use and serve as a harm reduction
measure (Grinspoon and Bakalar 1981). Oral cocaine is well-tolerated in
cocaine users (Johanson et al. 2007; Walsh et al. 2000) and although acute
doses of oral cocaine do not block the subjective or discriminative stimulus
effects of IV cocaine (Johanson et al. 2007), maintenance on oral cocaine has
been shown to decrease the subjective effects and craving for cocaine (Walsh
et al. 2000; Weil 1978). Therefore, by maintaining a cocaine-dependent
individual on daily doses of cocaine, the occurrence of risky behavior, criminal
activity, and health problems related to IV cocaine use could be reduced. This
treatment method is clearly not feasible due to many obvious societal concerns
both inside and outside of the United States (see Johns 1993; Sell et al. 1997),
so maintenance on other pharmacological treatments would be a more realistic
means toward harm reduction. Several medication strategies have been
employed in both preclinical and clinical studies. These include replacing cocaine with a similar (but less harmful) medication, restoring neuronal function after chronic cocaine exposure, blocking the reinforcing effects of cocaine, and relieving drug craving.

3. **AGONIST MEDICATIONS TO ALLEVIATE WITHDRAWAL EFFECTS**

3.1. **PHYSICAL DEPENDENCE ASSOCIATED WITH OPIATE AND NICOTINE ABUSE**

Physical dependence is a key feature of addiction that governs continual use of several drugs of abuse including opiates and nicotine. Opiate users become tolerant to many of the primary drug effects relatively quickly (Kreek 1987) and the frequency of drug use increases in parallel with the development of tolerance. At this point an opiate user is considered to be physically dependent because subsequent withdrawal of the drug can lead to physical symptoms that range from being merely “flu-like” to being severe within a few hours of the onset of opiate abstinence (Lindesmith 1980). More specifically, the opiate withdrawal syndrome is characterized by cholinergic effects including sweating, nausea/vomiting, lacrimation, diarrhea, rhinorrhea, pupil dilation, piloerection, yawning, tachycardia, hypertension, fever, and sleep disturbance (American Psychiatric Association 2000; Hasin et al. 2006; Saunders 2006; World Health Organization 2004). These aversive physical symptoms have long been thought to perpetuate drug-taking in opiate-dependent individuals. For instance, an early account by Alfred Lindesmith (1947) showed that
patients experiencing physical withdrawal symptoms from opiate pain medication frequently used their medication to alleviate these symptoms. Even so, if the physician were to attribute the aversive effects to a cause unrelated to the opiate medication (e.g., side effects of another medication or symptoms of another disease) the patients did not consider using the medication to attenuate their physical discomfort. More recently, both laboratory studies and personal accounts have confirmed a strong link between the physical withdrawal syndrome and drug-seeking behavior (Carrera et al. 1999; Koob et al. 1989).

The progression to nicotine dependence is much like that for opiates as positive reinforcement is responsible for early drug administration and tolerance to the reinforcing effects develops rapidly. A withdrawal syndrome involving lethargy, fatigue, sleep disturbances, anxiety, dysphoric mood, increased appetite, irritability, mild cognitive deficits and concentration problems (American Psychiatric Association 2000; Shiffman et al. 2004; World Health Organization 2004) occurs upon abstinence. As with opiates, dependent nicotine users typically continue taking the drug in order to avoid the unpleasant withdrawal syndrome (Kenny and Markou 2001).
3.2. REPLACEMENT THERAPY TO ALLEVIATE WITHDRAWAL SYMPTOMS IN OPIATE AND NICOTINE DEPENDENCE

As mentioned above, the ultimate goal of drug dependence treatment is long-term drug abstinence. Unfortunately, abstinence-oriented treatment programs have proven to be unsuccessful in practice (Paraherakis et al. 2000). Agonist therapy (or "replacement therapy") is a pharmacological treatment strategy used to achieve a long-lasting decrease in drug use by assisting the process of detoxification from an abused drug and preventing future relapse. Originally proposed in the 1960s with regard to opiate dependence (Dole et al. 1966a; 1966b), this treatment approach involves replacing the drug on which the patient is dependent with a similar, but less-harmful, drug.

3.2.1. FIRST-LINE REPLACEMENT MEDICATIONS

Methadone is a long-acting opiate agonist that has proven its clinical value in detoxifying heroin addicts and reducing subsequent heroin-seeking behavior and it is currently accepted as a first-line pharmacotherapy for opiate dependence (O’Connor 2005). Methadone binds to the mu opiate receptors, thus creating a "narcotic blockade" which prevents the subsequent binding of heroin (Dole et al. 1966a). The relatively long half-life of methadone (approximately 24 hours) allows it to counteract the physical symptoms of heroin withdrawal while also creating cross-tolerance to the reinforcing effects of heroin. In addition, methadone maintenance has been shown to normalize
the stress reactivity response in heroin-dependent patients, thereby decreasing the chance of stress-induced relapse (Kreek 2000). Methadone maintenance has shown the most benefit when administered in an inpatient setting, which allows for the dose to be tapered as the patient is detoxified from heroin (Amato et al. 2002) but it has also aided abstinence in outpatient settings when psychosocial treatment and/or contingency management are in place (Amato et al. 2008). Levo-alpha-acetylmethadol (LAAM) is another prescription opiate medication available for replacement therapy. It is typically reserved for patients that have continually relapsed to heroin use during methadone maintenance (Schwetz 2001), as its longer half-life of 48-72 hours allows for only two or three weekly doses (Freedman and Czertko 1981). Other agonist therapy medications, including codeine and slow-release oral morphine, have been shown to be equivalent to methadone and LAAM in terms of treatment retention and reduction in opiate use, but they are only available in a few countries outside of the United States at this time (Krausz et al. 1998; Mitchell et al. 2004).

The concept of replacement therapy has also been applied to the treatment of nicotine dependence. As the link between cigarette smoking and lung cancer was revealed (Doll and Hill 1950; Wynder and Graham 1950), alternate forms of nicotine administration were investigated for casual use. In the 1960s, it was found that nicotine delivered through an aerosol inhaler produced equivalent physical effects as tobacco cigarettes in humans (Herxheimer 1967), but this finding was not applied to treatment development
until the creation of a nicotine replacement gum in the 1970s (Brantmark et al. 1973; Ferno 1973). Today nicotine replacement therapy (NRT) is available in several forms (e.g., transdermal patch, chewing gum, sublingual tablet, oral lozenge, inhaler and nasal spray), and regardless of the route of administration NRT has been associated with a 50-70% increase in the rate of tobacco smoking cessation (Stead et al. 2008). Although there has been some debate about the role of behavioral therapy and/or support groups in this result (see Foulds et al. 2006 for review), the effectiveness of NRT has mostly been attributed to the alleviation of withdrawal symptoms (Gross and Stitzer 1989; Hughes et al. 1984). A more recent advancement in NRT is a "step-down" dosing regimen in which several doses of nicotine are available. This allows a person to control the pace at which he decreases his tobacco smoking and it is intended to enhance compliance (Shiffman et al. 2009).

3.2.2. Partial Agonist Medications

Partial agonist medications have gained more support as agonist pharmacotherapies for both opiate and nicotine dependence in recent years. Buprenorphine is a partial mu receptor agonist that has been used for painless heroin detoxification (Kutz and Reznik 2001; 2002), and has also shown promise as a maintenance medication in clinical studies (Fiellin et al. 2002; O'Connor et al. 1996). Although the half-life of buprenorphine (24-60 hours) is on par with methadone and LAAM, it has slower dissociation kinetics which allow its effects to last longer (Mattick et al. 2008). Accordingly, some physicians believe that
buprenorphine is a superior agonist medication for opiate dependence because it need only be administered once every two days (see Mattick et al. 2008), and its abuse potential is substantially decreased when combined with naloxone in a sublingual tablet (Fudala et al. 2003).

Varenicline is a partial agonist at the alpha-4-beta-2 nicotinic acetylcholine receptor that was developed in 1997 and approved as a smoking cessation treatment in 2006 (Aubin et al. 2008). Similar to buprenorphine, varenicline halts the physical withdrawal syndrome after nicotine is taken away, while also blocking the reinforcing effects of any subsequent nicotine administration (Coe et al. 2005). Although clinical studies with varenicline are in their infancy, by and large the treatment has been associated with decreases in nicotine use (see Cahill et al. 2008 for review). In addition, reports of nicotine craving and use were lower with varenicline treatment than with NRT (Stapleton et al. 2008).

3.3. Cocaine Dependence is Qualitatively Different than Opiate and Nicotine Dependence

The progression from casual cocaine use to cocaine dependence and addiction is qualitatively different from that of opiate and nicotine addictions. The Diagnostic and Statistical Manual of mental disorders (DSM-IV) describes two stages of cocaine use—abuse and dependence (American Psychiatric Association 2000). As opposed to opiate abuse, cocaine is initially used for
secondary reasons (e.g., enhancement of social and personal interactions), but continued use and abuse is attributed to its direct pharmacological effects as dependence develops. A person is considered to be dependent on cocaine when he or she exhibits signs of tolerance, withdrawal, or preoccupation with obtaining cocaine (Gawin 1989) and the interval between initial use and dependence is typically between two and four years (Gawin and Kleber 1985). During this time a cocaine user tends to administer cocaine in binges to overcome the short half-life of cocaine, and with time overall intake is substantially increased by extending the length of each binge to between eight and 24 hours (Gawin 1989; Riley 1997). Human cocaine users prefer this pattern of intake to the point that they will voluntarily take large doses of cocaine which augment anxiety and paranoia merely to extend the time until they “crash” (Gawin and Kleber 1986). In contrast to opiate and nicotine dependence, cocaine users do not typically progress to daily use. Instead, they tend to decrease the number of days of cocaine use each week in favor of longer individual binges (Gawin 1989; Riley 1997). A reason for this is that cocaine abuse does not seem to be motivated by a desire to end withdrawal symptoms, even though patients with the most severe levels of cocaine dependence have reported using other drugs to avoid the cocaine withdrawal (Bryant et al. 1991).

Although the agonist treatment strategies used for opiate and nicotine dependence can be applied to cocaine dependence, a different approach must be used, as the withdrawal syndrome associated with cocaine abstinence is
qualitatively different than that for opiates and nicotine. While sudden abstinence from high levels of cocaine use can cause physical symptoms (e.g., increases in heart rate, body temperature, sweating, and lacrimation; muscle problems, gastrointestinal disturbances, decreased appetite, and seizure; Cottler et al. 1993; Gawin and Ellinwood, Jr. 1989), these symptoms tend to be short-lived and more bearable than those associated with the opiate withdrawal syndrome (Satel et al. 1991; Weddington et al. 1990). These physical symptoms have even been considered to be long-lasting signs of cocaine intoxication instead of withdrawal symptoms per se (Cottler et al. 1993). Cocaine users tend to progress through a distinct stages, each with its own set of symptoms, during abstinence (Gawin 1991; Gawin and Kleber 1986). The initial "crash" period begins at the onset of abstinence and can last between nine hours and four days. This crash period is characterized by high cocaine craving with depression, agitation, and anorexia and ends with feelings of exhaustion as the cocaine craving subsides. At the beginning of the subsequent "withdrawal" period, subjects report feeling somewhat normal with regard to affective state, sleep patterns, motor ability, etc., but the craving for cocaine is intense for up to ten weeks. Other symptoms are of the "psychological" variety (Gawin and Kleber 1986) with depression, anxiety, irritability, concentration problems and sleep disturbances among the most reported symptoms (Soares et al. 2003).
3.4. Will Agonist Medications Attenuate Withdrawal Symptoms in Cocaine Dependence?

In order to apply the agonist approach to cocaine addiction, the concept of withdrawal must be reconsidered. The "withdrawal period" is generally thought to be a relatively short-term period that coincides with the beginning of abstinence. This idea clearly corresponds to the physical syndrome seen in abstinent opiate- and nicotine-dependent patients. However, there are other consequences of extensive drug use and/or removal of the drug that tend to persist for a longer period of time. With cocaine addiction, the psychiatric consequences can be rather extensive. Because cocaine (as well as other psychostimulants) enhances the reinforcing value of other stimuli, heavy users must relearn how to process natural reward during abstinence (Kalivas and O’Brien 2008).

In a similar manner as with opiates, long-term cocaine abuse could be a reflection of the drug's negative reinforcement value because administration can attenuate the chronic psychological symptoms during abstinence (Ahmed and Koob 2005; Khantzian 1985). Cocaine users have reported that they purposefully set aside small quantities of cocaine before a binge so that they will have some to take during the post-binge crash. This diminishes the negative impact that the drug has on their daily lives. By maintaining a low blood level of cocaine, the cocaine users were more likely to meet scheduled commitments and maintain employment (Gawin and Kleber 1986). Instead of
alleviating physical withdrawal problems, the low dose of cocaine allows the user to avoid the mental crash and associated psychological deficits of the cocaine abstinence syndrome. This self-medication behavior corresponds to agonist/replacement therapy and therefore suggests that maintaining a cocaine user on a low dose of a similar medication could serve a similar purpose. Just as agonist therapies assist opiate- and nicotine-dependent individuals to break the cycle of negative reinforcement, a successful medication for cocaine dependence could facilitate the patient's choice to maintain abstinence from cocaine by relieving the enduring psychological withdrawal symptoms.

4. **MEDICATIONS FOR "NEUROCHEMICAL NORMALIZATION” AFTER EXTENDED COCAINE ABUSE**

   The psychological symptoms that persist in abstinent cocaine users long after the crash and initial withdrawal period are manifestations of altered neurotransmission and/or neuron structure. "Neurochemical normalization therapy” (Negus et al. 2007; Rothman et al. 2005) suggests that medications could be extremely useful for counteracting the neurochemical dysregulation caused by continued cocaine use. The role of the treatment drug in "neurochemical normalization therapy" for cocaine dependence would be different than in "replacement/substitution therapy” for opiate and nicotine dependence, in that the treatment drug would be used for restoring a patient's non-withdrawal baseline neurochemical state instead of replicating the
neurochemical effects of cocaine. Theoretically, when a patient's "normal" neurochemical levels and patterns of neurotransmission have been re-established, he will be more capable of maintaining abstinence by ignoring internal cues and feelings of craving for cocaine. Although the opiate agonist medications are mainly used to alleviate physical withdrawal symptoms, Dole and Nyswander (1967) proposed an idea several decades ago regarding opiate treatment that is similar to the "neurochemical normalization" theory for treating psychostimulant dependence (see Rothman et al. 2002). They believed that the success of a replacement medication was related to its ability to counteract the permanent metabolic deficiency in the central nervous system caused by extended heroin use (Dole and Nyswander 1967).

In order to evaluate a medication's ability to restore baseline neurochemistry in cocaine-dependent patients, the neurochemical dysregulation associated with chronic cocaine abuse must be understood. Due to cocaine's action at dopamine (DA), serotonin (5-HT) and norepinephrine (NE) transporters (Koe 1976; Reith and Selmeci 1992), it is reasonable to expect deficits in each of these monoamine systems in terms of their extracellular levels, metabolism rates, receptor and transporter densities, and overall function of the monoamine-specific neuron populations in the brain after chronic cocaine exposure.
4.1. **Dysregulation of the Dopamine System**

The reinforcing strength of cocaine has largely been attributed to its inhibition of the DA transporter (DAT; Ritz et al. 1987; Volkow et al. 1997a; Wilcox et al. 2000; Wise 1984). Behavioral studies have shown that the resulting increase in extracellular DA is both "necessary and sufficient" for cocaine reinforcement to occur (Pettit and Justice, Jr. 1989; Pierce and Kumaresan 2006), and conversely, reinforcement does not appear to occur when DA has been depleted (Dackis and Gold 1985). Humans develop tolerance to the reinforcing euphoric effects of cocaine with longer cocaine binges (Fischman and Schuster 1982; Gawin and Kleber 1986) and this indicates that a change in DA activity occurs during the progression from cocaine abuse to cocaine dependence. There are conflicting data from both in vivo and in vitro studies concerning whether chronic cocaine exposure leads to neurotoxicity of DA neurons throughout the brain, with some studies reporting a decrease in DA cell numbers (Ellison 1992; Little et al. 2009), and others reporting no DA cell loss (Bennett et al. 1993; Goodman and Sloviter 1993). However, several other measures reveal a dysregulated DA system following chronic cocaine exposure in humans and animals.

Depletion of extracellular DA after long-term cocaine use brings about withdrawal symptoms (e.g., anhedonia and anergia; Dackis et al. 1986; Dackis and O'Brien 2001) and the corresponding dysfunction in reward processing has been proposed to underlie craving for cocaine that might lead an abstinent
Cocaine user to relapse (Dackis and Gold 1985). Cocaine-induced overstimulation of DA neurons can result in inefficient storage and release of DA or even the loss of functional DA neurons in striatal and midbrain structures (Ellison 1992; Little et al. 2009). DA depletion has been demonstrated in humans and animals during the time of chronic cocaine exposure as well as during various stages of withdrawal. In non-abstinent cocaine users, decreased amphetamine-induced DA release was seen in the striatum and subjects with lower DA release were more likely to choose a subsequent cocaine injection over a monetary reward (Martinez et al. 2007). Microdialysis studies in rats have demonstrated a decrease in extracellular DA levels in the ventral striatum after 24 hours of abstinence that can last for several days (Parsons et al. 1991; Rossetti et al. 1992). Even after seven days of cocaine withdrawal, DA and homovanillic acid (HVA) levels in the nucleus accumbens (NAc) were decreased by 36-38% in rats (Robertson et al. 1991). Furthermore, cocaine-dependent individuals abstinent for three to six weeks show reduced DA release in the striatum when given IV methylphenidate (Volkow et al. 1997b). These data indicates that changes in intracellular DA stores and/or DA-releasing capabilities are linked to the progression from cocaine abuse to cocaine dependence.

With regard to the ability of a medication to restore normal levels of DA, the time course of DA dysfunction during cocaine abstinence is an important consideration because cocaine users go through somewhat distinct stages during an extended abstinence period (Gawin and Kleber 1986). The DA-
mediated symptoms (e.g., anhedonia, anergia, motor disturbances) that occur during each stage would seemingly correspond to the efficiency of the DA system at that particular point in time (Dackis and Gold 1985). In addition, the fluctuation in craving intensity that commonly occurs throughout the stages of withdrawal (Gawin and Kleber 1986) is also associated with DA function (Dackis and O'Brien 2001).

Cocaine differentially affects DAT densities in the striatum and prefrontal cortex as well as cocaine binding sites on these proteins based on the level of cocaine exposure. Preclinical studies have shown decreased cocaine binding sites on DATs following several non-contingent cocaine injections (Izenwasser and Cox 1990) or the initiation of self-administration (Letchworth et al. 2001). However, this effect seems to move in the opposite direction with increased voluntary cocaine intake, thereby making increased DAT density a marker for cocaine-dependence (Mash et al. 2002). Radioligand binding studies in human postmortem tissue from heavy cocaine users show increased DAT densities in the striatum and NAc, both in cocaine-overdose (Staley et al. 1994) and non-overdose victims (Little et al. 1993; 1999; Mash et al. 2002). Furthermore, imaging studies using single photon emission computed tomography (SPECT) have confirmed increased DAT density and availability in acutely abstinent cocaine-dependent subjects (Crits-Christoph et al. 2008; Malison et al. 1998). This result has been replicated in tissue from rats that were continuously exposed to cocaine (Hitri et al. 1996; Kuhar and Pilotte 1996) and non-human primates after extended cocaine self-administration
(Letchworth et al. 2001). Furthermore, an association between DAT density and the level of cocaine exposure in terms of dose and duration were seen in both human and non-human primates (Letchworth et al. 1997; Little et al. 1999). Because axonal degradation can occur in DA neurons after extended cocaine exposure (Ellison 1992) the augmented density is most likely due to an increased number of DATs on each terminal (Little et al. 1999). Together, these studies have shown that the increased DAT density is not a pre-existing condition in human cocaine users prior to their first exposure to cocaine. Functionally, the augmented density of DATs is related to an increase in DA uptake and decreased basal levels of extracellular DA (Mash et al. 2002).

Changes in DA receptors from both D1-like and D2-like families (Civelli et al. 1991; Sibley and Monsma, Jr. 1992) have been linked to chronic cocaine abuse. The most prominent finding from positron emission tomography (PET) studies in humans is that abstinent cocaine users exhibit decreased D2 levels as well as lower D2 availability when compared to non-using controls (Martinez et al. 2004; Volkow et al. 1990; 1993; 1997b). Although it is difficult to distinguish between causality (i.e., whether a pre-existing low level of D2 availability renders an individual vulnerable to cocaine dependence) and consequence (i.e., D2 levels are reduced as a compensatory response to cocaine-induced DA overflow) in human studies, autoradiography binding studies in non-human primates have also shown a downregulation of D2 receptors in several areas including the NAc, caudate, and putamen after 18-22 months of cocaine self-administration (Moore et al. 1998a; Nader et al. 2002). Also, the significant
decrease in D2 availability can be seen even after seven months of abstinence (Nader and Czoty 2005).

Several preclinical studies have also shown deficits in D1 receptors after extended cocaine self-administration. Decreases in D1 density have been found to be more regionally specific than with D2 receptors, although there is disagreement as to whether the greatest decrease is seen in the caudate (Farfel et al. 1992) or the NAc shell (Moore et al. 1998b). Findings of decreased density of D1 receptors following cocaine self-administration are supported by behavioral data showing that rats with the greatest cocaine self-administration history exhibited the greatest deficits in subsequent testing (after three weeks of withdrawal) on behavioral measures regulated by both D1 and D2 receptors (Edwards et al. 2007). However, more recent studies have shown increased D1 binding in monkeys that had less cocaine self-administration (Nader et al. 2002) or were withdrawn from cocaine self-administration for 30 days (Beveridge et al. 2009), thus stressing the importance of cocaine history and withdrawal stage in assessing measures of DA dysfunction.

4.2. Dopamine Agonist Medications

An ideal medication for treating cocaine dependence will normalize neurochemical deficits without contributing additional adverse effects to the withdrawal syndrome. In addition to stabilizing deficits in the DA system, DA agonist medications would seemingly be well-accepted by patients because of
their inherent reinforcing effects (Dackis et al. 1986). While this would enhance patient compliance, it has the potential to become problematic in that the patient could become dependent on the medication itself. Therefore, as with methadone maintenance and NRT, the most clinically useful agonist medications would have a slower speed of onset and/or longer duration of action than that of cocaine (Carroll et al. 1999; Howell et al. 2000). This is an important factor because the rapid entry into the brain and short duration are key contributors to the abuse liability of cocaine and other DA-ergic drugs (Balster and Schuster 1973; Gorelick 1998; Panlilio et al. 1998).

4.2.1. Direct Dopamine Agonists

In accordance with their "DA depletion hypothesis", Dackis and Gold (1985) suggested the use of the non-selective direct DA agonist, apomorphine, to ameliorate the DA dysfunction associated with cocaine dependence. The increased extracellular DA levels after apomorphine treatment have been found to be beneficial in studies of Parkinson's disease (Schwab et al. 1951), but this and several other non-selective DA agonists, was found to increase cocaine-reinforced responding and reinstatement behavior in animals (Caine and Koob 1993; De Wit and Stewart 1981; Spealman et al. 1999). Additionally, non-selective DA agonists also have significant side effect profiles (Bukofzer and Livesey 2001; Kleber 1995; Malcolm et al. 1997) which would most likely contribute to compliance problems in cocaine-dependent patients (Soares et al. 2003).
Several other direct DA agonists with varying affinities for D1-like and D2-like receptors have also been investigated for their therapeutic potential. Agonists for D1-like receptors (i.e., D1 and D5) were found to decrease cocaine-reinforced responding and reinstatement in animals (Caine et al. 1999; 2000; Khroyan et al. 2000; 2003; Platt et al. 2001; Self et al. 1996; 2000) as well as subjective ratings of "high" and "liking" in humans (Haney et al. 1999). Although D1-like agonists had a better overall side effect profile than the non-selective agonists (Platt et al. 2002), the clinical usefulness of these drugs is limited because they also decreased food-reinforced responding (Platt et al. 2001). Many D2-like agonists were likewise proven to be inadequate either because they non-selectively decreased the reinforcing effects of cocaine, or because they augmented the reinforcing effects of cocaine in both preclinical and clinical studies (Caine et al. 1999; 2000; De Vries et al. 1999; Gorelick and Wilkins 2006; Khroyan et al. 2000; Kleven and Woolverton 1990; Moscovitz et al. 1993; Platt et al. 2003; Preston et al. 1992; Self et al. 1996). D3-selective agonists are believed to have more potential as agonist therapy medications than their other D2-like counterparts. 7-OHDPAT is a D3 agonist that has been shown to decrease cocaine conditioned place preference (Khroyan et al. 1999) as well as cocaine self-administration at doses that are not themselves self-administered (Caine and Koob 1993), and this and other similar drugs are currently under development.
4.2.2. **Partial Dopamine Agonists**

Similar to buprenorphine and varenicline (for treating opiate and nicotine dependence respectively), partial agonist medications have been considered for the treatment of cocaine dependence (Pulvirenti and Koob 1994). Partial DA agonists including aripiprazole (Lile et al. 2008) and BP897 (Pilla et al. 1999) have been shown to suppress cocaine self-administration in preclinical studies and further investigation is currently underway (see Bergman 2008 for review). As partial agonists, these medications exhibit both agonist and antagonist properties (e.g., BP897 is known to act as a D3 agonist in vitro but it acts as both agonist and antagonist in vivo). Because these drugs can block cocaine’s euphoric effects in addition to serving as replacement medications, partial agonists are thought to have more clinical utility than full agonists or antagonists (Ariens 1983; Childress and O’Brien 2000; Pilla et al. 1999).

4.2.3. **Indirect Dopamine Agonists**

Another approach to DA normalization uses indirect DA agonists to increase the extracellular levels of DA. Just as methadone and LAAM mimic the actions of heroin at the synapse, cocaine-like monoamine reuptake inhibitors have been investigated as potential treatments for cocaine dependence. The first FDA-approved psychostimulant to be tested as a treatment for cocaine dependence was methylphenidate. Patients with attention deficit hyperactivity
disorder (ADHD) benefit from methylphenidate treatment due to its high affinity for the DAT (Gatley et al. 1996) and similarly, cocaine users with comorbid ADHD decreased their cocaine intake with methylphenidate treatment (Khantzian et al. 1984). This finding was later supported by studies demonstrating decreases in both the positive subjective effects of cocaine and cocaine choice (Collins et al. 2006; Somoza et al. 2004). Similar decreases in the positive subjective effects of were seen in patients without ADHD after methylphenidate administration (Somoza et al. 2004), but ADHD subjects were more likely to reduce cocaine use after receiving the same treatment (Levin et al. 2007). Other studies have shown that methylphenidate is ineffective in human cocaine abusers either with (Schubiner et al. 2002) or without (Grabowski et al. 1997) comorbid adult ADHD. Bupropion is another monoamine reuptake inhibitor with inconclusive results in clinical populations with and without ADHD (Levin et al. 2002; Margolin et al. 1995; Shoptaw et al. 2008). Although bupropion has a high affinity for the DAT, its DAT occupancy is only about 22% after administration of an approved oral dose (Meyer et al. 2002) and therefore it may not be an optimal medication. Several other DAT inhibitors have been found to decrease food intake in addition to cocaine intake, thereby limiting their clinical utility (Howell et al. 2000; Kleven and Woolverton 1993; Mansbach and Balster 1993; Negus et al. 1999).

GBR120909 (vanoxerine) and other related novel DAT inhibitors were able to selectively decrease cocaine self-administration for an extended period of time in animals (Glowa et al. 1995; 1996; Lindsey et al. 2004), but the highly
anticipated clinical trials were terminated in Phase II due to adverse effects on cardiac function. Currently, new monoamine reuptake inhibitors, such as RTI-336, have fared well in preclinical studies and are expected to be effective in clinical populations (Carroll et al. 2006).

Monoamine releasers, of both the amphetamine and non-amphetamine variety, have also had mixed results in preclinical and clinical trials. d-Amphetamine is a psychostimulant currently available for the treatment of ADHD and narcolepsy. In self-administration studies, the reinforcing effects of cocaine were only diminished when d-amphetamine was delivered continuously for several days (Chiodo et al. 2008; Negus 2003; Negus and Mello 2003a; 2003b; Peltier et al. 1996), as this effect was absent when it was given as an acute pretreatment (Barrett et al. 2004; Ferrario and Robinson 2007; Horger et al. 1992; Mendrek et al. 1998). Methamphetamine is a stronger monoamine releaser, but the side effect profile (as well as the high abuse potential) precludes its use as a treatment medication. Cocaine users treated with amantadine, a non-amphetamine DA releaser, had a greater number of cocaine-free urine screens after two weeks (Alterman et al. 1992). In addition, amantadine also appears to be the most effective in patients with severe withdrawal symptoms (Alterman et al. 1992; Kampman et al. 2000; Shoptaw et al. 2002).

Several other unconventional indirect DA agonists have been tested as treatments for cocaine-dependent patients. Supplementation with levodopa (a
precursor to DA) indirectly affects DA transmission by increasing the production of DA inside the presynaptic neuron. In one clinical study, levodopa was found to be safe for cocaine-dependent patients, but showed no difference from placebo with regard to cocaine use or measures of subjective cocaine effects (Mooney et al. 2007). However, a more recent study found levodopa to be therapeutic when combined with abstinence-based reinforcement therapy (Schmitz et al. 2008). Selegiline is a monoamine oxidase B inhibitor that maintains elevated DA levels by slowing down its metabolism (Felner and Waldmeier 1979). In addition, selegiline itself is metabolized into l-amphetamine and l-methamphetamine (Yasar et al. 1996), which could potentially enhance its effects as an agonist pharmacotherapy. Although a recent study using a transdermal delivery method did not demonstrate a therapeutic effect of selegiline (Elkashef et al. 2006), it has not been excluded from future study. Disulfiram is another medication that can be considered an indirect DA agonist. It has been proposed for the treatment of cocaine dependence because its primary metabolite of disulfiram inhibits dopamine-beta-hydroxylase (DBH), thereby slowing down the conversion of DA into NE (Goldstein et al. 1964; Petrakis et al. 2000). Thus far, disulfiram treatment has been associated with decreases in cocaine use in clinical trials (Carroll et al. 1998; 2004; George et al. 2000). While the restoration of a “normal” level of DA may be partially responsible, the therapeutic effects of disulfiram are more likely due to the adverse effects it perpetuates when cocaine is administered (see Section 5 below).
4.3. **Dysregulation of the Serotonin System**

Although the validity of the DA hypothesis of addiction has been demonstrated in the literature over the last several decades, more recent evidence has shown that it is not, in itself, a complete explanation. Agonist therapy for stimulant dependence is most often directed toward DA effects (i.e., since DA is believed to be the neurotransmitter most centrally involved in the reinforcing effects of psychostimulants), but the 5-HT and NE systems may actually be a better target for pharmacotherapies. The serotonergic innervation of the NAc indicates that 5-HT plays a role in reward (see Brown and Molliver 2000). Considering the fact that cocaine has a high affinity for the 5-HT transporter (SERT), chronic cocaine exposure would naturally lead to decreased synaptic levels of 5-HT and other dysfunctions in 5-HT neurotransmission that are similar to those seen with the DA system. Indeed, many of the long-lasting psychological disturbances associated with cocaine abstinence (i.e., depressed mood, obsessive thoughts, impulsivity, and suicide attempts) result from 5-HT dysfunction (Barr et al. 2002; Garlow et al. 2003; Gawin and Kleber 1986). Cocaine withdrawal is even used as an animal model of depression because rats show decreased intracranial self-stimulation thresholds and decreased responding for natural rewards (see Barr and Markou 2005 for review).

Similar to the dysregulation seen in the DA system, chronic cocaine exposure has been associated with decreased extracellular levels of 5-HT.
(Parsons et al. 1995; Taylor and Ho 1977). This effect has been inconsistent in the preclinical literature, however, as several other studies have failed to find decreases in 5-HT levels. This difference may be due to variability in the level of cocaine exposure and amount of time off before the assessment of 5-HT (Kleven et al. 1988; Yeh and De Souza 1991). Diminished 5-HT function can be attributed in part to an increased density of SERTs after chronic cocaine use. One preclinical study found an increased SERT density in conjunction with decreased 5-HT levels (Cunningham et al. 1992). Also in a clinical study using SPECT, acutely-abstinent cocaine users were shown to have increased SERT availability (Jacobsen et al. 2000). The similarities between the cocaine withdrawal syndrome and major depressive disorder extend to 5-HT receptors as well. 5-HT2 receptors have been shown to be upregulated whereas 5-HT1 receptors were downregulated after chronic cocaine exposure (Simms and Gallagher 1996). However, other studies have not shown a change in either receptor subtype after chronic cocaine (Javaid et al. 1993; Johnson et al. 1993).

4.4. **Serotonin Agonist Medications**

Although antidepressant medications that improve 5-HT function seem to be a logical antidote to the depressive symptoms associated with cocaine abstinence, the findings from preclinical and clinical studies have been inconsistent. The most common medications currently used for treating depression (i.e., SERT inhibitors) have been shown to either be ineffective at
decreasing cocaine self-administration (Porrino et al. 1989; Tella 1995), or non-selective (Carroll et al. 1990a; Peltier and Schenk 1993). The results from human studies have been similarly disappointing (Batki et al. 1996; Covi et al. 1995). A potential reason for the overall ineffectiveness of SERT inhibitors is that most of these medications take several weeks to reach their peak effectiveness. By that time, the immediate withdrawal syndrome should already be finished (Kosten and O'Connor 2003). However, these medications may still be beneficial for relapse prevention, as they are known to increase DA levels and enhance DA transmission (Collu et al. 1997; Dewey et al. 1995; Ichikawa and Meltzer 1995). L-Tryptophan, the precursor to 5-HT, reduced cocaine-reinforced responding in rats (Carroll et al. 1990b) but did not reduce cocaine use in a clinical population that also received reward vouchers that were contingent on abstinence (Jones et al. 2004). Other 5-HT agonist medications have reportedly reduced craving for cocaine, but have been unable to affect cocaine intake thus far.

4.5. THE "DUAL DEFICIT" MODEL OF COCAINE WITHDRAWAL

As both DA and 5-HT dysfunction are responsible for the emergence of cocaine withdrawal symptoms, the "dual deficit" model of stimulant withdrawal proposed by Rothman, Blough and Baumann (2002) has become the basis for the development of new agonist therapy medications (Negus et al. 2007; 2008). This group found beneficial effects of a combination of phentermine (DA releaser) and fenfluramine (5-HT releaser) on cocaine intake (Glowa et al.
1997; Rothman et al. 1994; 1998; Shoaib et al. 1997). This led them to hypothesize that a combination of medications to restore both DA and 5-HT levels might be more beneficial during cocaine abstinence than any single medication that is currently available. It is important to note that the balance between DA and 5-HT activity determines the therapeutic and side effects of a treatment medication. For instance, a medication (or combination of medications) that has a greater effect on DA than 5-HT will likely attenuate the reinforcing effects of cocaine but it will also come with a high abuse liability and psychomotor side effects. Conversely, a treatment with a greater amount of 5-HT activity will not be very effective at decreasing cocaine intake, but it will dampen the unfavorable effects of the DA action (Porrino et al. 1989; Ritz and Kuhar 1989; Rothman et al. 2006).

Ideally, a single compound that merges the positive effects of DA and 5-HT stimulation (i.e., reducing the reinforcing strength of cocaine without affecting natural reward) without the aversive side effects of currently-available drugs (i.e., abuse liability, cardiac effects, anxiety) will soon be created. This idea of "selectively non-selective medications" has already been applied to other psychiatric conditions that share some neurochemical aspects of cocaine addiction, including depression, bipolar disorder and schizophrenia (Roth et al. 2004). A recently-developed compound, PAL-287, seems to have an accurate balance of DA and 5-HT releasing effects and it is a promising agonist therapy candidate for treating cocaine dependence (Rothman et al. 2005; Rothman and Baumann 2006).
4.6. Dysregulation of the Norepinephrine System

Cocaine increases extracellular NE by inhibiting the NET, but the exact role of NE in producing the reinforcing effects of cocaine is still being debated in the literature. Early research on the maintenance of cocaine self-administration behavior had concluded that the NET-blocking activity of cocaine does not play a role in its reinforcing properties (Woolverton 1987), despite its key role in the discriminative stimulus effects of cocaine (Spealman 1995). However, more recent studies examining responding previously reinforced by cocaine and other behavioral measures now provide evidence that increases in NE may contribute to the positive subjective effects of cocaine (see Weinshenker and Schroeder 2007 for review). Clonidine (an alpha2 adrenergic agonist) has reinforcing effects and is self-administered by animals (Shearman et al. 1981; Woolverton et al. 1982), thus supporting the claim that NE is involved in reinforcement. NE has also been implicated in the production of stress-related withdrawal symptoms in abstinent cocaine users and related stress-induced relapse (see Smith and Aston-Jones 2008 for review). Continued cocaine exposure results in similar dysfunction in the NE system as in the DA system, thereby supporting the idea that NE plays an important role in reinforcement learning. An increased density of NETs has been observed in post-mortem brain tissue from heavy cocaine users (Mash et al. 2005) and monkeys with a history of cocaine self-administration (Letchworth et al. 2001; Macey et al. 2003; Nader et al. 2002).
4.7. **Norepinephrine Agonist Medications**

Several drugs with NE agonist effects have been studied for their ability to decrease cocaine intake, with limited success. Although indirect NE agonists, including NET inhibitors and NE releasers, could improve extracellular levels of NE, several of these drugs have had mixed results in preclinical and clinical studies. NET inhibitors with varying selectivity, including desipramine and nisoxetine, did not decrease cocaine self-administration in preclinical studies when administered as an acute pretreatment (Tella 1995) or added directly to the self-administered cocaine (Wee et al. 2006). In contrast, desipramine (McDowell et al. 2005) and reboxetine (Szerman et al. 2005) have shown promise in clinical studies, but this has been largely attributed to the corresponding alleviation of depressed mood as opposed to any cocaine-specific action. Subsequently, clinical trials with desipramine have been terminated (Vocci and Ling 2005).

Newer medications that cause NET inhibition might be beneficial for cocaine-dependent individuals. Venlafaxine is a 5-HT/NE reuptake inhibitor that has been shown to be somewhat effective in reducing (but not eliminating) cocaine use in dependent users with severe depression (McDowell et al. 2000) but it has not demonstrated this success in the general population of cocaine abusers (Ciraulo et al. 2005; Foltin et al. 2003). Atomoxetine is a NE reuptake inhibitor that also increases DA levels in the PFC. It is approved for the treatment of ADHD, and like d-amphetamine and methylphenidate, has been
considered as a replacement therapy option in cocaine-dependent individuals. A recent study confirmed that the combination of atomoxetine is safe and tolerable in cocaine-dependent subjects (Stoops et al. 2008) and clinical studies are currently underway to reveal its therapeutic potential. Additionally, the therapeutic effects of modafinil on human cocaine self-administration (Hart et al. 2008) and reports of euphoria after cocaine in clinical studies (Dackis et al. 2003; Malcolm et al. 2006) have partially been attributed to the drug’s NE-augmenting activity (Duteil et al. 1990; Madras et al. 2006; Stone et al. 2002).

5. **The Use of Medications to Prevent or Block the Reinforcing Effects of Cocaine**

Treatment medications can also be used to prevent or block the reinforcing effects of cocaine. This method is supposed to help cocaine-dependent patients avoid relapse because they will learn that cocaine is no longer as reinforcing as it once was. One way to block the reinforcing effects of cocaine is with a DA antagonist medication. Neuroleptic drugs are DA antagonists that are known for creating many adverse effects in patients. Therefore, although several preclinical studies demonstrated decreased cocaine intake after treatment with DA antagonists (Khroyan et al. 2000; Mello and Negus 1996), compliance in the clinic has been extremely poor (Gawin 1986).
The typical neuroleptics used to treat cocaine dependence have mainly been D1 and D2 antagonists. Recently, however, medications that act through D3 antagonism have been investigated and may have greater potential as therapeutic agents in cocaine-dependent patients (Engleman et al. 2008; Vorel et al. 2002). Activation of 5-HT3 receptors normally increases DA activity in NAc (Dremencov et al. 2006), thus it is thought that 5-HT3 antagonists could block the reinforcing effects of cocaine. Ondansetron is a member of this class of medications, and has shown promise in a recent preclinical study (Johnson et al. 2006). Since ondansetron is already approved for treating nausea and vomiting, some have speculated that it will not only block some of the reinforcing effects of cocaine but also relieve some of the nausea associated with HIV infection in cocaine users (Montoya and Vocci 2008). Overall, the antagonist treatment approach is promising in theory, but the practice of it could present difficult obstacles in a clinical setting. Even if a patient is compliant with taking his/her medication, the blockade could potentially lead a cocaine-dependent patient to overdose on cocaine in an attempt to overcome the medication’s capacity to block the reinforcing effects of cocaine.

Agonist medications can also be used to prevent the reinforcing effects of cocaine by directly interfering with cocaine’s action in the synapse. For example, a long-acting agonist medication could occupy or alter the cocaine binding site on the DAT and prevent cocaine from binding. Ideally, cocaine would be metabolized by the time the long-acting agonist medication is removed from the DAT, thereby precluding cocaine from eliciting its euphoric
effects. This outcome could also be achieved with continuous administration of an agonist medication with a shorter duration of action. As with antagonist treatment, treatment with an agonist medication could put a patient at risk for overdose if he/she attempts to overcome the pharmacological blockade. However, the interaction between a high dose of cocaine and a treatment drug would have a greater impact on the patient if the treatment were an agonist instead of an antagonist. On its own, cocaine becomes aversive and anxiogenic after the initial euphoric effects have subsided (Ettenberg 2004), but when combined with a similar agonist medication the aversive aspects of cocaine could be amplified.

Likewise, disulfiram has been proposed for the treatment of cocaine dependence. As an approved medication for the treatment of alcohol dependence, disulfiram is used to prevent relapse by producing ethanol intolerance. The combination of disulfiram and alcohol creates excessive amounts of acetylcarndehyde which causes nausea and illness. Thus, in theory, disulfiram is able to change alcohol from a reinforcer to a punisher and thereby decrease the rewarding effects of alcohol. A similar effect occurs when cocaine is combined with disulfiram (see Grassi et al. 2007). As disulfiram can be considered an indirect DA agonist (see above), the combination of cocaine and disulfiram produces excessive DA and hyperstimulation, consequently magnifying the unpleasant effects of cocaine. Disulfiram treatment has been shown to decrease cocaine use in humans (Carroll et al. 1998; 2004; George et
al. 2000), although it is more effective in male subjects (possibly due to polymorphisms in the DBH gene; Nich et al. 2004).

6. **The Treatment of “Drug Dependence” Instead of “Cocaine Dependence”**

6.1. **Cocaine Abusers Typically Use Other Drugs as Well**

Poly-drug use is an important consideration when treating cocaine dependence because most cocaine users also abuse various combinations of other drugs of abuse (Carroll et al. 1993; Kenna et al. 2007). There is some difficulty associated with determining the principal drug of abuse in poly-drug users, as the principal drug of abuse is not necessarily that which the patient uses the most often, the preferred drug, or the drug which has caused the most detriment to the patient (Griffin et al. 2009; Rounsaville et al. 2003). Accordingly, poly-drug use may be responsible in part for discrepant findings in clinical medication studies. Although researchers recognize confounds from poly-drug use, controlling for this in clinical studies is not always feasible. It can be very difficult to find a sufficiently large sample of cocaine-dependent subjects that abstain from all other substances. Also, by limiting the use of all other drugs, clinical studies would subsequently be limiting the generalizability of their findings.
The high prevalence of poly-drug use in the general population raises the issue of whether a cocaine-specific medication is even necessary. Not only will a cocaine-specific pharmacotherapy be ineffective for a poly-drug user, it could potentially harm patients who are abstaining from multiple illicit drugs. For example, poly-drug users who are treated with clonidine to alleviate opiate withdrawal symptoms are susceptible to seizures from untreated alcohol or benzodiazepine withdrawal symptoms which are masked by the clonidine (Kosten and O'Connor 2003). Similarly, a replacement medication which would counteract the DA dysfunction in an abstinent cocaine user might impede their recovery from dependence on another substance. If a pharmacotherapy were to address more universal addiction-related problems, it may be more effective in poly-drug users (see Kenna et al. 2007 for review).

6.2. Craving is a Universal Feature of Drug Dependence

As mentioned above, several cocaine-specific medications have not been successful in the clinic. Since many of the criteria for drug dependence listed in the DSM-IV and ICD-10 are common among several drugs of abuse (American Psychiatric Association 2000; World Health Organization 2004), medications which target universal features of "drug dependence" rather than "cocaine dependence" may be more successful. Craving is a common aspect of drug dependence which has been described as a “memory for the pleasant aspects of the drug” (Koob and Bloom 1988). The drug craving phenomenon can be broken down into three main aspects which include an internal drive to acquire
euphoric effects from the drug (i.e., positive reinforcement), a need to use the drug to counteract the unpleasant effects of withdrawal (i.e., negative reinforcement), and an uncontrollable compulsion toward the drug of abuse (Dackis and O'Brien 2001; Nutt and Lingford-Hughes 2008). Drug craving has been shown to affect the same brain regions that are involved in the feelings of hunger and natural reward (Childress et al. 1999; Wise 1996) and it has also been conditioned to drug-associated cues (Childress et al. 1999; Ehrman et al. 1992).

A somewhat unique aspect to cocaine craving is that its intensity waxes and wanes during the withdrawal period. During a time of low craving a patient may feel a sense of control over his/her cocaine use, only to be overwhelmed by an unpredicted surge in craving for cocaine a short time later. Gawin and Kleber (1986) showed that cocaine-dependent patients were capable of an initial period of abstinence but tended to relapse with the onset of intense craving. This usually resulted in a perpetual three- to ten-day cycle of cocaine use, a “crash” period, normalization, cocaine avoidance, and eventual relapse. In rats, measures of cocaine craving appeared to be “incubating” for a period of about two months after the onset of withdrawal and only became stronger over time. This indicates that the most vulnerable time for a recovering cocaine user to relapse might actually occur after the acute withdrawal symptoms have subsided (Grimm et al. 2001).
6.3. Medications Which Target Drug Craving

The rationale behind “anti-craving” medications involves interfering with neurobiology associated with the various components of craving, instead of treating based on the pharmacology of the specific abused drug. Although the acute effects of cocaine administration are related to its effects on DA, NE and 5-HT transmission, chronic cocaine exposure has been shown to dysregulate other neurotransmitters systems as well, including gamma-aminobutyric acid (GABA) and glutamate (Kalivas et al. 2003; Shoji et al. 1997). Drugs that normalize these neurotransmitter systems are especially important for relieving craving because they can regulate the prefrontal cortex—an area of the brain that has been specifically linked to the craving phenomenon (Streeter et al. 2005). The leading drugs in this category enhance GABA activity while inhibiting glutamate activity. Topiramate is an anti-epileptic drug that acts as an agonist at GABA-A receptors and an antagonist at ionotropic glutamate receptors. It is considered to be a forerunner as a relapse-prevention medication (Kampman et al. 2004). Baclofen, a GABA-B agonist, has effectively decreased cocaine reinforcement in several preclinical self-administration studies (Brebner et al. 2000; Roberts et al. 1996; Roberts and Andrews 1997; Roberts and Brebner 2000; Weerts et al. 2007) and has been associated with decreased cocaine use in a small clinical population (Shoptaw et al. 2003). Vigabatrin is a GABA-ergic drug that prevents the metabolism of GABA by interfering with GABA transaminase. A recent clinical study reported decreased subjective measures of cocaine craving after three weeks of
treatment with vigabatrin (Brodie et al. 2005). N-acetylcysteine normalizes glutamate levels by balancing the cystine-glutamate exchange and has been beneficial in a reinstatement model of relapse (Baker et al. 2003; Kalivas 2007). Finally, modafinil has GABA and glutamate effects in addition to its effects on NE transmission and it is currently being investigated in several clinical trials (Dackis et al. 2005).

Some opiate medications have the potential to be used as anti-craving medications in people who are dependent on cocaine and other drugs of abuse. Mu-opioid receptor levels are increased in abstinent cocaine users—a finding that has been linked to cocaine craving (Schroeder et al. 2003; Zubieta et al. 1996). Opiate medications are able to indirectly increase DA in the NAc by removing the tonic inhibition from GABA-ergic neurons (Kreek and Vocci 2002). This is promising for treating cocaine dependence since many treatment-seeking cocaine users are already participating in a methadone maintenance program. Naltrexone was tested as an anti-craving medication for several drugs of abuse, but has not been beneficial for treating cocaine or nicotine dependence thus far (Vocci and Ling 2005). Buprenorphine (a mu agonist and kappa antagonist) has been shown to attenuate cocaine self-administration in monkeys (Mello and Negus 2007) and decrease both cocaine and heroin use in poly-drug users (McCann 2008; Montoya et al. 2004). Interestingly, buprenorphine may be exerting some of its therapeutic effects by counteracting the kappa opioid receptor system which has been shown to be upregulated after chronic cocaine use (Shippenberg et al. 2007).
7. **CONCLUSIONS**

The last few decades have brought about great progress in the search for an ideal medication for treating cocaine dependence. However, there are many non-pharmacological aspects to a successful medication strategy that must be considered. Perhaps the largest obstacle to a successful pharmacotherapy today is the reality that only a small fraction of the drug-abusing population is actively seeking treatment and ready to commit to a treatment program (Substance Abuse and Mental Health Services Administration 2008). More often than not, cocaine-dependent patients suffer from comorbid psychiatric disorders (Swartz and Lurigio 2006) and/or poly-drug dependence (Carroll et al. 1993; Kenna et al. 2007) which tend to lead to poor patient compliance. Individualized treatment plans have the potential to overcome these obstacles, however financial barriers including unemployment, poor insurance coverage for substance abuse treatment, and the large investment required to bring a single new medication to market (DiMasi et al. 2003) can impede treatment progress. Finally, the incorporation of behavioral interventions is a crucial factor in the success of any new medication strategy, as treatment outcomes are known to be much better with a combination of some form of behavioral therapy and medication (Carroll and Onken 2005; Grassi et al. 2007; Rawson et al. 2006) than with any medication alone.


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CHAPTER II

COCAINE SELF-ADMINISTRATION REINFORCED ON A PROGRESSIVE RATIO SCHEDULE DECREASES WITH CONTINUOUS D-AMPHETAMINE TREATMENT IN RATS

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The following manuscript was published in *Psychopharmacology* 200:465-473, November 2008, and is reprinted with kind permission from Springer Science + Business Media.

Funding provided by Grants F30DA18014, R01DA14030, and P50DA06634-14.
ABSTRACT

**Rationale:** To date, there is no medication specifically approved for cocaine addiction. Agonist medications are used clinically in the treatment of other addictions, which suggests that this method of drug therapy could potentially be successful in treating cocaine addiction as well. **Objectives:** The objective of this study was to determine the effect of extended *d*-amphetamine treatment on responding on a progressive ratio (PR) schedule reinforced by cocaine. **Materials and methods:** Rats were trained to self-administer cocaine (0.19, 0.38, 0.75, or 1.5 mg/kg/injection) or food on a PR schedule. After stable baseline breakpoints (the number of reinforcers earned in one session) were established over 3 days, animals were implanted with osmotic mini-pumps that continuously delivered *d*-amphetamine (5 mg/kg/day) for a duration of either 7 or 14 days. Breakpoints were then determined during and/or after this treatment period. **Results:** Rats demonstrated dose-dependent decreases in cocaine-reinforced responding over the *d*-amphetamine treatment period. Breakpoints for doses of 0.75 mg/kg/injection and below decreased significantly when compared to baseline and remained decreased for up to 14 days after mini-pump removal whereas those for the highest dose of cocaine remained unchanged. Additionally, *d*-amphetamine treatment during a 14-day abstinence period from cocaine self-administration had no effect on breakpoints when tested the day after mini-pump removal. **Conclusions:** These data suggest that the reduction in cocaine-reinforced responding after continuous *d*-amphetamine treatment cannot be accounted for by tolerance alone. Instead, the roles of learning and the interaction between cocaine and *d*-amphetamine must be considered and examined in future studies.

**Keywords:** Addiction, Agonist therapy, *d*-Amphetamine, Cocaine, Osmotic Mini-pump, Progressive ratio
INTRODUCTION

Currently, there is no medication approved by the FDA for the treatment of cocaine addiction (Preti 2007; Vocci et al. 2005); however, considerable effort continues to be directed toward the development of a pharmacological intervention to aid addicts in abstaining from cocaine use. A number of drugs and novel immunotherapies have shown promising results in clinical studies (O’Brien 2005; Vocci and Elkashef 2005) and further confirmatory trials are underway (Karila et al. 2008; Preti 2007).

One strategy that has been proposed is an agonist-like, replacement therapy for psychostimulant dependence (Grabowski et al. 2004b; Shearer 2008). This approach involves the use of a long-acting agonist with similar actions to cocaine in order to decrease cocaine craving and reduce cocaine use. This concept draws on studies showing beneficial effects of methadone or levo-alpha-acetyl-methadol (LAAM) treatment in the control of opiate dependence (Kreek and Vocci 2002) and the use of the nicotine patch to help with tobacco smoking cessation (Fiore 2000). To date, three double-blind randomized trials have provided promising clinical evidence that d-amphetamine may reduce cocaine intake and/or craving in cocaine-dependent individuals (Grabowski et al. 2001; Grabowski et al. 2004a; Shearer et al. 2003).

Preclinical studies have helped to clarify that the route and duration of d-amphetamine administration determines whether increases or decreases in
cocaine-reinforced responding are observed. Repeated intermittent exposure to psychostimulants, such as \( d \)-amphetamine, has been shown to lead to increased locomotor activity, termed behavioral sensitization (Kalivas and Stewart 1991). This activity has been thought of as a manifestation of increased sensitivity to the drug and may underlie certain aspects of psychostimulant addiction (Robinson and Berridge 2001; Wise and Bozarth 1987). Treatment regimens that produce a sensitized locomotor response are also associated with increases in cocaine self-administration reinforced on a progressive ratio (PR) schedule (i.e. higher breakpoints), suggesting that \( d \)-amphetamine treatment actually increases the reinforcing strength of cocaine (Lorrain et al. 2000; Vezina et al. 2002). Furthermore, acute \( d \)-amphetamine treatment produces a leftward shift in the cocaine dose-response curve in discrimination (Li et al. 2006) and fixed ratio (Barrett et al. 2004) self-administration studies and acts as a ‘priming’ stimulus to reinstate cocaine-reinforced responding (Lynch et al. 1998; Schenk and Partridge 1999). While rodent studies using acute IP treatments show an augmentation of the reinforcing effects of cocaine, other studies using different routes of administration or more prolonged treatments have found that \( d \)-amphetamine leads to a decrease in cocaine intake. For example, twice daily SC injections for 7 days decreased responding for cocaine in rats (Peltier et al. 1996). In monkeys, oral \( d \)-amphetamine pretreatment decreased responding for a sweetened cocaine fluid (Foltin and Evans 1999). Similarly, both IM (Glowa et al. 1995) and IV (Mansbach and Balster 1993) \( d \)-amphetamine pretreatment
decreased responding for IV cocaine. Thus, it appears that route and duration of \textit{d}-amphetamine treatment have substantial influences on cocaine-reinforced responding and should be considered when studying the therapeutic potential of \textit{d}-amphetamine.

Negus and Mello have examined the effect of slow IV infusions of \textit{d}-amphetamine on cocaine self-administration in rhesus monkeys in order to test the hypothesis that constant blood levels of \textit{d}-amphetamine over a prolonged period of time might have a therapeutic effect. They showed that \textit{d}-amphetamine treatment decreased not only cocaine preference in a food-drug choice procedure (Negus 2003) but also cocaine self-administration reinforced under progressive ratio (PR) or second-order schedules (Negus and Mello 2003a, 2003b). Food intake was only transiently decreased by this treatment method (Negus and Mello 2003b), suggesting the possibility that the effect on cocaine might be relatively specific.

The present experiments were designed to adapt the primate model used by Negus and Mello (2003a) to rodents and we confirm that slow delivery of \textit{d}-amphetamine via osmotic mini-pump for 7 or 14 days decreases cocaine-reinforced responding under a PR schedule. To further characterize this treatment effect, we also assessed cocaine-reinforced responding in animals that had gone through a 14-day \textit{d}-amphetamine treatment period in the absence of cocaine self-administration.
MATERIALS AND METHODS

ANIMALS

Male Sprague-Dawley rats (Harlan, Indianapolis, Ind., USA) weighing approximately 350 g at the start of the experiments were used as subjects. Throughout the experiments, rats were maintained on a reverse 12 h light/dark cycle (lights on at 3 pm) with food and water available ad libitum. All rats were habituated to this schedule for a minimum of 3 days before entering the experiment. Animals in the cocaine self-administration experiments were housed individually in stainless steel custom made experimental chambers (30 x 30 x 30 cm), whereas animals in the experiment using food as a reinforcer were pair-housed in polycarbonate cages.

SURGERY

Prior to the beginning of the study, rats in the cocaine self-administration experiment were anesthetized via an IP injection of ketamine (100 mg/kg) and xylazine (8 mg/kg) and implanted with a chronically indwelling Silastic® jugular catheter (CamCaths, Cambridgeshire, UK). The catheter was connected to a plastic anchor that exited through the skin on the dorsal surface in the region of the scapulae. Tygon® tubing (enclosed by a stainless steel protective tether), was used to connect the catheter with a counterbalanced fluid swivel (Instech Laboratories, Inc., Plymouth Meeting, Pa., USA) mounted above the experimental chamber. Tygon tubing also
connected the swivel to an infusion pump (Razel Scientific Instruments, Inc., Stamford, Conn., USA). Animals were allowed 3-5 days to recover from surgery before beginning self-administration. Catheters were flushed daily throughout the experiments with heparinized saline in order to maintain patency.

After stable self-administration behavior was established (as described in the experiments below), all animals were implanted SC with an osmotic mini-pump (Alzet Model 2001, Durect Corp. Cupertino, CA; see Theeuwes and Yum 1976) filled with d-amphetamine or saline. Briefly, animals were anesthetized with a mixture of oxygen, nitrogen and halothane (4%) and ventilated during surgery using halothane (1.5%). An incision in the skin was made between the scapulae (rostral to the plastic catheter anchor) and the mini-pump was inserted with the flow moderator pointing rostrally. The wound was closed using nylon sutures. The pump was removed 7 days later using the same procedure. Animals receiving 14 days of treatment were implanted with a second mini-pump which was removed after 7 additional days.

**Behavioral Training**

*Cocaine Self-Administration*

Experiments were conducted 7 days per week and each daily session started at 10 am. The start of a session was indicated by extension of a single active lever into the experimental chamber. During training, cocaine (1.5 mg/kg/injection) was available on a fixed ratio 1 (FR1) schedule of
reinforcement and was injected over approximately 4-5 s (depending on body weight) upon depression of the lever. Following each response, the lever was retracted and a light was illuminated for a 20-s post-response time-out period. Each training session lasted until 40 injections had been self-administered within 6 h, at which time the lever was retracted until the start of the next daily session. Self-administration training was termed complete following 5 consecutive daily sessions during which the animal self-administered all 40 injections while maintaining consistent post-infusion pauses in responding between each of the injections.

**FOOD-REINFORCED RESPONDING**

Rats were removed from their home cages to be tested individually in experimental chambers (see above) 7 days per week. The extension of a single lever signaled the beginning of each session and upon depression of this lever one 45 mg sucrose pellet (Noyes Inc.) was delivered into a food hopper. The lever was then retracted and a light was illuminated for a 20-s time-out period. Rats were trained to respond for sucrose pellets on a FR1 schedule and training was considered to be complete following 5 consecutive daily sessions during which the animal obtained 100 sucrose pellets within a 2 h testing period.

**PR Schedule of Reinforcement**

The PR schedule has proved useful in the study of treatments which might affect the reinforcing strength of cocaine. Responding on a PR schedule
is sensitive to dose (0.19-1.5 mg/kg/inj), producing an ascending dose-response curve (Ward et al. 2005) which can be shifted by pharmacological, neurotoxic, and hormonal manipulations (Negus 2003; Roberts et al. 1989a; Roberts et al. 1989b). Following training, rats responded for cocaine or food (sucrose pellets) under a PR schedule. Under these conditions, delivery of the reinforcer was contingent upon an increasing number of responses incremented through the following ratio progression: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, etc. (Richardson and Roberts 1996). Breakpoints were defined in the 6 h cocaine experiments as the number of ratios completed (i.e. the number of reinforcers delivered) before a 1 h period in which no reinforcement was delivered. While animals typically reach breakpoints for the highest dose of cocaine (1.5 mg/kg/inj) within 3 h, pilot studies showed that animals normally reached breakpoints for sucrose pellets (45 mg) within 30 to 60 min regardless of session length. For this reason, each food reinforcement session lasted only 2 h and breakpoints were defined as the number of ratios completed (i.e. the number of sucrose pellets obtained) before a 15 min period in which no reinforcement was delivered. In all cases, animals reached their breakpoints before the end of the session.

**Experiment 1: Effect of 7 Days of d-Amphetamine Treatment on the Dose-Response Curve for Self-Administered Cocaine**

The effect of 5 mg/kg/day SC infusion of d-amphetamine via osmotic mini-pump on cocaine-reinforced responding was investigated. After the
training criterion was reached, animals were assigned to 1 of 4 groups (N = 8), each having access to a different unit dose of cocaine (0.19, 0.38, 0.75 and 1.5 mg/kg/inj) for the remainder of the experiment. Immediately following completion of a 3-day baseline period on the PR schedule, during which self-administration behavior did not vary by more than 3 breakpoints, animals were implanted with an osmotic mini-pump as described above (see Surgery). The mini-pumps were filled with d-amphetamine at a concentration which would result in the delivery of 5 mg/kg over 24 h, as determined by each subject’s body weight at the time of implantation. Following a 7-day treatment period (during which cocaine self-administration took place), the mini-pump was removed. Animals then completed daily PR testing for an additional 7 days to monitor the effect of discontinuation of d-amphetamine treatment on cocaine intake.

**Experiment 2: Effect of 14 Days of D-Amphetamine Treatment on Cocaine Self-Administration**

**Experiment 2A: Cocaine Self-Administration Before, During and After an Extended D-Amphetamine Treatment Period (14 Days)**

This experiment extended Experiment 1 by lengthening both the treatment period and the post-treatment period from 7 to 14 days and by incorporating saline mini-pump control groups. After the completion of training, baseline breakpoints on the PR schedule for either 0.75 or 1.5
mg/kg/inj cocaine were established in 4 groups of rats as in Experiment 1. All rats were then implanted with 2 consecutive 7-day mini-pumps containing either d-amphetamine (0.75 group: N = 9; 1.5 group: N = 8) or saline (0.75 group: N = 10; 1.5 group: N = 10). Breakpoints were evaluated during this 14-day treatment period. During the post-treatment period, rats self-administering 1.5 mg/kg/inj cocaine were evaluated for 7 days (as in Experiment 1) and rats self-administering 0.75 mg/kg/inj cocaine were evaluated for 14 days.

**Experiment 2B: Cocaine Self-Administration Before and After, But Not During, a 14-Day d-Amphetamine Treatment Period**

Similar to Experiment 2a, 2 groups of rats (N = 8) were trained and stable baseline breakpoints for 0.75 mg/kg/inj cocaine were determined prior to the implantation of 2 consecutive 7-day mini-pumps containing either 5 mg/kg/day d-amphetamine or saline. During the 14-day treatment period, rats remained in their home experimental chambers where their catheters were flushed daily with heparinized saline (to maintain patency), but they did not have access to cocaine at this time. Cocaine self-administration resumed the morning after the second mini-pump was removed and breakpoints for 0.75 mg/kg/inj cocaine were then evaluated throughout a 14-day post-treatment period.
**Experiment 3: Effect of Continuous d-Amphetamine Treatment on Food-Reinforced Responding**

The effect of 5 mg/kg/day SC infusion of d-amphetamine on food-reinforced responding on a PR schedule was investigated. Following training, baseline breakpoints reinforced by one 45 mg sucrose pellet on a PR schedule were determined in 2 groups of rats (N = 8). One group was then implanted with d-amphetamine (5 mg/kg/day) mini-pumps and one group was implanted with saline mini-pumps. All mini-pumps were replaced after 7 days and the subsequent mini-pumps were removed after an additional 7 days (see above). Breakpoints were assessed for 14 days during the treatment period and 14 days after removal of the second mini-pump.

Testing was conducted under conditions of 20 h food deprivation. Following each daily food-reinforcement session, rats were allowed unlimited access to rat chow for 2 h before being returned to the home cage. This procedure results in modest weight gain and a consistent deprivation state during each daily test (Roberts et al. 1996). The amount of food consumed as well as daily body weights were recorded for each animal.

**Drugs**

Cocaine HCl was obtained from the National Institute on Drug Abuse, Rockville, MD, USA. Cocaine was dissolved in sterile 0.9% saline (containing heparin, 10 USP units/ml) in concentrations of 0.625, 1.25, 2.5 and 5 mg/ml
(expressed as the salt) and passed through a microfilter. \textit{d}-Amphetamine sulfate (Sigma-Aldrich, St. Louis, MO) was dissolved in sterile 0.9% saline.

**DATA ANALYSIS**

Conceptually, the final ratio (i.e. the response requirement for the last obtained reinforcer) is the measure of interest; however, final ratios taken from an exponential series usually violate the assumption of homogeneity of variance required of an analysis of variance (ANOVA; see Richardson and Roberts 1996). A log transformation can be performed on the final ratio values in order to conform to the assumptions of parametric statistics. In essence, this transformation simply yields values equivalent to the ordinal values of the final ratios (i.e. the number of reinforcers delivered). For this reason, the number of injections or sucrose pellets (breakpoint) was used as the dependent measure. All PR data were analyzed using a two-way ANOVA with repeated measures (SYSTAT Software Inc.). Paired \textit{t}-tests were used to analyze weight changes in each group of animals. Values of \( p < 0.05 \) were considered statistically significant and Bonferroni tests were used in \textit{post hoc} analyses.
RESULTS

Animals acquired cocaine self-administration behavior after an average of 6.8 (± 0.6) days on an FR1 schedule (data not shown).

EXPERIMENT 1

Figure 1a illustrates the effect of a constant infusion of d-amphetamine (5 mg/kg/day) via an osmotic mini-pump for 7 days on the cocaine dose-response curve. The mean breakpoints during the 3-day baseline testing period were compared to the mean breakpoints of the final 3 days of d-amphetamine treatment in this analysis. Repeated measures ANOVA revealed a significant effect of cocaine DOSE \( [F(3,28) = 6.42, \ p < 0.01] \) and d-amphetamine TREATMENT \( [F(1,28) = 24.7, \ p < 0.001] \) as well as a DOSE x TREATMENT interaction \( [F(3,28) = 4.40, \ p < 0.05] \), thus indicating that the effect of d-amphetamine depended on the dose of cocaine self-administered. Inspection of Figure 1a reveals that self-administration was greatly reduced at the lowest dose tested and was unaffected at the highest dose. Post-hoc Bonferroni analysis confirmed a significant difference at the 0.19 mg/kg/inj dose (\( p < 0.001 \)).

Figure 1b illustrates the effect of d-amphetamine mini-pumps on cocaine self-administration over time. Repeated measures ANOVA including the last day of baseline and the 7-day treatment period revealed a significant effect of cocaine DOSE \( [F(3,28) = 5.86, \ p < 0.01] \). The main effect of DAY was
**FIGURE 1**

Effect of a continuous 7-day infusion of *d*-amphetamine (5 mg/kg/day) on the dose-response curve for cocaine self-administration reinforced on a PR schedule. a) Points represent the mean (± SEM) breakpoints for cocaine averaged from a 3-day baseline period (open circles) or the last 3 days of *d*-amphetamine treatment (closed circles). Asterisk (*) indicates a significant difference from baseline. b) Points represent the mean (± SEM) breakpoints measured in groups of animals (N = 8) self-administering various doses of cocaine. Days 1-3 represent a baseline period. Shaded portion (days 4-10) indicates the period during which the animals received a constant infusion of *d*-amphetamine. Days 11-17 represent the post-treatment period. The final ratio values corresponding to breakpoints are represented on the right y-axis.
**Figure 1A**

![Graph showing the relationship between cocaine dose (mg/kg/inj) and breakpoint/number of reinforcers, with baseline and d-amphetamine mini-pump conditions indicated.](image-url)
**FIGURE 1B**

The figure shows a graph illustrating the breakpoint and final ratio over days for different cocaine doses self-administered. The graph includes data for 1.5 mg/kg/inj, 0.75 mg/kg/inj, 0.38 mg/kg/inj, and 0.19 mg/kg/inj. The shaded area indicates the d-Amphetamine mini-pump treatment period.
statistically significant \[ F(7,196) = 7.33, p < 0.001 \] as well as the DOSE \times \text{DAY} \] interaction \[ F(21,196) = 2.12, p < 0.01 \], indicating that the magnitude of change produced by 7 days of \( d \)-amphetamine depended on the unit injection dose of cocaine. In agreement with Figure 1a, Figure 1b suggests that the \( d \)-amphetamine mini-pump produced a gradual decrease in breakpoints at the lower cocaine doses whereas responding reinforced by the highest dose of cocaine (1.5 mg/kg/inj) was not significantly affected. In the post-treatment recovery analysis, there was a significant effect of DOSE \[ F(3,28) = 8.30, p < 0.001 \] and DAY \[ F(7,196) = 9.02, p < 0.001 \], but no interaction between the 2 factors.

\textbf{Experiment 2}

Figure 2a depicts the effect of extended treatment (14 days) with continuous \( d \)-amphetamine or saline on responding for 1.5 mg/kg/injection cocaine. Repeated measures ANOVA including the last day of baseline and the 14-day treatment period failed to find significant main effects of either TREATMENT or DAY, but revealed a significant TREATMENT \times \text{DAY} interaction \[ F(14,224) = 2.58, p < 0.01 \] for groups self-administering 1.5 mg/kg/inj cocaine. This is accounted for by the small transient increase in breakpoints for the \( d \)-amphetamine group on days 2-4 of the treatment period. There were no significant findings in the post-treatment recovery analysis for groups self-administering 1.5 mg/kg/inj cocaine.
Figure 2b shows the effect of a 14-day treatment period with continuous d-amphetamine or saline on responding for 0.75 mg/kg/inj cocaine that occurred during and/or after the treatment period. As expected, all 4 groups had statistically similar baseline breakpoints. Repeated measures ANOVA including the last baseline day and the 14-day treatment period for groups self-administering cocaine during the mini-pump treatment period revealed a significant effect of mini-pump TREATMENT \(F(1,17) = 28.72, \ p < 0.001\]. The main effect of DAY was statistically significant \(F(14,238) = 8.68, \ p < 0.001\] as well as the TREATMENT x DAY interaction \(F(14,238) = 7.58, \ p < 0.001\], indicating that breakpoints decreased more over time in animals treated with d-amphetamine. Repeated measures ANOVA during the post-treatment period for all 4 groups of animals revealed a significant effect of GROUP \(F(3,28) = 9.33, \ p < 0.001\] and a GROUP x DAY interaction \(F(39,364) = 2.17, \ p < 0.001\]. Inspection of Figure 2b shows that animals that did not self-administer cocaine while being treated with d-amphetamine were unaffected by the d-amphetamine alone, as their breakpoints were similar to those reached by animals that received saline mini-pumps. Post hoc analysis revealed that post-treatment breakpoints were significantly lower in animals that self-administered cocaine while being treated with d-amphetamine.
**FIGURE 2**

Effect of a continuous 14-day infusion of *d*-amphetamine (5 mg/kg/day) on cocaine self-administration reinforced on a PR schedule. Points represent the mean (± SEM) breakpoints measured in groups of animals self-administering either a) 1.5 mg/kg/inj cocaine or b) 0.75 mg/kg/inj cocaine. Days 1-3 represent a baseline period. Shaded portion (days 4-17) indicates the period during which the animals received either a constant infusion of *d*-amphetamine (closed symbols) or saline (open symbols). Days 18-31 represent the post-treatment period. Circles and squares represent animals that self-administered cocaine throughout the experiment and triangles represent animals that did not have access to cocaine during the 14-day treatment period. The final ratio values corresponding to breakpoints are represented on the right y-axis.
FIGURE 2A

1.5 mg/kg/inj Cocaine

Mini-pump treatment:
- ● d-Amphetamine
- ○ Saline

Breakpoint / # Reinforcers

Days

Final Ratio

Mini-pump treatment period
Figure 2b

0.75 mg/kg/inj Cocaine

Mini-pump treatment:
- □ d-Amphetamine
- ▲ d-Amphetamine
- □ Saline
- △ Saline

Breakpoint / # Reinforcers

Final Ratio

Days

Mini-pump treatment period
**Experiment 3**

Figure 3 illustrates the effect of 14 days of continuous d-amphetamine treatment on food-reinforced responding on a PR schedule over time, as compared to saline treatment. Repeated measures ANOVA including the final baseline day and the 14-day treatment period found significant main effects of both d-amphetamine TREATMENT \[ F(1,14) = 7.98, \ p < 0.05 \] and DAY \[ F(14,196) = 2.16, \ p < 0.05 \], but no significant TREATMENT x DAY interaction \[ F(14,196) = 1.57, \ p < 0.10 \] which indicates that the d-amphetamine-treated animals consistently reached higher breakpoints than the saline-treated animals throughout the treatment period. Similarly, post-treatment recovery analysis revealed main effects for TREATMENT \[ F(1,14) = 5.37, \ p < 0.05 \] and DAY \[ F(14,196) = 2.37, \ p < 0.01 \], but no significant TREATMENT x DAY interaction \[ F(14,196) = 1.07, \ ns \], indicating that breakpoints for d-amphetamine-treated rats remained elevated above those for saline-treated rats for 14 days after mini-pump removal.

Body weights before implantation and after removal of the mini-pumps were compared. Paired t-tests did not reveal any change in weight as a result of 7 days of d-amphetamine treatment for animals self-administering any dose of cocaine in Experiment 1. As shown in Table 1, the baseline weights of d-amphetamine-treated animals self-administering 0.75 mg/kg/inj cocaine or food for 14 days did not significantly differ from their corresponding saline control groups before mini-pump implantation. Paired t-tests revealed a significant increase in weight for animals self-administering 0.75 mg/kg/inj...
cocaine regardless of mini-pump treatment \([d\text{-}Amphetamine: t = -2.67, df = 8, p < 0.05; Saline: t = -5.34, df = 9, p < 0.001]\). For animals responding for food, there was a significant decrease in weight only in animals treated with \(d\text{-}amphetamine [t = 4.27, df = 7, p < 0.01]\).
**Figure 3**

Effect of a continuous 14-day infusion of d-amphetamine (5 mg/kg/day) or saline on food intake reinforced on a PR schedule. Points represent the mean (± SEM) breakpoints measured in 2 groups of animals (N = 8) responding for 45 mg sucrose pellets on a PR schedule. Days 1-3 represent a baseline period. Shaded portion (days 4-17) indicates the period during which the animals received either a constant infusion of d-amphetamine (closed circles) or saline (open circles). Days 18-31 represent the post-treatment period. The final ratio values corresponding to breakpoints are represented on the right y-axis.
**Figure 3**

**Sucrose Pellet**

**Mini-pump treatment:**
- ● d-Amphetamine
- ○ Saline

*Breakpoint / # Reinforcers vs. Days*

*Final Ratio*

*Mini-pump treatment period*
**TABLE 1**

Body weights of animals before and after extended treatment with d-amphetamine or saline.

<table>
<thead>
<tr>
<th>Reinforcer</th>
<th>Mini-pump treatment</th>
<th>Body Weight ± SEM (g)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
</tr>
<tr>
<td>Cocaine 0.75 mg/kg/inj</td>
<td>d-Amphetamine</td>
<td>386.2 ± 8.0</td>
<td>399.0 ± 6.1 *</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>383.0 ± 6.0</td>
<td>399.5 ± 3.8 *</td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>d-Amphetamine</td>
<td>333.0 ± 4.0</td>
<td>307.6 ± 5.2 *</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>330.6 ± 6.0</td>
<td>335.0 ± 5.7</td>
<td></td>
</tr>
</tbody>
</table>

Animals were weighed before and after the 14-day treatment period. Data are represented as body weight (g) ± SEM. Asterisk (*) indicates a significant difference from mean baseline weight for the group.
**DISCUSSION**

The present experiments explored the effects of a slow subcutaneous infusion of \textit{d}-amphetamine, via osmotic mini-pump, on cocaine self-administration reinforced under a PR schedule. Breakpoints for only the lowest dose of cocaine (0.19 mg/kg/inj) were significantly decreased by 7 days of \textit{d}-amphetamine treatment. A trend was observed with moderate doses which failed to reach significance and the highest dose (1.5 mg/kg/inj) remained unaffected. Extending the treatment period to 14 days significantly decreased breakpoints for a moderately high dose of cocaine (0.75 mg/kg/inj), but again breakpoints for the highest dose of cocaine remained unchanged. Moreover, 14 days of \textit{d}-amphetamine treatment when animals did not have access to cocaine had no effect on subsequent cocaine self-administration.

The present experiments replicate, in rats, a phenomenon reported by Negus and Mello (2003a) in rhesus monkeys given extended treatment with \textit{d}-amphetamine. In that study, \textit{d}-amphetamine (0.01-0.1 mg/kg/h) was delivered every 20 min over 10 days using a double lumen IV catheter while subjects self-administered a single dose of cocaine (0.032 mg/kg/inj) under a PR schedule. \textit{d}-Amphetamine treatment was shown to produce a dose-dependent decrease in cocaine-reinforced responding. Negus and Mello (2003b) also showed a greater reduction in responding for cocaine doses on the low end of the dose-response curve during \textit{d}-amphetamine treatment using a second order schedule, a procedure that the authors consider to reveal similar information about
potential pharmacotherapies as PR (Negus and Mello 2003a). Cocaine-maintained responding in both of these studies appeared to progressively decrease over the d-amphetamine treatment period, similar to findings in the present study.

The results from the present study as well as those from the studies mentioned above (Negus and Mello 2003a, 2003b) raise a question as to how d-amphetamine treatment leads to decreased cocaine self-administration in animals. One possibility, suggested by Peltier and colleagues (1996), is that extended exposure to d-amphetamine causes cross-tolerance to the reinforcing effects of cocaine. In their study, d-amphetamine was administered by a subcutaneous injection (3.2 mg/kg) every 12 h for 7 days and post-treatment breakpoints for cocaine were reduced when compared to baseline. These results are similar to those from the present study; both sets of data showed that d-amphetamine treatment produced the greatest effect at the lowest unit injection dose of cocaine.

Strictly speaking, cross-tolerance implies that tolerance to a specific effect of one drug has an impact on the same effect of another drug. Neither of the studies mentioned above (Negus and Mello 2003a; Peltier et al. 1996) nor the present study specifically evaluated the reinforcing effects of continuously delivered d-amphetamine, so any reference to cross-tolerance is merely speculation. Regardless, the general idea of tolerance would not appear to completely account for the data from the present study. If exposure to d-
amphetamine created tolerance to the reinforcing effects of cocaine, one would expect all animals similarly treated with the same amount of d-amphetamine to demonstrate similar decreases in the reinforcing strength of the same dose of cocaine. In Experiment 2b, animals that self-administered cocaine during the treatment period showed decreased breakpoints but animals that were not permitted to self-administer cocaine during the treatment period did not show a change in cocaine-reinforced responding when subsequently assessed. Therefore, the explanation must necessarily involve a combination between d-amphetamine and cocaine. Intuitively, one would expect that the combination of d-amphetamine plus self-administered cocaine would create more tolerance than d-amphetamine alone. While this explanation may apply to Experiment 2b, one would expect to see the greatest amount of tolerance (i.e. greatest decrease in breakpoints) for animals self-administering the most cocaine, which would be at the highest dose. The results from Experiment 2a show that this was not the case.

A cocaine injection can be thought of as a compound stimulus having both positive and aversive properties and thus its reinforcing strength reflects the net effect of these competing features (Ettenberg 2004; Wheeler et al. 2008). Therefore, the observed reductions in breakpoints in the present study might be explained by either a decrease in the positive reinforcing effects (as mentioned above) or an augmentation of anxiogenic or other limiting/aversive effects, or a combination of the two. The gradual diminishment of breakpoints observed during the d-amphetamine treatment period appears to reflect the
process by which animals learn of a change in the net effect of cocaine. If d-amphetamine treatment primarily enhanced the aversive properties of self-administered cocaine in the present study, one would expect the greatest enhancement for the highest dose of cocaine. However, breakpoints for 1.5 mg/kg/inj cocaine did not decrease during the treatment period. As with the concept of decreased reinforcing strength mentioned above, this idea cannot fully explain the treatment effect.

It is important to note that breakpoints for 0.75 mg/kg/inj cocaine recovered only gradually after d-amphetamine treatment and never fully reached baseline levels. This could have important therapeutic implications. It is not clear what factors control this recovery process although it is unlikely that it is simply the clearance of d-amphetamine which would occur in a few days. We speculate that the long recovery process may have to do with negative associative effects that diminish only slowly after d-amphetamine is cleared.

Food-maintained responding has typically been assessed in many studies investigating the therapeutic potential of d-amphetamine as a pharmacotherapy for cocaine abuse (Barrett et al. 2004; Negus 2003; Negus & Mello 2003a; 2003b). It was included in the present study to evaluate the possibility that d-amphetamine might non-specifically disrupt responding or perhaps produce stereotyped behavior directed toward the lever. We chose to use 45 mg sucrose pellets as a food reinforcer because in previous studies we
have shown that they support similar baseline breakpoints as the moderately high dose of cocaine reported here (Roberts et al. 1996). Even though animals reached similar baseline breakpoints for food and cocaine (0.75 mg/kg/inj) in the present study, d-amphetamine treatment was found to have opposite effects on food- and cocaine-reinforced responding. These data, in combination with the body weight data, add useful information on the effects of extended treatment with d-amphetamine and suggest that the present dose delivered via mini-pump is not debilitating or overtly toxic. However, much has been written about the problems associated with comparing cocaine- and food-reinforced responding as a way to assess selectivity of a potential pharmacotherapy (Barrett et al. 2004). While the present food data is generally in agreement with the observations of Negus and Mello (Negus 2003; Negus and Mello 2003a; 2003b) we are reluctant to draw strong conclusions about specificity. In light of the unanticipated results of Experiment 2b which imply the necessity of the combination of d-amphetamine and cocaine self-administration in reducing the reinforcing effects of cocaine, it appears that the effect of d-amphetamine treatment alone on food-reinforced responding is tangential to the issue.

Further work is necessary to distinguish between the contribution of a learned component versus other pharmacological interactions that might occur with simultaneous cocaine and d-amphetamine administration (see Jayanthi and Ramamoorthy 2005; Scarponi et al. 1999; Ukairo et al. 2007). Whatever the mechanism, cocaine self-administration was shown here to be suppressed for up to 14 days after mini-pump removal. To the degree that decreases in
cocaine-reinforced responding in rats apply to the development of medications for cocaine addiction, it appears that there are interactions between cocaine and $d$-amphetamine that could be exploited.
REFERENCES


CHAPTER III

DECREASED REINFORCING EFFICACY OF COCAINE FOLLOWING TWO WEEKS OF CONTINUOUS d-AMPHETAMINE TREATMENT IN RATS

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The following manuscript was submitted to Psychopharmacology (March 2009).

Funding provided by Grants R01DA14030 and P50DA06634-14.
ABSTRACT

**Rationale:** Recent studies have investigated *d*-amphetamine as a potential agonist medication for cocaine dependence. In rats, a 14-day continuous infusion of *d*-amphetamine via osmotic mini-pump has been shown to decrease cocaine-reinforced responding under a progressive ratio (PR) schedule of reinforcement. **Objectives:** This study was designed to assess the influence of the *d*-amphetamine treatment dose and the self-administered cocaine dose on the magnitude of this effect. **Materials and methods:** Experiment 1: Rats were trained to self-administer 1.5 mg/kg/inj under a PR schedule, then implanted with *d*-amphetamine mini-pumps for 14 days (days 1-7: 5 mg/kg/day; days 8-14: 7.5 mg/kg/day). Breakpoints were evaluated throughout the treatment period and during a 14-day post-treatment period. Experiment 2: Rats were trained to self-administer cocaine under a PR schedule and initial dose-response curves were determined before implantation of *d*-amphetamine mini-pumps. During the 14-day *d*-amphetamine (5 mg/kg/day) treatment period, rats self-administered one of four cocaine doses (0.19, 0.38, 0.75 or 1.5 mg/kg/inj). A post-treatment dose-response curve and responding under a fixed ratio 1 (FR1) schedule were evaluated after mini-pump removal. **Results:** Experiment 1: Breakpoints for 1.5 mg/kg/inj cocaine did not decrease after an increased *d*-amphetamine dose. Experiment 2: The PR dose-response curve was shifted downward after the treatment period in rats that had self-administered 0.19 and 0.38 mg/kg/inj cocaine. In contrast, rats in the 0.75 and 1.5 mg/kg/inj groups demonstrated increased intake rates under an FR1 schedule after the treatment period. **Conclusions:** These data suggest that continuous *d*-amphetamine treatment attenuates the reinforcing efficacy of cocaine.

**Keywords:** Addiction; Agonist therapy; *d*-Amphetamine; Cocaine; Osmotic mini-pump; Progressive ratio
INTRODUCTION

The search for a successful pharmacological treatment for cocaine dependence has been a considerable focus of the National Institute on Drug Abuse for several decades. Two distinct research strategies have been termed “bottom-up” and “top-down” (Vocci and Ling 2005). The bottom-up approach involves developing novel medications based on basic research findings on the neurochemistry and genetics of cocaine addiction. Although several promising compounds (Montoya and Vocci 2008; Rothman et al. 2008a; 2008b) and vaccines (Kosten 2005; Orson et al. 2008) are being developed and evaluated in this fashion, the Food and Drug Administration (FDA) has yet to approve a medication for the treatment of cocaine addiction. For this reason, recent research efforts have adopted a “top-down” approach by investigating the utility of medications that are currently marketed for the treatment of other disorders. d-Amphetamine is a stimulant medication that has been approved by the FDA for the treatment of both attention deficit hyperactivity disorder and narcolepsy. In recent years, its therapeutic potential for treating cocaine dependence has been evaluated in both clinical and preclinical populations.

Clinical studies using d-amphetamine in both immediate release (Shearer et al. 2003) and extended-release (Grabowski et al. 2001; 2004a; Rush et al. 2009) oral formulations have demonstrated beneficial effects in cocaine-dependent individuals, including decreases in subject ratings of cocaine effects and craving, measures of cocaine use, and use-related crime. These results
have been attributed to the similarities between the effects of $d$-amphetamine and those of cocaine and the usefulness of $d$-amphetamine to serve as a replacement drug in cocaine-dependent individuals (Grabowski et al. 2004b; Shearer 2008). This idea of agonist replacement therapy was first proposed for the treatment of opiate dependence (Dole et al. 1966; Kreek 2000), and is currently a primary line of treatment for heroin and prescription opiate dependence (e.g., methadone maintenance and levo-alpha-acetyl-methadol treatment; Fiellin et al. 2006; Kreek and Vocci 2002) as well as tobacco smoking (i.e., nicotine replacement therapy; Fiore 2000; Stead et al. 2008).

In preclinical studies, whether $d$-amphetamine treatment produces an increase or decrease in the behavioral effects of cocaine has been shown to depend upon both the duration of treatment. Acute injections of $d$-amphetamine have been shown to augment the response to cocaine in studies of locomotor activity (Bonate et al. 1997; Ferrario and Robinson 2007; Schenk et al. 1991; Shuster et al. 1977) and conditioned place preference (Lett 1989; Shippenberg and Heidbreder 1995). In cocaine self-administration studies, acute pretreatment with $d$-amphetamine produces a similar leftward shift in the cocaine dose-response curve on measures of acquisition and maintenance of cocaine-reinforced responding (Barrett et al. 2004; Ferrario and Robinson 2007; Horger et al. 1992; Li et al. 2006; Mendrek et al. 1998), as well as reinstatement responding (Lynch et al. 1998; Schenk and Partridge 1999). By contrast, extended treatment with $d$-amphetamine appears to produce a reduction in the reinforcing effects. Treatment with $d$-amphetamine for at
least 7 days either by twice-daily SC injections (Peltier et al. 1996), slow IV infusions three times per hour (Negus 2003; Negus and Mello 2003a; 2003b), or constant infusion from an osmotic mini-pump (Chiodo et al. 2008) has been shown to decrease cocaine self-administration in rats and monkeys with minimal impact on food-reinforced responding.

Our laboratory has previously reported that breakpoints under a progressive ratio (PR) schedule were decreased following continuous d-amphetamine treatment (5 mg/kg/day) via SC osmotic mini-pump (Chiodo et al. 2008). The magnitude of this effect was dependent on the duration of treatment and the unit dose of cocaine. Seven days of continuous d-amphetamine infusion had the greatest effect on the lowest unit injection dose of cocaine (0.19 mg/kg/inj). Breakpoints associated with a dose in the middle of the curve (0.75 mg/kg/inj) were not significantly reduced by 7 days of d-amphetamine treatment; however a significant decrease was seen with 14 days of treatment. This longer duration of treatment did not affect the highest dose tested (1.5 mg/kg/inj).

These data raise an important concern regarding the potential usefulness of d-amphetamine treatment. Ideally a medication should produce a downward shift in the entire dose-response function (Grabowski et al. 2004b), although a rightward shift would also demonstrate a therapeutic treatment effect. To date we have been unable find a dose and duration of d-amphetamine treatment which reduces responding at the peak of the dose-effect curve. Data
from Chiodo et al. 2008 suggest that the slope of the curve had been affected but the position of the peak of the curve remained unchanged. A failure of any treatment to reduce the reinforcing efficacy of cocaine suggests that the putative therapeutic effects would be entirely surmountable.

Two approaches were used in the present study in order to document a possible reduction in cocaine's reinforcing efficacy. Negus and Mello (2003a) demonstrated in monkeys that higher doses of d-amphetamine augmented the degree to which cocaine-reinforced breakpoints were reduced. In addition, clinical studies using agonist therapies for treating both psychostimulant and opiate dependence have suggested that an increase in treatment dose is more effective based on several factors, including a patient’s drug history (Caplehorn et al. 1993; Fleming and Roberts 1994; Trafton et al. 2006). This prompted us to reassess whether a larger dose of d-amphetamine might have a significant effect on self-administration of higher doses of cocaine. In the present studies we escalated the dose of d-amphetamine during the second week of treatment and assessed the effect on self-administration of the most effective dose of cocaine (1.5 mg/kg/inj). We have also examined the dose-response relationship in animals that demonstrate a decrease in responding at lower doses of cocaine, as this was not completely addressed in our previous study. Chiodo et al. (2008) used a between subjects design in which each group of animals was tested on a single dose of cocaine. In the present study, dose response curves under a PR schedule were evaluated in every subject before and after 14 days of continuous d-amphetamine treatment. As the PR schedule does not yield a
complete explanation of drug intake behavior, we also examined changes in the rate of cocaine intake under a fixed ratio 1 (FR1) schedule of reinforcement. Here we report a substantial downward shift in the cocaine dose-effect curve as measured under a PR schedule (demonstrating a reduction in reinforcing efficacy) after continuous d-amphetamine infusion. Additionally, we show an increased rate of cocaine intake under an FR1 schedule in animals that did not show a change in PR breakpoints, thus revealing dissociation between responding under these two schedules of reinforcement.
MATERIALS AND METHODS

ANIMALS

Male Sprague-Dawley rats (Harlan, Indianapolis, Ind., USA) weighing approximately 350 g at the start of the experiments were used as subjects. Rats were maintained on a reverse 12 h light/dark cycle (lights on at 3 PM) with food and water available ad libitum. All rats were habituated to this schedule for a minimum of 3 days before entering the experiment. Throughout the experiments, rats were housed individually in stainless steel custom made experimental chambers (30 x 30 x 30 cm).

SURGERY

Each rat was anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (8 mg/kg) and implanted with a chronically indwelling Silastic® jugular catheter (CamCaths, Cambridgeshire, UK) prior to the beginning of the study. The tubing of the catheter extended from the jugular vein to a subcutaneous plastic anchor which exited through the skin on the dorsal surface in the region of the scapulae (Roberts and Goeders 1989). Tygon® tubing (enclosed by a stainless steel protective tether), was connected to the plastic anchor to attach the catheter to a counterbalanced fluid swivel (Instech Laboratories, Inc., Plymouth Meeting, PA., USA) mounted above the experimental chamber. The swivel was then connected to an infusion pump (Razel Scientific Instruments, Inc., Stamford, CT) outside of the experimental
chamber with Tygon tubing. Self-administration began after rats had 3-5 days to recover from surgery. During the course of the experiments, catheters were flushed daily with heparinized saline in order to maintain patency.

Rats were implanted SC with an osmotic mini-pump (Alzet Model 2001, Durect Corp. Cupertino, CA; see Theeuwes and Yum 1976) filled with d-amphetamine once their self-administration behavior was stable (as described in the experiments below). Briefly, animals were anesthetized with a mixture of oxygen, nitrogen and halothane (4%) and ventilated during surgery using halothane (1.5%). An incision in the skin was made between the scapulae (rostral to the plastic catheter anchor), the mini-pump was inserted with the flow moderator pointing rostrally, and the wound was closed using nylon sutures or surgical glue. The mini-pump was replaced after 7 days using the same procedure so that each rat received 14 continuous days of d-amphetamine treatment. The mini-pumps were filled with d-amphetamine at a concentration which would result in the delivery of either 5 or 7.5 mg/kg over 24 h, as determined by each subject’s body weight at the time of implantation.

**Cocaine Self-Administration**

For all experiments, self-administration occurred 7 days per week. A single active lever extended into the experimental chamber each day at 10 AM (i.e., in the dark phase of the light/dark cycle) to indicate the beginning of a self-administration session. The lever was linked to the infusion pump through a computer so that a 4-5 s (depending on body weight) injection of cocaine was
delivered once an animal pressed the lever and completed the response requirement. Initially, rats were trained to self-administer 1.5 mg/kg/injection under an FR1 schedule of reinforcement. A 20-s time-out period, signalled by retraction of the lever and illumination of a light, occurred after each lever press. After an animal self-administered 40 injections within 6 h, the lever was retracted until the start of the next daily session. Self-administration training was termed complete following 5 consecutive daily sessions during which the animal self-administered all 40 injections while maintaining consistent post-infusion pauses of 5-8 min between each of the injections.

Following training, rats began to self-administer cocaine (0.19-1.5 mg/kg/inj) in daily 6-h sessions under a PR schedule. On this schedule, response requirements were increased through the following ratio progression: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, etc. (Richardson and Roberts 1996). The breakpoint was defined as the number of completed ratios (i.e., the number of reinforcers delivered) before 1 h elapsed without completion of the next ratio. Each session lasted 6 h, but rats typically reached a breakpoint within 3 h and exhibited only minimal non-reinforced responding for the remainder of the session.
EXPERIMENT 1: EFFECT OF INCREASING THE DOSE OF CONTINUOUS D-AMPHETAMINE ON COCAINE SELF-ADMINISTRATION

This experiment investigated the effects of an increasing dose of d-amphetamine (i.e., from 5 to 7.5 mg/kg/day; SC) on self-administration of 1.5 mg/kg/inj cocaine under a PR schedule. After the completion of training under an FR1 schedule, animals (N = 8) self-administered 1.5 mg/kg/inj cocaine until they achieved a stable 3-day baseline period, wherein self-administration behavior did not vary by more than 3 breakpoints. At this point, animals were implanted with mini-pumps that delivered 5 mg/kg/day d-amphetamine. These mini-pumps were removed after 7 days and replaced with new mini-pumps that delivered 7.5 mg/kg/day d-amphetamine for 7 additional days. This dose of d-amphetamine was chosen because previous studies have shown that doses of 10 mg/kg/day and above (delivered via mini-pump) cause depressive-like symptoms, rapid weight loss, and neurotoxicity during and after the treatment period (Cryan et al. 2003; Eison et al. 1983; Martin-Iverson and Lodge 1991; Nielsen 1981; Robinson and Camp 1987; Ryan et al. 1990). Also, a gradual increase in d-amphetamine dose has been shown to protect against the neurotoxic effects associated with the higher dose (Robinson and Camp 1987). Self-administration of 1.5 mg/kg/inj cocaine occurred daily during this 14-day treatment period. After Day 14, mini-pumps were removed and post-treatment breakpoints were assessed for 14 additional days.
Experiment 2: Changes in the Dose-Response Curve for Self-Administered Cocaine After 14 Days of Continuous D-Amphetamine Treatment

The effect of 5 mg/kg/day SC infusion of d-amphetamine via osmotic mini-pump on cocaine-reinforced responding over a range of cocaine doses (0.19-1.5 mg/kg/inj) was investigated. After animals (N = 32) reached the training criterion, they self-administered 0.75 mg/kg/inj cocaine under a PR schedule of reinforcement until they reached 3 consecutive days of stable breakpoints. Next, animals were tested on each of 4 cocaine doses (0.19, 0.38, 0.75 and 1.5 mg/kg/inj) in a Latin square design during 4 consecutive testing days to determine an initial dose-response curve. At this point, animals were implanted with osmotic mini-pumps that delivered a continuous infusion of 5 mg/kg/day d-amphetamine over 14 days (see Surgery). During this 14-day treatment period, animals were divided into 4 groups (with equivalent baseline breakpoints; N = 8), and each group self-administered 1 of the 4 component cocaine doses of the initial dose-response curve (0.19, 0.38, 0.75 or 1.5 mg/kg/inj) in daily PR sessions. Mini-pumps were removed on Day 14 and the cocaine dose-response curve was reassessed in all animals by using the initial Latin square design. Following completion of the final dose-response curve, each animal self-administered 40 injections of 1.5 mg/kg/inj cocaine under an FR1 schedule.
**DRUGS**

Cocaine HCl (National Institute on Drug Abuse, Rockville, MD) was dissolved in sterile 0.9% saline (containing heparin, 10 USP units/ml) in concentrations of 0.625, 1.25, 2.5 and 5 mg/ml (expressed as the salt) and passed through a microfilter. *d*-Amphetamine sulfate (Sigma-Aldrich, St. Louis, MO) was dissolved in sterile 0.9% saline.

**DATA ANALYSIS**

Breakpoint was used as the main dependent measure for self-administration on the PR schedule. In Experiment 1, breakpoints from the final baseline testing day and the 14 days during *d*-amphetamine treatment were analyzed using a one-way ANOVA with repeated measures (SYSTAT Software Inc.). In Experiment 2, the initial PR dose-response curves before mini-pump implantation were compared between all 4 groups using a two-way ANOVA with GROUP (i.e., based on which of the 4 cocaine doses was self-administered during the *d*-amphetamine treatment period) and DOSE (i.e., the 4 cocaine doses in the dose-response curve; 0.19, 0.38, 0.75 and 1.5 mg/kg/inj) as factors. Breakpoints during the *d*-amphetamine treatment period for all 4 groups were compared using a two-way ANOVA with repeated measures with GROUP and DAY as factors. Total cocaine intake during the *d*-amphetamine treatment period was compared between groups using a one-way ANOVA. Finally, a two-way repeated measures ANOVA comparing the post-treatment dose-response curve to the initial dose-response curve was done for each group.
individually, using a two-way ANOVA with repeated measures with DOSE (i.e., the 4 cocaine doses in the dose-response curve; 0.19, 0.38, 0.75 and 1.5 mg/kg/inj) and TREATMENT (i.e., before d-amphetamine vs. after d-amphetamine treatment) as factors. Changes in rate of intake on the FR1 schedule were analyzed for each group of animals in Experiment 2. A two-way ANOVA with repeated measures was used to compare the mean inter-injection interval (in seconds) for Day 5 of training on the FR1 schedule to that of the FR1 session conducted after completion of the post-treatment dose-response curve. Group data was transformed into “percentage of baseline” values for clarity in Figure 4. Values of p<0.05 were considered statistically significant and Holm-Sidak tests were used in post hoc analyses.
RESULTS

EXPERIMENT 1

Figure 1 shows the effect of a constant infusion of d-amphetamine via an osmotic mini-pump for 14 days (7 days of 5 mg/kg/day and 7 days of 7.5 mg/kg/day) on breakpoints maintained by 1.5 mg/kg/inj cocaine. Comparison of the final baseline day and all 14 treatment days with a one-way ANOVA with repeated measures did not reveal a significant difference \[F(14, 98)=0.92, \text{ns}\] throughout the d-amphetamine treatment period.

EXPERIMENT 2

Figure 2a illustrates the initial breakpoint dose-response curve for all animals (N = 32) prior to d-amphetamine treatment. Two-way ANOVA revealed a significant effect of cocaine DOSE \[F(3, 127)=17.16, p<0.001\]. Animals were randomly assigned to 1 of 4 groups to be tested on 0.19, 0.38, 0.75 or 1.5 mg/kg/inj cocaine. Figure 2b illustrates the effect of 14 days of continuous d-amphetamine treatment on breakpoints associated with these 4 groups. Repeated measures ANOVA revealed a significant effect of GROUP \[F(3, 479)=10.83, p<0.001\], DAY\[F(13, 479)=10.28, p<0.001\], and a significant GROUP x DAY interaction \[F(42, 479)=12.89, p<0.001\].

The post-treatment cocaine dose-response curves for all 4 groups are shown in Figure 2c. ANOVA revealed a significant effect of GROUP \[F(3, 127)=18.17, p<0.001\] and DOSE \[F(3, 127)=6.84, p<0.001\]. Repeated measures
**FIGURE 1**

Effect of increasing the dose of continuous *d*-amphetamine on self-administration of 1.5 mg/kg/inj cocaine under a PR schedule. Points represent the mean (±SEM) breakpoints. Shaded portions represent the *d*-amphetamine treatment period (7 days of 5 mg/kg/day and 7 days of 7.5 mg/kg/day). The final ratio values corresponding to breakpoints are represented on the right y axis.
**Figure 1**

1.5 mg/kg/inj Cocaine

Breakpoint / # Reinforcers

Days

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</table>

- d-Amphetamine
- mini-pump treatment
- 5 mg/kg/day
- 7.5 mg/kg/day
FIGURE 2

Changes in the dose-response curve for self-administered cocaine after 14 days of continuous d-amphetamine (5 mg/kg/day) treatment. All points represent the mean (±SEM) breakpoints under a PR schedule. A) Initial dose-response curve established in all animals after training. B) 14-day d-amphetamine treatment period during which animals were divided into four groups to self-administer one of four cocaine doses (0.19, 0.38, 0.75, or 1.5 mg/kg/inj). C) Final dose-response curves for each of the four groups after removal of d-amphetamine mini-pumps. The final ratio values corresponding to breakpoints are represented on the right y axis.
Figure 2A

Initial dose-response curve

---

- **Figure 2A:**

  Initial dose-response curve

  - **Breakpoint / # Reinforcers vs. Cocaine Dose (mg/kg/inj):**
    - The graph shows a positive correlation between the cocaine dose and the breakpoint or number of reinforcers. As the cocaine dose increases, the breakpoint also increases.
  - **Final Ratio:**
    - The y-axis represents the final ratio, which seems to be on a logarithmic scale, as indicated by the range from 4 to 402.

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130
**Figure 2b**

**d-Amphetamine treatment period**

**Cocaine dose self-administered (mg/kg/inj):**

- ▲ 0.19
- ○ 0.38
- ■ 0.75
- ● 1.5

Breakpoint / # Reinforcers

Days

Final Ratio

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

Post-treatment dose-response curve

Cocaine dose during treatment period (mg/kg/inj):
- ▼ 0.19
- ◇ 0.38
- ■ 0.75
- ● 1.5

Breakpoint / # Reinforcers

Final Ratio

Cocaine Dose (mg/kg/inj)
ANOVA comparing initial and post-treatment dose-response curves within each group revealed a significant difference for the 0.19 mg/kg/inj group \([F(1, 63)=37.96, p<0.001]\) and post hoc Holm-Sidak analysis confirmed significant differences for all doses in the dose-response curve \([0.19 \text{ dose}: t=4.15, df=7, p<0.001; 0.38 \text{ dose}: t=5.61, df=7, p<0.001; 0.75 \text{ dose}: t=4.26, df=7, p<0.001; 1.5 \text{ dose}: t=4.71, df=7, p<0.001]\). For the 0.38 mg/kg/inj group, there was a significant effect of TREATMENT \([F(1, 63)=5.92, p<0.05]\), and this is accounted for by significant differences when tested on the 0.38 dose \([t=2.29, df=7, p<0.05]\) and the 0.75 dose \([t=2.20, df=7, p<0.05]\). There was not an effect of TREATMENT for the 0.75 mg/kg/inj group \([F(1, 63)=3.80, \text{ ns}]\) or the 1.5 mg/kg/inj group \([F(1, 63)=0.54, \text{ ns}]\).

The total amount of cocaine self-administered during the 14-day \(d\)-amphetamine treatment period is illustrated in Figure 3. One-way ANOVA revealed a significant difference between groups \([F(3, 31)=76.85, p<0.001]\). Holm-Sidak post hoc analysis revealed significant difference for all comparisons except between the 0.19 mg/kg/inj and 0.39 mg/kg/inj groups \([t=1.36, df=3, \text{ ns}]\).

The change in FR1 intake rate is expressed as the percent change from baseline in Figure 4. One animal from each group was excluded from the analyses due to catheter failure prior to the post-treatment FR1 testing session. There was no difference in intake rates for the final day of FR1 training between the 4 groups \([F(3, 27)=0.68, \text{ ns}]\). Two-way ANOVA with
FIGURE 3

Total cocaine intake (mg/kg) during the 14-day \textit{d}-amphetamine treatment period for groups of animals that self-administered various doses of cocaine. * significant difference from the 0.19 mg/kg/inj group (p<0.01). # significant difference from the 0.38 mg/kg/inj group (p<0.01). † significant difference from the 0.75 mg/kg/inj group (p<0.01).
Cocaine intake during 14-day treatment period
**FIGURE 4**

Changes in the rate of cocaine intake under an FR1 schedule after 14 days of continuous d-amphetamine treatment. Bars represent percent change from baseline taken prior to the initial dose-response curve. * significant difference from baseline (p<0.01).
**Figure 4**

**Cocaine intake rate on FR1 schedule**

[Bar graph showing cocaine intake rate (% of baseline) for different groups: 0.19, 0.38, 0.75, and 1.5. The graph indicates significant differences with asterisks (*) for groups 0.75 and 1.5.]
repeated measures revealed an effect of TIME \[F(2, 55)=16.75, \ p<0.001\] with significant differences for the 0.75 group \[t=2.89, \ df=6, \ p<0.01\] and the 1.5 mg/kg/inj group \[t=3.01, \ df=6, \ p<0.01\].

Figure 5 shows the cumulative records from a representative animal (from the 1.5 mg/kg/inj group) while self-administering 1.5 mg/kg/inj under a PR schedule before (i.e., PRE) and after (i.e., POST) the 14-day \(d\)-amphetamine treatment period. The breakpoint remained the same in both sessions, whereas increased rate of drug intake can be observed.
FIGURE 5

Cumulative records from an animal in the 1.5 mg/kg/inj group illustrating the increased rate of cocaine intake under a PR schedule after 14 days of continuous d-amphetamine treatment. Each drug infusion (i.e., breakpoint) is represented by a diagonal inflection. PRE: 1.5 mg/kg/inj test session from the initial dose-response curve. POST: 1.5 mg/kg/inj test session from the post-treatment dose-response curve.
Figure 5

1.5 mg/kg/inj Cocaine

POST

PRE

Responses

Time (hr)
In the present study we investigated the ability of continuous \(d\)-amphetamine treatment, delivered via osmotic mini-pump, to decrease cocaine-reinforced responding and the extent to which cocaine dose, the level of cocaine exposure and \(d\)-amphetamine treatment dose influenced the magnitude of this effect. The major finding of the present study is that continuous \(d\)-amphetamine treatment can produce a substantial downward shift in the cocaine dose-effect curve as measured under a PR schedule of reinforcement.

Our initial aim of this study was to decrease breakpoints for a dose of cocaine at the peak of the curve by increasing the treatment dose of \(d\)-amphetamine. We had previously found that 14 days of 5 mg/kg/day \(d\)-amphetamine was not strong enough to affect the reinforcing efficacy of 1.5 mg/kg/inj cocaine in our previous study (Chiodo et al. 2008). Therefore in Experiment 1 of the present study, animals that self-administered 1.5 mg/kg/inj cocaine received a higher \(d\)-amphetamine dose during the second half of the 14-day treatment period. The treatment dose was increased from 5 mg/kg/day to 7.5 mg/kg/day only after the first week in order to avoid the toxic effects (i.e., stereotypy, excessive weight loss, depressive-like behaviors and neurochemical deficits) seen with extended mini-pump delivery of higher \(d\)-amphetamine doses (Cryan et al. 2003; Eison et al. 1983; Martin-Iverson and Lodge 1991; Nielsen 1981; Ryan et al. 1990). We found that breakpoints for the
high dose of cocaine (1.5 mg/kg/inj) remained at the baseline levels during a week-long period of d-amphetamine infusion with 5 mg/kg/day (as expected) and remained at baseline levels throughout the week of 7.5 mg/kg/day d-amphetamine as well.

Using a second strategy to demonstrate a downward shift in the curve, we focused on our previous positive results in which 14 days of d-amphetamine treatment was shown to reduce breakpoints at low unit doses of cocaine. The present results replicated the principle findings of Chiodo et al. (2008), by showing that the decrease in breakpoints during the 14-day d-amphetamine treatment depends on the unit injection dose of cocaine. Again, breakpoints associated with lower unit doses of cocaine were reduced during the treatment period, whereas the higher doses were not. In our previous study a between subjects design was used in which each group of animals was tested on a different dose of cocaine. In the present study, dose-response curves under a PR schedule were evaluated within all groups before and after 14 days of continuous d-amphetamine treatment. This design clearly shows that the effect of d-amphetamine is not restricted to a specific unit dose but instead translates into a shift of the entire dose-response curve. A downward shift in the cocaine dose-response curve was seen after animals self-administered low to moderate doses of cocaine (0.19-0.75 mg/kg/inj) under a PR schedule during a 14-day d-amphetamine (5 mg/kg/day) treatment period, whereas the dose-response curve was unchanged for animals that self-administered a high dose of cocaine (1.5 mg/kg/inj) during the treatment period.
Extended d-amphetamine treatment has been shown to decrease responding under a PR schedule (in nonhuman primates and rats) using several paradigms (Chiodo et al. 2008; Negus and Mello 2003a; Peltier et al. 1996). Peltier et al. (1996) showed that 7 days of twice-daily IP d-amphetamine injections decreased breakpoints for cocaine in rats. Negus and Mello found similar decreases in the reinforcing efficacy of cocaine in monkeys when d-amphetamine was delivered every 20 minutes through an IV cannula (Negus and Mello 2003a) and this effect also applied to responding under a second-order schedule (Negus and Mello 2003b) and in a food-drug choice procedure (Negus 2003). Our present data and that from our previous study replicate this general finding, and also highlight differences that occur between these various methods of extended d-amphetamine treatment. It has been shown that the time between d-amphetamine injections can be very influential on subsequent behavioral responses to psychostimulants (Ellison and Morris 1981; Nelson and Ellison 1978). Moreover, when d-amphetamine is delivered via osmotic mini-pumps, animals do not exhibit sensitized behavioral responses that are typically seen after repeated intermittent injections (e.g., acoustic startle; Kokkinidis 1984). This could account for the differences in post-treatment responding seen between the studies. For example, breakpoints returned to baseline very quickly after the d-amphetamine treatment had ended in the studies by Peltier et al. (1996) and Negus and Mello (2003a). However, we have shown that breakpoints not only decrease after continuous d-amphetamine treatment delivered via an osmotic mini-pump but also remain below baseline
for up to two weeks after the $d$-amphetamine mini-pumps were removed (Chiodo et al. 2008).

Tolerance has conventionally been used to explain decreases in cocaine self-administration over time and extended $d$-amphetamine treatment has been thought to create cross-tolerance to the reinforcing effects of cocaine (Peltier et al. 1996). If tolerance were to account for our data, one would expect to see the greatest decrease in breakpoints in conjunction with a higher dose of the daily $d$-amphetamine infusion, a higher self-administered cocaine dose, or a greater amount of total cocaine exposure. However, the findings of the present study as well as those found previously (Chiodo et al. 2008) diminish the role of tolerance in decreasing cocaine self-administration under a PR schedule. First of all, breakpoints for 1.5 mg/kg/inj cocaine remained at baseline level throughout the $d$-amphetamine treatment period regardless of the $d$-amphetamine dose. Next, the greatest decrease in breakpoints after either 7 or 14 days of continuous $d$-amphetamine treatment was seen for the lowest dose of cocaine (0.19 mg/kg/inj), while breakpoints for the highest dose (1.5 mg/kg/inj) remained at baseline throughout and well beyond the treatment period. Finally, the results from the present study suggest that although the reinforcing efficacy of cocaine can be decreased after continuous $d$-amphetamine treatment, the magnitude of this effect cannot be entirely predicted by the total amount of self-administered cocaine during the $d$-amphetamine treatment period. As shown in Figures 2b and 3, animals that self-administered 1.5 mg/kg/inj during the $d$-amphetamine treatment period
not only received the greatest concentration of cocaine per each IV infusion, they also self-administered considerably more injections of cocaine over 14 days than animals in the other groups. Despite earlier studies linking high cocaine exposure to a decrease in the reinforcing efficacy of the drug (Hammer, Jr. et al. 1997), the present findings from the PR dose-response curves suggest the opposite when continuous d-amphetamine treatment is incorporated. Although the PR dose-response curve was unchanged in the 1.5 mg/kg/inj group, a nearly 8-fold downward shift can be seen in the 0.19 mg/kg/inj group after d-amphetamine treatment. This is somewhat unexpected considering that the average amount of cocaine taken by these animals over 14 days was almost 15 times lower than that of the 1.5 mg/kg/inj group. Additionally, the diminished breakpoints in the 0.19 mg/kg/inj group are not merely a demonstration of extinction responding over time, as we have previously shown that breakpoints for this dose of cocaine progressively increase for at least seven days after d-amphetamine treatment has been terminated (Chiodo et al. 2008).

It should be emphasized that high drug intake was associated with an increased rate of drug intake under an FR1 schedule; however a change in FR1 responding did not predict a change in responding on the PR schedule. In Experiment 2, the mean inter-injection interval under an FR1 schedule was substantially decreased (i.e., the rate of intake was increased) after the d-amphetamine treatment period whereas none of the doses on the breakpoint dose-response curve was changed in these animals (see Figures 2c and 4).
Conversely, animals in the 0.19 mg/kg/inj group demonstrated the largest downward shift in the PR dose-response curve but no change in the rate of intake under an FR1 schedule. This dissociation between breakpoints under a PR schedule and rate of intake under an FR schedule is not unique to the present study (see Brebner et al. 2000; Richardson and Roberts 1991; Roberts et al. 1989; 1996) thus indicating that the two measures reveal distinct information about the factors that control cocaine self-administration. The increased cocaine intake rate might be interpreted as an indicator of behavioral tolerance (Emmett-Oglesby and Lane 1992), but this tolerance is limited to a specific aspect of reinforcement because no evidence of tolerance was observed with breakpoints. In behavioral economic terms, breakpoints can be considered as a measure of “price” and FR rate as a measure of “consumption” (see Hursh 1991; 2005 for review). In a recent study, price and consumption were found to be weak predictors of one another (Oleson and Roberts 2009). The increased rate of consumption seen in animals that had self-administered the greatest amount of cocaine in the present study is in agreement with the escalation of cocaine intake over time demonstrated by Ahmed and Koob (1998; 1999).

Overall, our data suggest that continuous treatment with a low dose of d-amphetamine treatment could be of some benefit to human cocaine users. In clinical studies of d-amphetamine treatment, improvements in subjective ratings of cocaine use and craving as well as cocaine-related crime (Grabowski et al. 2001; 2004a; Shearer et al. 2003) can be viewed as measures of harm
reduction for both the cocaine user and society in general (Des Jarlais 1995). In preclinical self-administration studies, changes in the pattern of drug intake reveal similar information about the value of a pharmacological treatment. The present study demonstrated changes in intake pattern on two different schedules of reinforcement as well as a substantial downward shift in the cocaine dose-response curve.

With regard to the clinical application of these findings, several factors remain to be determined. First, we found that the magnitude to which \(d\)-amphetamine infusion affects cocaine-reinforced responding is dependent on the self-administered cocaine dose. Since the greatest decrease of breakpoints occurred in animals that had the least amount of cocaine exposure (i.e., the 0.19 mg/kg/inj group), this might suggest that any therapeutic effect of \(d\)-amphetamine would be limited to humans who use cocaine casually instead of those who are the most dependent on cocaine. This would appear to translate well to the clinic situation wherein a patient’s drug history plays a substantial role in the therapeutic effect of any treatment medication (McLellan and Alterman 1991). However, the results from this study are not a complete evaluation of the effectiveness of a slow continuous infusion of \(d\)-amphetamine and therefore cocaine self-administration under other schedules of reinforcement is needed in order to establish the parameters of this treatment method. In addition, we previously found that continuous \(d\)-amphetamine treatment had no effect in animals that did not have access to cocaine during the treatment period (Chiodo et al. 2008), suggesting that the therapeutic
effect requires a combination of $d$-amphetamine and cocaine. Further study is necessary to determine whether the cocaine must be self-administered to see this effect (indicating a psychological or associative explanation) or if a noncontingent delivery of cocaine in combination with $d$-amphetamine will also decrease subsequent cocaine self-administration (indicating a pharmacological explanation).


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Rothman RB, Baumann MH, Prisinzano TE, Newman AH (2008a) Dopamine transport inhibitors based on GBR12909 and benztropine as potential medications to treat cocaine addiction. Biochem Pharmacol 75: 2-16


CHAPTER IV

DISCUSSION
1. **INTRODUCTION**

As of 2009, the FDA has not approved a medication for the specific purpose of treating cocaine dependence. Considering the economic and social burdens on our society due to the growing number of cocaine-dependent individuals in America (see Substance Abuse and Mental Health Services Administration 2008), NIDA established its Medications Development Division and launched a powerful initiative for medication development in 1990. In the years since, many important advancements have been made in the fields of neuroscience, pharmacology, and psychiatry which have equipped researchers with a strong knowledge base concerning the neurobiology, genetic susceptibility, and behavioral manifestation of cocaine addiction. Researchers have adopted a “bottom-up” approach in which the discoveries guide the development of novel medications (Montoya and Vocci 2008), vaccines (Orson et al. 2008), and behavioral therapies (Vocci and Montoya 2009) for cocaine dependence. Although several treatments have shown promise in preclinical studies, the substantial investment of both time and money that is required to bring an investigational drug to market (DiMasi et al. 2003) has inspired another research strategy—the “top-down” approach. With this approach, medications that have been approved for other conditions are tested clinically in cocaine-dependent individuals (Vocci and Ling 2005).

Amphetamine is a synthetic psychostimulant medication that was originally approved by the FDA in the 1930s as an appetite suppressant and
wakefulness-promoting drug. The abuse potential of amphetamine was realized in the 1950s, thereby compelling the government to label it as a controlled substance (Miller 1997). Currently, amphetamine can be prescribed as a treatment for ADHD or narcolepsy and is available in both immediate- and sustained-release forms as either mixed amphetamine salts (e.g., Adderall) or as the active isomer, \(d\)-amphetamine (e.g., Dexedrine). Amphetamine was first proposed as a potential treatment for cocaine dependence based on its early success as an appetite suppressant. Researchers believed that amphetamine would also be effective at decreasing cocaine intake because both food- and drug-seeking are "homeostatic deficit-driven processes" (Rothman et al. 2002). Recently, \(d\)-amphetamine has been shown to be safe and tolerable in cocaine users (Rush et al. 2009) and results from the three clinical treatment studies suggest that \(d\)-amphetamine may be a helpful agonist pharmacotherapy for cocaine-dependent individuals (Grabowski et al. 2001; 2004; Shearer et al. 2003).

Although \(d\)-amphetamine has been tested in over 250 cocaine-dependent human subjects, there are inherent limits to clinical studies that can be surmounted in preclinical models. First, most clinical treatment studies are only 8-12 weeks in duration and a treatment effect may take longer to appear. Additionally, the cumulative effects of an extended pharmacological intervention cannot be assessed in that time period. Finally, protocols for clinical studies do not allow for individualized treatment based on factors such as history of cocaine use, preferred route of administration, symptoms
associated with their level of dependence and initial response to the treatment medication (McKay 2005). Therefore preclinical studies are necessary to get a better understanding of the utility of agonist therapy medications for the treatment of cocaine dependence.

The general theme from the preclinical literature suggests that the route of \textit{d}-amphetamine administration and the treatment schedule are crucial to determining the subsequent effect on behavior reinforced by cocaine. Pilot studies from our lab found that acute IP pretreatment with \textit{d}-amphetamine increased the reinforcing efficacy of cocaine as measured under a PR schedule (unpublished data). This was in agreement with several other preclinical studies in which cocaine self-administration was increased after acute \textit{d}-amphetamine treatment in rodents and monkeys (Ferrario and Robinson 2007; Horger et al. 1992; Lorrain et al. 2000; Valadez and Schenk 1994). By contrast, when \textit{d}-amphetamine was delivered repeatedly (Peltier et al. 1996) or infused continuously (Negus 2003; Negus and Mello 2003a; 2003b) over several days or weeks, cocaine self-administration was attenuated.

2. \textbf{Major Findings of This Research}

The experiments presented in Chapters II and III were designed to extend the preclinical assessment of extended \textit{d}-amphetamine treatment for cocaine dependence. We used a drug delivery method (i.e., SC osmotic mini-pump) that was novel to the literature in this field as a model of continuous
delivery of \textit{d}-amphetamine through an extended release oral formulation (Theeuwes et al. 1991; Theeuwes and Yum 1976). In Chapters II and III we demonstrated that continuous slow infusion of \textit{d}-amphetamine selectively decreased breakpoints reinforced by cocaine. The collective results from our experiments not only confirmed previous work by Peltier and colleagues (1996) and Negus and Mello (2003a), but they also extended the literature by offering new interpretations of the value of \textit{d}-amphetamine as an agonist therapy medication. Throughout the studies presented in Chapters II and III, three prevailing factors were found to influence the extent to which \textit{d}-amphetamine is able to decrease the reinforcing efficacy of cocaine:

1. Duration of \textit{d}-amphetamine treatment
2. Unit dose of self-administered cocaine
3. Occurrence of cocaine self-administration throughout the \textit{d}-amphetamine treatment period

\textbf{2.1. Duration of \textit{d}-Amphetamine Treatment}

In Chapter II, animals were treated with \textit{d}-amphetamine (5 mg/kg/day) for either seven or 14 days. A significant decrease in breakpoints only occurred for the lowest dose of cocaine (0.19 mg/kg/inj) after seven days of \textit{d}-amphetamine treatment. However, breakpoints for 0.38 mg/kg/inj (demonstrated in Chapter III) and 0.75 mg/kg/inj cocaine were decreased when an additional week was added to the treatment period. This effect was shown
to be selective, as the decreased breakpoints for cocaine were not mirrored by a decrease in food-reinforced responding under a PR schedule (Ch II; Fig 3).

The duration of d-amphetamine treatment was shown to affect post-treatment responding as well. Breakpoints for 0.75 mg/kg/inj cocaine showed a non-significant trend downward at the end of the seven-day treatment and returned to baseline within two days after the mini-pump was removed (Ch II; Fig 1b). After 14 days of d-amphetamine treatment, however, breakpoints for the same dose of cocaine still had not returned to baseline after 14 post-treatment test days (Ch II; Fig 2b). This is a novel finding, as the only other studies that investigated cocaine self-administration under a PR schedule after extended d-amphetamine treatment found that post-treatment breakpoints recovered almost immediately after the treatment period had ended (Negus and Mello 2003a; Peltier et al. 1996).

Clinical studies of both pharmacological and behavioral treatments for cocaine dependence have shown a correlation between treatment duration and positive treatment outcome (McKay 2005). Our data correspond well to the clinical situation in this regard because breakpoints for a larger range of cocaine doses were decreased when the d-amphetamine treatment period was extended from seven to 14 days. This suggests that a longer treatment regimen with d-amphetamine may especially benefit patients who have a greater history of cocaine use.
Any pharmacotherapy that leads to a reduction in cocaine use can be viewed as a harm reduction measure for both the cocaine-dependent patient and society in general (Fleming and Roberts 1994). However, clinicians view a truly successful treatment medication as one that continues to decrease drug use and drug craving long after treatment cessation (McLellan and Alterman 1991). Our data suggest that a longer treatment period may improve the effectiveness of an agonist pharmacotherapy for cocaine dependence. This concept has already been shown with methadone maintenance, as the greatest reduction in heroin use occurred for patients in longer treatment programs (Amato et al. 2002).

2.2. **Unit Dose of Cocaine**

A consistent theme throughout our studies was that the most robust effects of $d$-amphetamine treatment on PR breakpoints occurred with cocaine doses at the low end of the dose-response curve. In Chapter II, breakpoints were decreased for cocaine doses of 0.75 mg/kg/inj and below during the $d$-amphetamine treatment period, but breakpoints for 1.5 mg/kg/inj cocaine were unchanged by either seven or 14 days of 5 mg/kg/day $d$-amphetamine (Ch II; Figs 1, 2a). Since 1.5 mg/kg/inj cocaine is at the peak of the PR dose-response curve and is preferred over lower doses (Ward et al. 2005), we concluded that the dose of $d$-amphetamine (5 mg/kg/day) used in Chapter II might have been too low to overcome the reinforcing effects of this high dose of cocaine. In Chapter III, we tested this idea by increasing the $d$-amphetamine
dose to 7.5 mg/kg/day for the second week of treatment. We hypothesized that breakpoints would decrease during the second week, but we found that breakpoints for 1.5 mg/kg/inj cocaine remained at baseline level throughout the treatment period regardless of the $d$-amphetamine treatment dose (Ch III; Fig 1). This may indicate that one week was not long enough for the 7.5 mg/kg/day $d$-amphetamine dose to reduce the reinforcing effects of cocaine and a longer treatment period should be investigated with self-administration of 1.5 mg/kg/inj cocaine (see Section 3.1 below).

Although the data from Chapter II suggested that the dose-response curve was changed by $d$-amphetamine treatment, the results from these experiments were not a complete explanation of the entire phenomenon. This is because each point on the dose-response curve was generated from a unique group of animals. Each group had different self-administration histories in terms of the unit dose of cocaine to which they were given access throughout the experiment and subsequently, the total amount of cocaine exposure throughout that treatment period. Therefore, we were unable to confidently predict how animals would respond for a different dose of cocaine after the treatment period. Figure 1 of this chapter illustrates this point. We knew from the results of Chapter II that after 14 days of $d$-amphetamine treatment, breakpoints for 1.5 mg/kg/inj cocaine remained at the baseline level while those for 0.75 mg/kg/inj and below were decreased. Upon first glance, these data would appear to be part of the same curve (solid black line in Figure 1). This would indicate that all animals would show a significant decrease in
**FIGURE 1**

Hypothetical changes in the breakpoint dose-response curve for cocaine after 14 days of continuous *d*-amphetamine infusion. Gray line represents the initial dose-response curve (PRE). Solid black line represents a dose-dependent decrease. Dotted line represents a downward shift of the entire dose-response curve.
Figure 1
breakpoints for 0.19-0.75 mg/kg/inj but no change for 1.5 mg/kg/inj cocaine—regardless of the dose they self-administered during the 14-day treatment period. However, it is also possible that animals that had access to 0.19-0.75 mg/kg/inj cocaine during the treatment period would respond significantly lower for all doses of cocaine when tested after the treatment period (dotted line in Figure 1). This would indicate that each group has its own distinct post-treatment curve, with the greatest downward shift for the 0.19 mg/kg/inj group and no change in the 1.5 mg/kg/inj group.

The second experiment in Chapter III was designed to clarify the dose-dependent effects seen in Chapter II. Full dose-response curves were evaluated in four separate groups of animals before and after the d-amphetamine treatment period. We found that the dose-response curve for animals that had access to 1.5 mg/kg/inj during the treatment period was unchanged by d-amphetamine, whereas a downward shift in the entire curve was observed for animals that had access to 0.19 or 0.38 mg/kg/inj cocaine (Ch III; Fig 2c). This indicates that the attenuation of the reinforcing effects of cocaine directly corresponded to the amount of cocaine exposure during the d-amphetamine treatment period. Interestingly, self-administration under an FR1 schedule did not conform to this general theme. Animals in the 1.5 mg/kg/inj group showed an increased rate of intake, but animals in the 0.19 mg/kg/inj group showed no change (Ch III; Fig 4). This dissociation between PR and FR schedules has been shown previously in our laboratory (Brebner et al. 2000; Richardson and Roberts...
1991; Roberts et al. 1989; 1996), and our data highlight the subtle differences between what we can learn from each schedule of reinforcement.

These data bring up an important issue regarding the pharmacological treatment of cocaine dependence. The therapeutic effect of a medication is somewhat dependent on a patient’s drug history (e.g., years of regular cocaine use, preferred method of administration, and time between binges; McLellan and Alterman 1991). In self-administration studies, a subject’s history has a strong influence on future responding (Barrett 1977). In Chapter III, animals in the 1.5 mg/kg/inj group reached an average breakpoint of 17 for each session during the d-amphetamine treatment period, whereas animals in the 0.19 mg/kg/inj group only reached breakpoints of around nine. These breakpoints correspond to 713 and 94 total responses within each daily session, respectively. Regardless of the unit dose of cocaine, this large separation between the levels of responding in the two groups may play a role in post-treatment self-administration behavior. That is, after an animal presses the lever over 700 times every day for two weeks, his behavioral repertoire may be confined to this range of responses for an indefinite period of time. This idea may partially explain why animals in the 1.5 mg/kg/inj group still responded at a relatively high level for the lowest dose of cocaine (0.19 mg/kg/inj) after the d-amphetamine treatment period. Additionally, this is supported by the increased rate of intake on the FR1 schedule seen in this group of animals.
2.3. **Occurrence of Cocaine Self-Administration Throughout the D-Amphetamine Treatment Period**

The most noteworthy finding from our data is that an attenuation of the reinforcing efficacy of cocaine only occurs if animals have access to cocaine during the d-amphetamine treatment period. In Chapter II, the dramatic reduction of breakpoints for 0.75 mg/kg/inj after 14 days of d-amphetamine treatment seemed to indicate that d-amphetamine created cross-tolerance to the reinforcing effects of cocaine. This idea had previously proposed by Peltier et al. (1996), after they saw decreased breakpoints following an extended d-amphetamine treatment period without cocaine self-administration. Additionally, unpublished data from our laboratory showed that continuous treatment with d-amphetamine attenuated the locomotor-stimulating effects of cocaine, thereby indicating that d-amphetamine produced behavioral tolerance (see Appendix II). In an attempt to demonstrate this in our self-administration model, animals were trained with 0.75 mg/kg/inj under a PR schedule, but were not allowed to self-administer cocaine during the entire d-amphetamine treatment period. We expected to see a decrease in breakpoints for all animals that received d-amphetamine mini-pumps. Instead, breakpoints for these animals were not significantly different from their own baseline or saline controls (Ch II; Fig 2b).

In addition to this, the results from animals that self-administered 1.5 mg/kg/inj during the d-amphetamine treatment period do not suggest
tolerance as the main explanation for this phenomenon. As mentioned in Chapter II, if $d$-amphetamine and cocaine exposure were combined to create a general tolerance to psychostimulant effects, we would expect to see the greatest decrease in breakpoints for animals that self-administered 1.5 mg/kg/inj while receiving two weeks of $d$-amphetamine treatment. These animals generally had 15 times more cocaine intake than animals that had access to 0.19 mg/kg/inj cocaine. Even after the $d$-amphetamine treatment dose was increased to 7.5 mg/kg/day, these animals did not reduce their breakpoints.

If our model were translated to the clinical situation in a literal sense, it would predict better treatment outcomes for cocaine-dependent patients who are treated with $d$-amphetamine in an out-patient setting. This is because the patient would have to self-administer cocaine (preferably in the environment that has been conditioned to cocaine) in order to learn that cocaine has lost some of its reinforcing effects. It is highly unlikely that clinicians will supply cocaine to their patients or advise them to take daily visits to their individual drug-associated environments so that they may experience the combination of $d$-amphetamine and cocaine. However, there may be safer options to try in the clinic. For example, a combination of $d$-amphetamine with a cocaine-like monoamine reuptake inhibitor (e.g., bupropion or methylphenidate) may reduce cocaine use and craving. At this point in time, our data cannot speak to the potential success of this sort of treatment because it is still unclear whether breakpoints are reduced due to a learning phenomenon or an
interaction between cocaine and d-amphetamine in the synapse (I will address this in Section 3.2).

3. Questions to Address in Future Studies

3.1. Will Breakpoints for the Highest Cocaine Dose Decrease After a Longer Treatment Period with a High Dose of d-Amphetamine?

In Chapter III, we increased the d-amphetamine treatment dose from 5 to 7.5 mg/kg/day during the second week of treatment, but this did not affect breakpoints for 1.5 mg/kg/inj cocaine. We began the treatment with 5 mg/kg/day for the first week of treatment based on previous studies that showed toxic effects (i.e., stereotypy, weight loss, and neurochemical dysfunction) when higher doses of d-amphetamine (above 7.5 mg/kg/day) were continuously delivered to rats via mini-pumps (Eison et al. 1983; Martin-Iverson and Lodge 1991; Nielsen 1981; Ryan et al. 1990). Also, the treatment regimen used in our experiment corresponds to the clinical situation wherein the lowest effective dose of a medication is given to a patient and then gradually increase until the desired therapeutic effect is achieved. In methadone maintenance programs, the treatment dose is commonly elevated for users who don’t respond to the starting dose (Caplehorn et al. 1993) and behavioral therapy programs follow a comparable “stepped care” plan (McKay 2005). Similarly, a gradual increase in d-amphetamine dose has been associated with low
incidences of adverse symptoms and an improvement in treatment outcome in preclinical and clinical studies (Grabowski et al. 2001; 2004; Robinson and Camp 1987).

As suggested in Section 2.2, a 14-day d-amphetamine treatment period might not be long enough to see a change in breakpoints for 1.5 mg/kg/inj cocaine. Just as animals that self-administered 0.75 mg/kg/inj cocaine required an extra week of d-amphetamine treatment to decrease breakpoints, animals that self-administer 1.5 mg/kg/inj cocaine may demonstrate a therapeutic treatment effect with longer exposure to the high treatment dose of d-amphetamine. Therefore, in a future experiment, the treatment period should be extended so that animals receive seven days of 5 mg/kg/day and 14-21 days of 7.5 mg/kg/day d-amphetamine. If breakpoints for 1.5 mg/kg/inj cocaine decrease within this time period, then we will know that both the dose and duration of d-amphetamine treatment determine the subsequent effects on cocaine self-administration. If breakpoints remain at baseline, then our data will be in agreement with several other studies showing that DA agonist treatments tend to be selective for low doses of cocaine (Glowa and Fantegrossi 1997; Peltier et al. 1996; Roberts et al. 2003).

3.2. Will Post-Treatment Breakpoints Still Decrease if Non-Contingent Cocaine is Administered During the D-Amphetamine Treatment Period?
The results from Chapter II have shown that the concurrent presence of both d-amphetamine and cocaine is necessary for reducing post-treatment cocaine-reinforced responding under a PR schedule. We know that the subjective effects of cocaine have not been completely eliminated by d-amphetamine treatment because the animals do not show extinction responding. However, the steady reduction of breakpoints associated with 0.19-0.75 mg/kg/inj cocaine suggests a learning process plays a primary role in this behavior. A decrease in responding under a PR schedule could indicate that the animal is learning that the positive effects of cocaine have been diminished or that the aversive effects of cocaine have been magnified after mini-pump implantation. Either way, the animal forms a new association between their behavior and the altered drug effects. Alternately, the decrease in breakpoints may result from a pharmacological interaction between cocaine and d-amphetamine.

In theory, we could answer this question by comparing post-treatment breakpoints between animals that self-administered cocaine during the d-amphetamine treatment period and animals that received the same amount of IV cocaine in a non-contingent method. However, non-contingent IV delivery of cocaine typically introduces new confounds into an experiment because it is more stressful and potentially lethal to animals than self-administered cocaine (Dworkin et al. 1995; Jacobs et al. 2003). Instead, cocaine could be delivered through a separate mini-pump or daily IP injections. These options are by no means ideal controls for cocaine self-administration, but if the treatment
effect is robust enough, it will occur with these relatively slow routes of administration.

3.3. How Will Continuous d-Amphetamine Treatment Affect Other Characteristic Behaviors of Cocaine Dependence?

The DSM-IV lists several criteria for drug dependence (American Psychiatric Association 2000) and drug-dependent individuals may exhibit any combination of these. Therefore a successful medication should affect more than one type of behavior associated with drug dependence. This is an important consideration with regard to preclinical evaluation of potential pharmacotherapies. The PR schedule is considered to be a model of "increased motivation to acquiring the drug" and has been used in several laboratories to assess changes in the reinforcing efficacy of a drug (Arnold and Roberts 1997; Richardson and Roberts 1996; Rowlett 2000). However, it is limited in its applicability to other aspects of human drug-taking behavior. For instance, the PR schedule cannot be used to examine changes in the initiation of drug-taking behavior because the dependent measure (i.e., breakpoint or final ratio) is only determined once the animal has already self-administered several injections of cocaine. Although the level of cocaine in the blood and brain at the time when an animal reaches its breakpoint may be affected by several factors (e.g., cocaine dose, number of injections, and inter-injection interval),
clearly a moderate level of cocaine circulates through the body and brain throughout the self-administration period. Even rats that reach very low breakpoints typically self-administer the first few injections on the PR schedule very quickly. Some researchers have surmised that animals deliberately take the first few injections of a session in short succession until a certain desired blood level of cocaine (Gerber and Wise 1989; Yokel and Piekens 1974) and/or extracellular DA (Oleson et al. 2008; Pettit and Justice, Jr. 1989) is achieved. For the remainder of the session, responding occurs when the amount of cocaine or DA falls below the preferred level (Ahmed and Koob 1998; Wise et al. 1995). This can be achieved on the PR schedule due to the low ratio requirements for the first five injections (i.e., below an FR10). Other schedules of reinforcement can be used to measure responding at the beginning of intake as opposed to the end of a self-administration session.

A discrete trials (DT) procedure can be used to measure the effect of d-amphetamine treatment on cocaine intake without the confound of drug-loading. Under a DT3 schedule, animals have three opportunities to self-administer an injection of cocaine each hour for a total of 72 daily reinforcement opportunities. Animals tend to develop a circadian pattern of cocaine intake on the DT3 schedule during which a greater number of injections is taken during the dark phase of the light/dark cycle (Fitch and Roberts 1993; Roberts et al. 2002). When rats are given only three opportunities per hour to receive a cocaine injection, they cannot drug-load in the beginning of a session as they typically do under a PR schedule. Unlike
breakpoint measures, the initiation of a period of cocaine intake can be observed when the animal is not under the influence of multiple overlapping doses of cocaine.

We conducted a pilot study to examine the effects of \(d\)-amphetamine treatment on self-administration under a DT3 schedule (see Appendix I). Animals that self-administered 1.5 mg/kg/inj under a DT3 schedule reduced their intake during the dark phase of the light/dark cycle (Appendix I; Fig 1). When all injection opportunities are collapsed into a single daily measure, the animals demonstrate a downward trend after only seven days of \(d\)-amphetamine treatment. This is interesting because the DT3 schedule revealed decreased intake of 1.5 mg/kg/inj cocaine with only seven days of \(d\)-amphetamine infusion whereas the PR schedule did not show an effect after 14 days of treatment. Despite the fact that animals in the DT3 experiment had more cocaine exposure during the \(d\)-amphetamine treatment period than animals in the PR experiments (approximately 35 inj vs. 17 inj), each injection was separated by at least 20 minutes, so this did not allow the animals to build up a preferred level and maintain it with subsequent injections.

Another DSM-IV characteristic of drug dependence which is very common in cocaine addicts is "increased intake over time" (American Psychiatric Association 2000). This behavior may simply reflect neurochemical tolerance or it may demonstrate compulsive behavior that emerges when the role of cocaine shifts from positive to negative reinforcement over time. In order for a
medication to be beneficial to the clinical population, this "increased intake" must be attenuated. The PR procedure we used in the experiments in Chapters II and III does not cause animals to increase their cocaine intake over time. In fact, our training procedure (40 injections of 1.5 mg/kg/inj cocaine for 5 days) has been shown to lead to stable responding under a PR schedule. From a behavioral pharmacology perspective this is an ideal situation in which to study potential pharmacotherapies since changes in reinforcing efficacy can be easily observed. However, from a translational perspective this does not accurately model a key aspect to human drug dependence—loss of control over drug intake after continued drug use. Ahmed and Koob began to model this aspect of cocaine dependence in rats over a decade ago with their seminal "escalation" procedure (Ahmed and Koob 1998; 1999; Koob et al. 2004). In rats, they found that the stable level of responding under an FR1 schedule from one-hour self-administration sessions was disrupted when the session length was increased to six hours. Specifically, animals that had longer access to cocaine continued to increase their daily rate of intake over 12 days (Ahmed and Koob 1998). Research from our lab has extended this phenomenon to self-administration under a PR schedule by demonstrating a training procedure which causes breakpoints under a PR schedule to increase over time (Liu et al. 2005; Morgan et al. 2006). These experiments showed that when rats were given limited access to cocaine during training under an FR1 schedule (i.e., 20 injections of 0.75 mg/kg/inj cocaine for 3 days), their breakpoints gradually increased over 14 days. This PR procedure should be incorporated into future studies of
continuous \(d\)-amphetamine treatment to address whether \(d\)-amphetamine can interrupt the progressive increase in cocaine-reinforced responding.

Human cocaine users tend to increase their cocaine intake by extending the length of binges (Gawin and Kleber 1986). This behavior is difficult to model in self-administration studies because unlimited access to cocaine can lead to lethal overdose (Bozarth and Wise 1985; Deneau et al. 1969). Binge-like behavior can be modeled in rats with a DT5 procedure. The chance of overdose is substantially decreased under a DT5 schedule because reinforcement opportunities are separated by 12 minutes. This time period is short enough to allow the effects of subsequent cocaine injections to overlap but long enough to prevent an animal from self-administering a lethal dose. Animals typically self-administer nearly every available cocaine injection during the first 48 hours under a DT5 schedule before reducing their intake to about 50% (Roberts et al. 2002). The initial high frequency of drug intake can be reinstated under a DT5 schedule after a forced abstinence period (Morgan and Roberts 2004), thus allowing us to assess the effect of continuous \(d\)-amphetamine treatment on an animal’s drive to initiate a cocaine binge.

4. **Conclusions**

Our data show that \(d\)-amphetamine is able to reduce some aspects of cocaine reinforcement in rats. Preclinical models, such as the one used here, are valuable tools in the field of medication development. Although our rodent
model of self-administration allowed us to control for confounding variables such as poly-drug use, social hierarchy and environmental cues, this may actually limit the extent to which our findings can be literally translated to the clinical situation for human cocaine abusers. The overall question as to whether d-amphetamine should be given to cocaine-dependent patients is difficult to answer with animal studies alone because many of the variables that can be controlled for in these models are actually central to human drug-seeking and -taking behavior. The abuse potential of d-amphetamine is well-known in our society and many people believe that the legal prescription of psychostimulants to a psychostimulant abuser may be more harmful than helpful. However, as demonstrated with opiate medications, contingency management programs and alternative means of administration (e.g., depot injection or combination with a DA antagonist) may be able to reduce the abuse potential of d-amphetamine. Overall, d-amphetamine treatment may benefit a segment of the cocaine-dependent population and serve as a harm reduction measure until more suitable medications are developed and made available.
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APPENDIX I

CONTINUOUS D-AMPHETAMINE INFUSION DECREASES COCAINE SELF-ADMINISTRATION UNDER A DISCRETE TRIALS PROCEDURE IN RATS
**FIGURE 1**

Effects of a continuous infusion of *d*-amphetamine on the circadian pattern of cocaine self-administration reinforced under a DT3 schedule of reinforcement. Points represent the mean percentage of available injections (1.5 mg/kg/inj cocaine) self-administered during each part of the 24-h session (Error bars not shown for clarity). White diamonds represent 10 baseline days. Black squares represent 7 treatment days during which an SC osmotic mini-pump delivered 5 mg/kg/day *d*-amphetamine. The circadian cycle of drug intake was unchanged by *d*-amphetamine treatment, although there was a trend toward decreased intake during the dark phase.
**Figure 1**

![Graph showing % injections taken over Time of Day with markers for Baseline and d-Amphetamine.](image-url)
FIGURE 2

Effects of a continuous infusion of \textit{d}-amphetamine on cocaine self-administration reinforced under a DT3 schedule of reinforcement. Points represent the mean (± SEM) number of reinforcers obtained in each daily 24-h session. Days 1-10 are baseline days. The shaded portion represents 7 days of mini-pump treatment. Gray diamonds represent the saline mini-pump group and black triangles represent the \textit{d}-amphetamine mini-pump group. The two groups are not significantly different during treatment. The first day after mini-pump removal (Day 18), the saline group self-administered more injections than the \textit{d}-amphetamine group but this difference dissipated by Day 19. There is a trend toward a decreased number of injections from the final baseline testing day to the final treatment day in the \textit{d}-amphetamine group.
Figure 2
APPENDIX II

CONTINUOUS D-AMPHETAMINE INFUSION ATTENUATES COCAINE-INDUCED LOCOMOTOR ACTIVITY IN RATS
Rationale: Due primarily to its DA activity, cocaine has dramatic locomotor-activating properties in rats. Locomotor behavior has been used extensively in psychostimulant research and has come to be associated with and even predictive of the reinforcing properties of such drugs. While increased stimulant-induced locomotor activity can represent sensitization, decreased activity can serve as a visible measure of tolerance.

Objectives: Here we examined the effects of continuous d-amphetamine treatment on cocaine-induced locomotor activity in order to understand the role of tolerance in the therapeutic effect of d-amphetamine treatment.

Materials and methods: Two groups of rats (N = 8) were habituated to locomotor testing chambers during the dark phase of the light/dark cycle for 2 days before locomotor testing began. Figure 1 shows the general timeline of the testing procedure. During each testing session animals received four separate doses of cocaine (0/saline, 3, 10 and 30 mg/kg/inj; IP) in a cumulative dosing procedure. Locomotor activity was recorded for 20 min after each cocaine injection and each session lasted 80 min. Baseline activity was collected for one day before implantation of mini-pumps containing either d-amphetamine (5 mg/kg/day) or saline for seven days. Locomotor activity was testing on days 1 and 6 of the treatment period. Mini-pumps were removed after testing on day 7 and locomotor activity was evaluated again on days 9 and
13. Ambulatory counts were analyzed by comparing cocaine-induced activity to saline baselines throughout the course of testing.

**Results:** Total ambulatory counts during the 60 min of cocaine exposure were significantly reduced on days 1 and 6 in the d-amphetamine treatment group when compared to saline baseline activity at the beginning of the session. The saline treatment group demonstrated a trend toward increased ambulatory counts during the treatment period (see Figure 2a). The locomotor responses to saline during the mini-pump treatment period (i.e., days 1 and 6) were significantly higher in the d-amphetamine treatment group than in the saline treatment group (see Figure 2b).

**Conclusions:** Continuous infusion of d-amphetamine (via osmotic mini-pump) resulted in a progressive decrease in locomotor behavior which returned to baseline following the seven-day treatment period. These data indicate that continuous infusion of d-amphetamine produces some level of cross-tolerance to the locomotor stimulating effects of cocaine.
Locomotor activity testing - experimental design

**Figure 1**

[Diagram showing the experimental design for cocaine administration and time periods for baseline, mini-pump treatment, and post-treatment periods.]
FIGURE 2

Effects of a continuous infusion of \textit{d}-amphetamine on cocaine-induced locomotor activity in rats. A) Points represent total ambulatory counts during the 60 min of cocaine administration (3, 10 and 30 mg/kg/inj; IP). Saline baselines (i.e., the first 20 min of each session) for the two groups were normalized in order to adequately compare the changes between the groups. Black triangles/solid lines represent the saline mini-pump group and gray squares/dotted lines represent the \textit{d}-amphetamine mini-pump group. Shaded region represents the seven-day mini-pump treatment period. B) Bars represent ambulatory counts after a saline injection (i.e., the first 20 min of each session). Black bars represent the saline mini-pump group and gray bars represent the \textit{d}-amphetamine mini-pump group.
Figure 2b

Ambulatory counts

- Saline
- d-Amphetamine

Testing Day

Mini-pump treatment period

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<tr>
<th>Day</th>
<th>Saline</th>
<th>d-Amphetamine</th>
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<td>500</td>
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</tr>
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</table>
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EDUCATION

Ph.D. in Neuroscience 2009
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• David C.S. Roberts, Ph.D. (Advisor, Department of Physiology & Pharmacology)
  - Dissertation project: Effects of continuous subcutaneous d-amphetamine
treatment on cocaine self-administration in rats.

B.S. in Biological Psychology 2003
The College of William & Mary, Williamsburg, VA
• Pamela S. Hunt, Ph.D. (Advisor, Department of Psychology)
  - Research project: Effects of opioid antagonists on ethanol intake and
social learning in preadolescent rats.

RESEARCH EXPERIENCE

Graduate Research Assistant 2003-2009
Dept. of Physiology & Pharmacology, WFUSOM
• David C.S. Roberts, Ph.D. (dissertation advisor)
  - Effects of continuous subcutaneous d-amphetamine treatment on cocaine
self-administration in rats.
  - How site-specific damage (via ibotenic acid lesions) to the prefrontal cortex
changes cocaine-reinforced responding on a progressive ratio schedule in rats.
  - Assisted in manuscript preparation and review
• Anthony Liguori, Ph.D.
  - Cognitive impairments associated with withdrawal from marijuana and/or
nicotine in drug-dependent humans.
• Allyson J. Bennett, Ph.D.
  - Role of postural instability in lateral hand preference in rhesus monkeys.
Dept. of Neurobiology & Anatomy
• Mary Lou Voytko, Ph.D.
  - Effectiveness of estrogen replacement in delaying cognitive impairments
seen in a non-human primate model of menopause.

Undergraduate Research Assistant 2002-2003
Dept. of Psychology, The College of William & Mary
• Pamela S. Hunt, Ph.D. (research advisor)
  - Effects of opioid antagonists on ethanol intake and social learning in
preadolescent rats.

Research Intern (Summer Research Opportunities Program) 2002
Department of Physiology & Pharmacology, WFSOM
- James E. Smith, Ph.D. (research advisor)
  - Evaluation of extracellular monoamine levels during speedball (cocaine +heroin) self-administration in rats.

**Undergraduate Intern for Clinical Trials**
Health Research of Hampton Roads Clinical Research Center, Newport News, VA
- Chester L. Fisher Jr., M.D., M.P.H. (mentor)
  - Investigational medications for the treatment of chronic obstructive pulmonary disease.

**Teaching Experience and Training**

**Lecturer**
Winston Salem State University, Winston-Salem, NC 2007-2008
- Physical Therapy program (Course director: Allyn C. Howlett, Ph.D.)
  - Physiology PHT5401, Topics: Neurotransmission; Autonomic Nervous System
  - Pharmacology PHT6203, Topics: Antipsychotic Drugs; Opioid Pain Regulation

Wake Forest University 2007
- Undergraduate Department of Psychology (Course director: Wayne Pratt, Ph.D.)
  - Physiological Psychology PSY320, Topic: Drug Abuse

North Carolina Central University 2006
- Graduate biology program (Course director: Allyn C. Howlett, Ph.D.)
  - Physiology & Pharmacology BIOG5104, Module: Autonomic Nervous System

**Assistant course coordinator, WFUSOM** 2008
- Introduction to Professional Development GRAD700

**Research Intern Mentor, WFUSOM** 2005
- Kate Friedenberg (Salem Academy High School), Lab: Allyson J. Bennett, Ph.D.

**Teaching Courses/Conferences**
- Skills in Biomedical Teaching, WFUSOM 2008
- Teaching of Physiology and Pharmacology, WFUSOM 2007
- Teaching Advancement Program, WFUSOM 2007-2008
- Lilly Conference on College and University Teaching, Greensboro, NC 2008

**Honors and Fellowships**

**Research presentation awards**
- Mary A. Bell Award (First place poster-behavioral category), WFUSOM 2008
  - Western North Carolina Chapter of the Society for Neuroscience (WNCSN) research poster competition
- Neuroscience Graduate Student Tutorial Award, WFUSOM 2006
  - Second place, WFUSOM
  - First place, WFUSOM 2004 & 2005
Public outreach awards
- WNC SN Synapse Award, WFUSOM 2008
  - Leadership & service in communication of neuroscience to the public
- SFN Next Generation Award, WNC SN Nominee 2007 & 2008

NIH Pre-doctoral Training Grant in Neuroscience, NIH 2003-2005

PUBLIC OUTREACH ACTIVITIES

Brain Awareness Council (BAC), WFUSOM 2003-present
- Co-founder; Co-director
  - Instituted the BAC organization with three other students in 2003; recruited and trained volunteers; secured funding from local businesses; partnered with a local biotechnology company to promote our public events during Brain Awareness Week.
- Education coordinator
  - Developed and organized educational materials and lesson plans for monthly BAC visits to local public schools; planned, and carried out Brain Awareness Week activities (i.e., activity stations at local science and children’s museums; public neuroscience seminars that are pertinent to adults in the community).

SciTech Summer Technology Institute 2006
- Learning session director
  - Organized various lectures and activities to teach middle school students about drug abuse research and career options in science.

Science Fair Judge, Hanes Middle School, Winston-Salem, NC 2004

PROFESSIONAL MEMBERSHIPS

American Association for the Advancement of Science (AAAS) 2008-present
Society for Neuroscience 2006-present
Western North Carolina Chapter of the Society for Neuroscience 2005-present

RESEARCH SKILLS

Behavioral testing
- Rodents: drug self-administration (psychostimulants and alcohol); food self-administration; locomotor activity testing.
- Non-human primates: cognitive testing with touch-screen computers, laterality testing, behavioral event observation.
- Humans: cognitive testing; subject screening and interviewing.

Other laboratory skills
- Rodent surgery: intrajugular catheter implantation, administration of electrolytic and excitotoxic brain lesions; subcutaneous osmotic mini-pump implantation.
- Histology: transcardial perfusion, cryostat sectioning, thionin staining.
- Limited experience with: microdialysis; high performance liquid chromatography; immunohistochemical staining; stereology.
EXTRAMURAL REVIEWS

Neuroscience and Biobehavioral Reviews

PUBLICATIONS


Chiodo KA and Roberts DCS. The reinforcing efficacy of cocaine is attenuated following two weeks of continuous d-amphetamine treatment in rats. (submitted to Psychopharmacology).

Bennett AJ, Chiodo KA. Freestanding bipedal posture significantly influences lateralized hand use in rhesus monkeys. (submitted to Am J Primatology)

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ABSTRACTS

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Chiodo KA and Roberts DC. (2006) Ibotenic acid lesions of the medial prefrontal cortex increase cocaine self-administration reinforced under a progressive ratio schedule. *WNCSN Annual Poster Session, WFUSOM.*

Chiodo KA and Roberts DC. (2006) Ibotenic acid lesions of the medial prefrontal cortex increase cocaine self-administration reinforced under a progressive ratio schedule. *Society for Neuroscience Annual Meeting, Atlanta, GA.*


Chiodo KA and Roberts DCS. (2005) Ibotenic acid lesions in the prelimbic and infralimbic areas of the medial prefrontal cortex differentially affect cocaine self-administration reinforced on a progressive ratio schedule. *WNCSN Annual Poster Session, WFUSOM.*

**Research Seminars**

**Graduate Student Summer Tutorial,** Neuroscience Program, WFUSOM

Chiodo KA. (2008) “The agonist treatment approach and cocaine addiction: should amphetamine be given to cocaine addicts?”


Chiodo KA. (2005) “Sensitization to the reinforcing effects of cocaine: the role of the prefrontal cortex.”


**Graduate Student Seminar Series,** Dept. of Physiology & Pharmacology, WFUSOM


Chiodo KA. (2006) “How is the prefrontal cortex involved in cocaine addiction?”


**Invited Lectures**

Chiodo KA. (2008) “Welcome to the world of graduate school.” Orientation for WFUSOM first-year biomedical graduate students, WFUSOM.

