ABSTRACT

Cocaine addiction can be defined as a psychological disease that is characterized by uncontrollable, compulsive drug-seeking and drug use despite negative health and social consequences. One obstacle for the treatment of cocaine addiction is the susceptibility to relapse which can persist several years despite prolonged periods of abstinence. Relapse can be triggered by drug predictive stimuli such as environmental context, drug associated cues, and addictive drug itself. The conditioned place preference (CPP) and self-administration behavioral paradigms are useful models for studying the ability of these drug predictive stimuli to reinstate drug-seeking behavior. Previously, we have reported that treatment with the D-serine, a NMDAR coagonist, is effective in facilitating the effects of extinction to reduce cocaine-primed reinstatement. Therefore, we sought to investigate the dose-dependent effects of D-serine on extinction training and drug-primed reinstatement in cocaine-conditioned rats. We showed that D-serine had no effect on the acquisition or development of cocaine-induced locomotor sensitization or CPP. Additionally, we demonstrated that the combination of extinction training
along with D-serine treatment resulted in a significant reduction of drug-seeking behavior; this decrease was evident after up to 4 weeks abstinence. Furthermore, we assessed D-serine’s effectiveness in reducing drug-primed reinstatement under conditions in which extinction training is conducted in a novel environment. From this set of experiments we concluded that D-serine’s ability to reduce drug-prime reinstatement is not transferable to a novel context, rendering it ineffective as an adjunctive cognitive enhancement treatment as exposure therapy typically takes place in a novel environment. To conclude my dissertation work, the aim of my last report was to assess the effects of exposure to the cocaine self-administration environment on drug-seeking behavior and on Fos protein expression in the hippocampal formation. Exposure to the cocaine environment significantly increases Fos protein expression only in the ventral subiculum. However, there was a trend toward a significant effect in the ventral CA1 pyramidal cell layer. Although more work must be done to add to the clarity of the current results, these result suggest that the ventral subiculum and perhaps, the ventral CA1 pyramidal cell layer play a critical role in context-induced drug-seeking behavior.

INDEX WORDS: Addiction, Cocaine, Reinstatement, Extinction, D-serine, hippocampus, Fos
BRAIN REGIONS AND MECHANISMS INVOLVED IN EXTINCTION AND
REINSTATEMENT OF COCAINE SEEKING BEHAVIOR IN RATS

by

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My Fiancé, My Family, My Friends, and Faithful Prayer Warriors
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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1. Introduction

Generally people begin taking drugs for recreational proposes however repeated consumption of addictive substances can lead to serious unwanted effects including tolerance (decrease in the effect of a drug despite a constant dose or a need for increased dosage to maintain a stable effect) to some drug effects, sensitization (enhancement of drug response) to others, and dependence (an adapted state of cells, circuits or organ systems that occurs in response to excessive drug stimulation) [1]. Another serious consequence of repetitive drug taking, however, is addiction. Although some tend to use addiction and dependence interchangeably, they are scientifically different. Dependence refers the physical symptoms of chronic drug use, while addiction can be defined as a psychological disease that is characterized by uncontrollable, compulsive drug-seeking and drug use despite negative health and social consequences [2]; the focus of my dissertation is on the latter of the two. One obstacle for the treatment of cocaine addiction is the susceptibility to relapse which can persist several years despite prolonged periods of abstinence [3]. In 2012, the National Survey on Drug Use and Health reported that cocaine was the third most used illicit substance in the United State with an estimated 1.6 million cocaine users. As the overall use of cocaine has declined between the years of 2002 and 2009, cocaine use among individuals 12 years and older remains to number in the millions, with estimates of 639,000 new users in 2012, approximately 1,800 initiates per day. To date the development of a treatment for cocaine addiction has proven to be problematic and currently there are no FDA approved
treatments available. As a result of the continued use and ineffective treatment for cocaine addiction, many researchers have dedicated their time and resources to develop treatment for this addiction.

Cocaine is an extract from the Erythroxylum coca plant, which is a native plant of South America, Mexico, Indonesia, and the West Indies. Several ancient civilizations have exploited cocaine rewarding effects for religious, medical and ceremonial purposes. In 1855, the cocaine alkaloid was first isolated. However, it was not until 1884 that cocaine’s properties as a local anesthetic and vasoconstrictor were presented at an ophthalmology conference by Carl Koller. In the late 1880’s cocaine was introduced into the recipe of several popular beverages such as Vin Mariani’s Coca Wine and the original Coca-Cola recipe. Additionally, cocaine was sold over the counter for a wide variety of ailments, becoming a popular patent medicine taken for conditions such as depression and toothaches. In 1914 Congress passed the Harrison Narcotics Act banning the use of cocaine in the United States. The Controlled Substance Act passed 1970 classified cocaine as a schedule II drug. Schedule II drugs are considered to have a strong potential for abuse but that have legitimate medical use. The only legal use for cocaine was as a local anesthetic. In the 1980’s and 1990’s illegal cocaine use amongst people skyrocketed and as a result many fell victim to the addictive properties of cocaine [for review see,4, 5].
Adapted from Russo, S. et al., Nature Neuroscience (2012)

Figure 1.1 Neurocircuitry of the reward pathway.

Depicted is a mid-sagittal illustration of a laboratory rat brain. Shown are the major reward nuclei and reward-related circuits involved in drug-seeking /taking behavior and relapse. The primary circuit facilitating drug-induced reward is the dopaminergic projections from the ventral tegmental area and to the nucleus accumbens. The red solid lines represent excitatory glutamatergic projects. GABAergic innervation is shown in purple and dopaminergic connections are blue. The principal circuitry mediating cue-induced relapse to drug-seeking behavior includes glutamatergic cell groups in the amygdala and hippocampus and their projections to the meso-accumbens dopaminergic pathway. These brain loci are color-coded to indicate the neurotransmitter system involved: glutamatergic neurons (red), GABAergic neurons (purple), and hypothalamic peptidergic neurons (black). Abbreviations of brain loci: VTA, ventral tegmental area; NAc, nucleus accumbens; mPFC, medial prefrontal cortex; CP, caudate-
putamen; DMT, dorsomedial thalamus; SC, superior colliculus; IC, inferior colliculus; VP, ventral pallidum; SNr, substantia nigra; PAG, periaqueductal gray; DR, dorsal raphe; LC, locus coeruleus.
2. Reward Pathway

The reinstatement of drug-seeking has been observed in rats exposed to addictive substances such as stimulants, nicotine, ethanol and opioids, triggered by drug predictive stimuli such as environmental context, stress, drug-associated cues, as well as the addictive drug itself [6]. The rewarding effects of all addictive substances can be attributed to synaptic and neuronal changes in the mesolimbic (or reward/dopaminergic) pathway.

By the use of the intra-cranial self-stimulation protocol, previous research has found a robust relationship between stimulation of the medial forebrain bundle and several associated areas and the reinforcement of paired behaviors. Studies had shown that animals will repeatedly stimulate this collection of axon fibers. Furthermore, data from clinical trials report that humans describe stimulation of the medial forebrain bundle as pleasurable. This axonal bundle contains descending and ascending fibers that extend from the basal forebrain, connecting to the midbrain. Enhancement of dopaminergic activity in the nucleus accumbens (NAc) is a commonality of all addictive drugs. These addictive substances including amphetamines, cocaine, opiates, nicotine, phencyclidine (PCP) and ketamine, cannabinoids, benzodiazepines, barbiturates and ethanol. Activation of the mesocorticolicimbic dopamine system is essential for the reinforcing properties of addictive drugs and natural rewards. From the ventral tegmental area, where the mesocorticolicimbic pathways originate, dopaminergic projections are sent through the medial forebrain bundle to key reward structures including, nucleus accumbens, prefrontal cortex, hippocampus and the amygdala.
3. **Ventral tegmental area**

The ventral tegmental area (VTA) is located in the midbrain and lies rostral to the pons and the hindbrain. The dopaminergic neurons within the VTA are thought to alert and make ready an animal for noteworthy experience and are thought to be activated for two primary purposes. One, to notify an animal that a novel motivationally significant event is occurring and to imitate the appropriate behavioral response, for example, to move toward a reward such as food or to flee a stressful situation such as seeing a ferocious dog. Secondly, dopamine is triggered by environmental cues (previously associated with a behavioral response) in anticipation of a motivationally relevant event. Once approach or avoidance behavioral responses to a particular cue have been learned, dopamine release ceases. In the case of addiction, addictive substances increase dopaminergic activity. With every administration of an abused drug, dopamine is released and during each of these instances an organism is continuing to make further association between the environment and cocaine, thereby making environmental stimuli more effective in triggering craving and promoting drug-seeking. As the activity of the dopaminergic neuron in the VTA increases as a result of continues drug use, the brain regions to which these neurons project are also altered. The relevance of several of these essential regions to cocaine addiction is discussed below.

4. **Nucleus accumbens**

Microdialysis studies in rodents and imaging studies in humans have confirmed that after injections of cocaine and presentations of drug associates cues, respectively; there is an increase in dopamine levels in the nucleus accumbens (NAc). The NAc is located rostral to the preoptic area, in the basal forebrain. It is one component of the ventral striatum and is itself composed of
two divisions, the core and the shell. The core is an extension of the ventromedial striatum and is bordered by the NAc shell, caudate and putamen around its ventral and medial edges. It is thought that the shell is more connected with the limbic system than the core. The connectivity of the NAc links the limbic system with motor action. At the center of the reward pathway are the interactions between the VTA and the NAc. It is linked to reinforcing properties of both natural and drug reward.

5. **Amygdala**

The amygdala is a group of nuclei that are positioned deep and medially to the temporal lobes. It is comprised of 3 main areas the medial, basolateral and central amygdala. The amygdala had long been considered a structure for mediating emotional memory. Recent work has associated the amygdala with aversive processing associated with the negative emotions of fear and anxiety and aversive learning. Additionally, the amygdala has been linked to appetitive processing and positive emotions.

The amygdala innervates the NAc and these connections are believed to be important for the development of stimulus-reward associations. The central nucleus of amygdala has been implicated in aversive effects of drug withdrawal. Whereas the basolateral amygdala is extensively innervated by cortical areas and it is these projections that are linked with perception of anxiety and fear. Furthermore, there exists a special relation between amygdala and hippocampus by the direct and indirect link through fibers of the entorhinal cortex. Receiving dopaminergic fibers from the VTA via connections with the hippocampus, the amygdala may have a role in acquisition and retrieval of emotional memories in the context of drug addiction.
8. Hippocampus

The hippocampus is located in the medical temporal lobe of each side of the brain. H.M., a famous patient who surgically had both parts of his medial temporal lobes removed for the treatment of epilepsy, sparked interest in the hippocampus. As a consequence of having both his hippocampi removed, H.M. was not able to form new long-term declarative memories [7], however he could remember his remote past. This discovery that the loss of the medial temporal lobe affected memory without interrupting other cognitive functions significantly helped to define the function of the hippocampus as a region that is crucial in encoding memories.

The hippocampus has a distinct laminar organization and a well-defined circuit for the transmission of information. The hippocampus is primarily composed of glutamatergic pyramidal and granule cells, the remaining cells are GABAergic interneurons [8]. It includes many regions, including the entorhinal cortex, the dentate gyrus, the hippocampus proper (which is subdivided into CA3, CA2 and CA1), and the subiculum. The entorhinal cortex provides the majority of input to the dentate gyrus through the perforant pathway. Via granule cells, the dentate gyrus sends projects called mossy fibers to the CA3. CA3 pyramidal cells send axons called the Schaffer collaterals to the CA1, and send a projection to the contralateral hippocampus via commissural pathway. The pyramidal cells of the CA1 send projections to the subiculum, and the subiculum sends axons back to entorhinal cortex completing the circuit. Contrary to other brain regions, the hippocampus transmits information largely in unidirectional manner [9]. The subiculum, made up of pyramidal cells and interneurons, is the main output of the hippocampus [10].
9. Hippocampus and reward

The hippocampus can be divided along its septotemperal axis into two anatomically and functionally separate structures, the dorsal hippocampus and ventral hippocampus. Generally, the dorsal hippocampus has been implicated in contextual reinstatement of cocaine seeking [11], while the ventral hippocampus plays a key role in cue-induced reinstatement and cocaine-primed reinstatement [12]. Regardless of its septotemperal division the hippocampus is plays a crucial role in relapse.

It was observed that ventral subiculum enhances dopamine neuron firing in the VTA [13]. Additionally, the literature suggest that stimulating the ventral subiculum of the hippocampus with theta burst stimulation induces drug seeking behavior in rats [14] In this study to implicate the role of hippocampus in relapse, Vorel et al. (2001) found that microinjections of kynurenic acid to the VTA but not the vehicle blocked the reinstatement after ventral subiculum stimulation. This suggests the involvement of ionotrophic glutamate receptors in reinstatement. Based on these findings, and published work from fear conditioning studies (see below), we decided to examine the effect of D-serine, a full agonist at the glycine modulatory site of the NMDA receptor, on relapse. The majority of the work for my dissertation is focused investigating the efficiency of D-serine to drug-primed reinstatement in the CPP behavior paradigm and “ABA” self-administration model. For the final portion of my dissertation, we elucidate the role of the hippocampus in contextual cue-primed reinstatement by examining Fos protein expression. Discussed below are the behavioral paradigms employed in this study.
10. Animal models of drug addiction

Three of the most widely utilized animal models employed to assess addictive-like behaviors in animals are locomotor sensitization, conditioned place preference (CPP), and the self-administration behavioral paradigms. The locomotor sensitization behavioral assay typically involves repeated exposure of an animal to a low dose of a psychostimulant, resulting in a progressive increase in locomotor activity, a phenomenon termed behavioral sensitization. In the following set of experiments we used this paradigm to verify that the reduction in drug-seeking seen in animals treated with D-serine was due the facilitation of the NMDA receptor and not a result of decreased locomotor activity and to ensure that D-serine did not alter response novelty or cocaine.

The CPP behavioral paradigm is a protocol in which the motivational properties of a drug are repeatedly paired with a neutral environment. After sufficient conditioning, these environmental cues can serve as conditioned stimuli, thereby eliciting approach from an animal in a drug-free state [15]. Using the CPP paradigm we were able to quickly (as the protocol lasts only a few days) and easily (no surgical procedures involved) generate animals to find the optimal dose range for D-serine. Furthermore, we incorporated this behavioral model into my study to investigate how persistent effects of D-serine were.

The self-administration experimental protocol differs from the previously mentioned behavioral paradigms in that operant responding (lever pressing or nose poking) must occur in order for the animal to receive drug. Additionally, the amount of drug taken throughout a session and the rate at which the drug is administered is controlled by the animal rather than the experimenter. To date, the self-administration experimental protocol is considered to be more comparable to human addiction, having the highest face validity among the addiction behavioral
assays [16]. In the current report we have used lever pressing as the instrumental response required for drug delivery. The reinforcement contingencies can vary including, Fixed ratio (FR) schedule, progressive ratio (PR) schedule, fixed interval, variable ratio or variable interval [16].

The most commonly utilized schedule of reinforcement is the fixed ratio reinforcement schedule and the reinforcement schedule employed in my studies. Under the FR schedule of reinforcement, the animal must emit response according to the FR response. Therefore, under a FR-1 schedule an animal must press the lever (or nose poke) one time in order for one infusion to be delivered. In our model of cocaine self-administration, a FR-3 reinforcement schedule is used, 3 responses for one infusion. The animals self-administer cocaine in an operant chamber that houses distinct visual cues (house light, levers, rod flooring etc.). Upon completion of the reinforcement schedules an infusion of cocaine is given and paired with the activation of a tone and a cue light. Through classical conditioning, the cue light and tone act as a conditioned reinforcer. Therefore when these stimuli are presented alone, they can induce drug-seeking behavior.

Generally, addiction researchers utilize a limited access protocol. This assay in some ways mirror human addiction in that is allows for the animals to control their drug intake however, it is limited as it only results in a stable pattern of drug-intake and not the binge pattern observed in humans. The extended access, or long assess, protocol allows for the emulation of a hallmark characteristic of cocaine addiction in humans, an escalation of drug-intake over time. We have chosen to incorporate extended access into our rodent self-administration protocol, as it is thought to resemble more closely the state of addiction in humans
11. Background

The use of the aforementioned preclinical animal models has allowed the mechanisms that underlie reinstatement behavior to be explored [16]. The current rationale for the treatment of addiction has been modeled after existing anxiety disorders therapy protocols. In the treatment of anxiety disorders, exposure therapy has been shown to be an effective treatment for reducing the frequency and intensity of episodes [17]. The N-Methyl-D-aspartate (NMDA) receptor has been implicated in extinction learning [18] and several conditioned fear studies illustrate that antagonism of NMDA receptors during extinction impairs the effects of such training [19]. In a complementary manner, enhancement of NMDA receptor activity with D-cycloserine (DCS), a partial agonist at the glycine site of the NMDA receptor, facilitates fear extinction [20]. Furthermore, in an experiment done by Ledgerwood et al. [21], rats fear conditioned with 5 light-foot shock pairings received DCS or saline treatment immediately after extinction training (6 light-pairings, one session). A reduction of fear-induced freezing upon light presentation was evident in the rats treated with DCS when compared to the saline control group. This effect was only seen in animals that had undergone extinction training. These results were confirmed by later work in the same laboratory and by other researchers [22-24]. DCS treatment of fear disorders has also been assessed in clinical trials. In a study by Hofmann et al [25] individuals with public speaking anxiety were treated with 50 mg of DCS one hour prior to each of the five therapy sessions. The individuals receiving DCS reported lower levels of anxiety up to one month after treatment as compared to individuals given a placebo. Such reported success with DCS treatment in clinical trials have been described for panic disorders, phobias, social disorders and obsessive compulsive disorders [for review see,26]. The translational success of this line of investigation from an understanding of preclinical mechanisms in animals to promising clinical
results in humans has prompted a strong interest in using a similar rationale for the treatment of addiction, but the effectiveness of exposure therapy in this context has been unclear [27].

Accounting for the effectiveness of DCS treatment in fear extinction, several labs have adopted this same principle for the treatment of cocaine addiction. Botreau [28] demonstrated that DCS when given immediately before extinction training, significantly enhanced extinction when compared to saline controls. In a study done by Paolone [29], giving DCS in conjunction with extensive extinction training did not result in a significant effect. However, when combined with a sub-maximal extinction protocol, DCS treatment was efficient in decreasing the total number of extinction trials needed to reduce CPP. Yet in another study [30], DCS was tested to examine its effects on the context specificity of extinction training. In this experiment, cocaine was self-administered in context A, extinguished in an alternate environment (context B), and animals were retested in the original context (A). The results of this study demonstrate that DCS treatment reduced cue-induced drug-seeking in animals extinguished in a secondary environment rather than the acquisition environment.

Using a cocaine self-administration model, we have previously described a requirement for NMDA receptor activity during extinction training to reduce subsequent drug-primed reinstatement [31]. In addition, we have examined the actions of D-serine, a full agonist at the glycine modulatory site of the NMDA receptor and its effects on cocaine-primed reinstatement. By employing sub-optimal extinction protocols in rats allowed either limited access [32] or extended access [33] to cocaine self-administration, the enhancing effects of D-serine treatment during or immediately following extinction training resulted in reduced drug-primed reinstatement. This only occurred when D-serine is given in conjunction with extinction training; a finding also reported using DCS [34].
In recent years addiction has been viewed as a learning and memory disease state. It is thought that addiction “hijacks” the neural mechanisms of learning and memory which is responsible, under normal conditions, to shape survival behaviors related to the pursuit of rewards and the cues that predict them [35-37]. Cue-drug memories are persistent, triggering relapse after years of abstinence. Thus, drug-associated stimuli can invoke memories of the drug, and induce craving during periods of abstinence in addicts, and induce relapse [38]. These findings implicate mechanisms of associative learning and memory systems that can be used to later retrieve drug-associated memories and induce drug-seeking and drug-taking. Additionally, several studies have shown that chronic use of cocaine is associated with deficits in cognitive functioning, including decision-making, response inhibition, planning, working memory, and attention [39, 40]. It appears that these deficits in cognitive functioning are due to cocaine induce modifications in the prefrontal cortex, amygdala and hippocampus (for review see [41]). Various studies have shown a link between cognitive function to treatment outcome in stimulant users. In a series of studies, Aharonovich et al. have demonstrated that cocaine-dependent individual with cognitive impairments are less able to benefit from behavioral treatment [42, 43]. The fact that the success of exposure therapy (or extinction in preclinical studies) may be hindered by drug-induced deficits in cognitive functioning has led to the study of has led to the study cognitive enhancers (drugs that have the potential to enhance attentional control and memory) for the treatment of cocaine addiction. As stated previously, DCS had been every effective in treating fear related conditions and has been successful in facilitating extinction training in preclinical studies. However, DCS has not been as effective in clinical trials (for review see [44]). Thus, the greater part of my dissertation involves investigating the effects of D-serine, as opposed to DCS, for the treatment of cocaine addiction.
12. Summary

The aim of this chapter is to acquaint the reader with the drug addiction literature, and about the animal models of addiction and drug relapse. Animal reinstatement models of drug seeking are well accepted to model relapse in clinical situations and thereby are important in translational medicine. From the background information provided here, it is evident that the use of cognitive enhancers for the treatment of cocaine addiction is understudied, hence the need for more exploration in this area. There are no studies that address D-serine’s efficiency to reduce drug-primed reinstatement in the CPP or the “ABA” self-administration model. Furthermore, there are no reports to date that study the mechanisms underlying the reinstatement of drug-seeking behaviors induced by contextual cues in animals that are allowed extended access to cocaine.

13. Specific Aims

In light of the aforementioned studies, the set of experiments in this report contains five aims:

1. To investigate the dose-dependent effects of D-serine (10 mg/kg, 30 mg/kg and 100 mg/kg) on extinction training and drug-primed reinstatement in cocaine-conditioned rats.

2. To characterize the effects of D-serine on drug-primed reinstatement using the CPP behavioral paradigm.

3. To assess the duration of D-serine’s ability to reduce cocaine-induced drug-seeking behavior.
The results of aims 1-3 are summarized in chapter one. From the results presented in chapter two, I next considered gaps in current literature on the effectiveness of D-serine to reduce cocaine–primed reinstatement in an alter extinction environment, hence my next aim.

4. To further the D-serine self-administration experiments already conducted in my lab and examine D-serine’s ability to reduce the context specificity of extinction training in an “ABA” self-administration protocol.

The findings from aim 4 are presented in chapter three.

Following this set of experiments I decided to take an immunohistological approach to understanding the neural mechanisms of cocaine addiction in an effort for develop more successful treatments, therefore leading me to my fifth and final aim.

5. To examine the effect of exposure to cocaine-paired environmental cues on Fos protein expression in the hippocampal subregions.

Here the aim was to dissect out which hippocampal subregions are critical for context-induced reinstatement in animals with a history to extended access to cocaine. Several studies have been conducted that have investigated the roles various limbic structures in environmental-induced increases in Fos protein expression. However, none to date have examined this relationship under a long access self-administration protocol. From this experiment we sought to elucidate the role of the hippocampal formation in context-induced reinstatement in an animal model of drug relapse that is more analogous to human addiction.
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CHAPTER 2

D-SERINE FACILITATES THE EFFECTIVENESS OF EXTINCTION TO REDUCE DRUG-PRIIMED REINSTATEMENT OF COCAINE–INDUCED CONDITIONED PLACE PREFERENCE

D-SERINE FACILITATES THE EFFECTIVENESS OF EXTINCTION TO REDUCE DRUG-PRIMED REINSTATEMENT OF COCAINE–INDUCED CONDITIONED PLACE PREFERENCES

Abstract

Addiction is a disease that is characterized by compulsive drug-seeking and use despite negative health and social consequences. One obstacle in treating addiction is a high susceptibility for relapse which persists despite prolonged periods of abstinence. Relapse can be triggered by drug predictive stimuli such as environmental context and drug associated cues, as well as the addictive drug itself. The conditioned place preference (CPP) behavioral model is a useful paradigm for studying the ability of these drug predictive stimuli to reinstate drug-seeking behavior. The present study was designed to investigate the dose-dependent effects of D-serine (10 mg/kg, 30 mg/kg and 100 mg/kg) on extinction training and drug-primed reinstatement in cocaine-conditioned rats. In the first experiment, D-serine had no effect on the acquisition or development of cocaine-induced locomotor sensitization or CPP. In experiment 2, D-serine treatment resulted in significantly decreased time spent in the drug-paired compartment following completion of an extinction protocol. A cocaine-primed reinstatement test indicated that the combination of extinction training along with D-serine treatment resulted in a significant reduction of drug-seeking behavior. The third experiment assessed D-serine’s long-term effects to diminish drug-primed reinstatement. D-serine treatment given during extinction was effective in reducing drug-seeking for more than four weeks of abstinence after the last cocaine exposure.
These findings demonstrate that D-serine may be an effective adjunct therapeutic agent along with cognitive behavioral therapy for the treatment of cocaine addiction.

Key words: cocaine, D-serine, reinstatement, extinction, place preference
1. Introduction

Addiction can be defined as a psychological disease that is characterized by uncontrollable, compulsive drug seeking and drug use despite negative health and social consequences [1]. One obstacle for the treatment of addiction is the susceptibility to relapse which can persist several years despite prolonged periods of abstinence [2]. The use of preclinical animal models such as self-administration, behavioral sensitization and conditioned place preference [3] has allowed the mechanisms that underlie the priming of reinstatement behavior to be explored. The reinstatement of drug-seeking has been observed in rats exposed to addictive substances such as psychostimulants, nicotine, ethanol, opioids, and may be triggered by drug predictive stimuli such as environmental context, stress, drug-associated cues, as well as the addictive drug itself [4].

In the treatment of anxiety disorders, exposure therapy has been shown to be an effective treatment for reducing the frequency and intensity of episodes [5]. The N-Methyl-D-aspartate (NMDA) receptor has been implicated as being involved in extinction learning [6], and several conditioned fear studies illustrate that antagonism of NMDA receptors during extinction impairs the effects of such training [7]. In a complementary manner, enhancement of NMDA receptor activity with D-cycloserine, a partial agonist at the glycine site of the NMDA receptor, facilitates fear extinction [8]. The translational success of this line of investigation from an understanding of preclinical mechanisms in animals to promising clinical results in humans has prompted a strong interest in using a similar rationale for the treatment of addiction, but the effectiveness of exposure therapy in this context has been unclear [9].

Using a cocaine self-administration model, we have previously described a requirement for NMDA receptor activity during extinction training to reduce subsequent drug-primed
reinstatement [10]. In addition, we have examined the actions of D-serine, a full agonist at the glycine modulatory site of the NMDA receptor and its effects on cocaine-primed reinstatement. By employing sub-optimal extinction protocols in rats allowed either limited access [11] or extended access [12] to cocaine self-administration, the enhancing effects of D-serine treatment during or immediately following extinction training resulted in reduced drug-primed reinstatement. This only occurred when D-serine is given in conjunction with extinction training; a finding also reported using D-cycloserine [13].

Another effective paradigm for evaluating the effects of drugs of abuse in preclinical studies is the conditioned place preference (CPP) experimental design. The CPP paradigm is one in which the motivational properties of a drug is repeatedly paired with a neutral environment. After sufficient conditioning, these environmental cues can serve as conditioned stimuli and elicit approach in the drug-free state when reintroduction occurs [14]. D-cycloserine is effective in facilitating cocaine-induced CPP in both rats and mice [15, 16]. As is the case for self-administration, CPP behavior can be extinguished and reinstated following drug-priming, stress, or conditioned cues [17]. A significant feature of the CPP protocol is the practical advantage of being able to test relatively large numbers of animals that allow dose-response studies to be efficiently conducted without the use of more labor intensive protocols.

The present study was designed to investigate the dose-dependent effects of D-serine (10 mg/kg, 30 mg/kg and 100 mg/kg) on extinction and drug-primed reinstatement in cocaine-conditioned rats. When combined with extinction training, D-serine was effective in facilitating extinction and in reducing cocaine-primed reinstatement; an effect that persisted for more than 4 weeks. These results suggest that D-serine is a promising adjunct treatment to be combined with exposure therapy for the treatment of cocaine addiction.
2. Materials and Methods

2.1 Drugs

D-serine was purchased from Sigma (St. Louis, MO, USA). Cocaine hydrochloride was obtained from NIDA (RTI International, NC, USA).

2.2 Animal Maintenance

Sprague–Dawley male rats (Harlan, Indianapolis, IN, USA) were housed in pairs in clear plastic cages. They were maintained on a 12 h light/dark cycle (0700 hr/1900 hr) with food and water available ad libitum. Animals were allowed to acclimate to their home cages for one week and were habituated to handling for 3 days before behavioral testing began. Sessions were conducted daily between 0900hrs and 1500hr. These studies were approved by the University of Georgia Institutional Animal Care and Use Committee and conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

2.3 Apparatus

Gosnell [18] gave a detailed account of the chambers and its dimensions. Behavioral testing was carried out in 43.2 × 43.2 cm chambers with clear plastic walls and a solid smooth floor (Med Associates, St. Albans, VT, USA). Each chamber was housed in a sound-attenuating box equipped with two house lights (20 lx) and a ventilation fan. Two banks of 16 infrared photo beams and detectors detected horizontal activity. Activity Monitor software (Med Associates) was used to count beam breaks.

Conditioned place preference (CPP) testing occurred in a two compartment insert (Med Associates) that modified the open field chamber. The modified chamber was divided into two identical compartments (42.8 × 21.3 cm) separated by a black partition containing a guillotine door. If removed, the rat had access to the entire chamber and was put in place to confine the
animal’s activity to one compartment during conditioning. The compartments differed by floor type (grid, wire mesh vs. rod, steel bars) and by ceiling color (transparent vs. black). The compartment with the rod-floor had the black ceiling which darkened that side of the chamber insert; preliminary studies indicated that this arrangement yielded an equal preference between compartments.

2.4 Experimental design

2.4.1 Experiment 1

Seymour and Wagner [19] describe in detail the experimental design of our first set of experiments. Rats were tested in a Pretest session (protocol day 1) where each animal was placed in the light/grid compartment and allowed free access to both compartments of the CPP chamber for 15 min (Fig. 2.1A). The time spent in each compartment was analyzed to assure that none of the animals had a strong bias toward either side of the compartment. Any rat spending >65% of its time in either compartment was excluded from this unbiased CPP study. The following day the place preference inserts were removed and rats were placed in the center of the open field chamber for 30 minutes to monitor baseline activity (Activity, protocol day 2). An i.p. injection of either 0.9% saline (n=45) cocaine (10 mg/kg, n=34) was given and the animals placed back in the open field where activity was monitored for an additional 60 minutes.

Conditioning sessions were begun the next day (protocol days 3-6). Saline-paired and cocaine-paired animals were given either an i.p. injection of saline or 100 mg/kg of D-serine 1-2 hours prior to conditioning sessions, in the home cage. In all, four groups were tested saline/saline (n=29), saline/cocaine (n=24), D-serine/saline (n=16), and D-serine/cocaine (n=10). Rats were placed in one of the two compartments for 15 minutes and then returned to their home
cage. Four hours later, animals were injected with either saline or cocaine and confined to the opposite compartment for the second daily conditioning session. Following the completion of conditioning, a second drug-free place preference test was administered (Post Test, protocol day 7). One week later, the open field Challenge test (protocol day 13) was conducted in the same manner as the Activity test to assess sensitization.

2.4.2 Experiment 2

The protocol for Experiment 2 (Fig. 2.1B) did not include open field assessments of locomotor activity. Following the CPP Pretest, cocaine conditioning preceded with saline and cocaine (15mg/kg) pairings as previously described, except no home cage pretreatments occurred. Following the CPP Post Test, a passive extinction protocol (days 7-10) was carried out in which groups received their respective treatments of saline (n=10), 10 mg/kg D-serine (n=6), 30 mg/kg D-serine (n=7), or 100 mg/kg of D-serine (n=14) immediately before being confined to their former cocaine-paired compartment. As during the conditioning phase, animals were also confined to the opposite compartment and given the vehicle treatment (saline) immediately prior to the second extinction session. Following the end of extinction training, a third CPP test was conducted (Extinction Test, protocol Day 11). Animals were placed in their saline-paired compartment and explored the CPP chamber for 15 minutes. The next day, all rats were given an i.p. injection of 10 mg/kg cocaine and again placed in their saline-paired compartment to explore the CPP chamber for 15 minutes (Reinstatement Test, protocol day 12).

2.4.3 Experiment 3

Experiment 3 followed the exact procedures of experiment 2 except some of the former rats were held abstinent in their home cage environment for an additional 30 days following the Reinstatement Test. On protocol day 43, a second reinstatement test (4 wk Reinstatement Test)
was conducted. Once again, all animals received an i.p. injection of 10 mg/kg of cocaine immediately prior to being placed into the saline-paired compartment of the CPP chamber and allowed free access for 15 minutes. Two combined groups were analyzed in this experiment, a saline & 10 mg/kg group, n = 11; and a 30 & 100 mg/kg group, n = 16.

2.5 Statistical Analysis

Statistics were done using SigmaStat software, version 3.1. One-way repeated measures (RM) ANOVAs were used to analyze time spent in the drug-paired compartment across multiple CPP tests and one-way ANOVAs were used to analyze between groups for the shift in preference, locomotor activity, and locomotor sensitization results. Post-hoc analysis used in all cases was Holm-Sidak.

3. Results

3.1 Experiment 1 (Fig. 2.1A) The effects of pretreatment with systemic D-serine on locomotor activity and the development of conditioned place preference or locomotor sensitization.

3.1.1 D-serine pretreatment does not affect the response to a novel environment or cocaine.

D-serine has undergone substantial testing as an antipsychotic agent in human trials [20] and investigated for activity in several preclinical rodent models of schizophrenia (reviewed by [21]. However in some respects, relatively little characterization of the behavioral effects of D-serine has been reported in preclinical studies. For example, the measurement of locomotor behavior is used extensively in the assessment of rewarding and sensitizing properties of drugs of abuse such as cocaine [22, 23]. Although D-serine alone does not have obvious behavioral consequences when administered i.c.v. [24] or systemically [25] the potential interactive effects
of systemic D-serine on measures such as the acquisition and development of cocaine-induced sensitization or CPP have not been described.

Following the protocol for the first experiment (Fig. 1A), 4 groups of rats were pretreated with either saline (n=29 saline group, n=24 cocaine group) or D-serine (100 mg/kg; n=16 saline group, n=10 cocaine group) in their home cage prior to exposure to their first conditioning session on day 3. D-serine pretreatment had no effect on the locomotor responses of either saline- or cocaine-treated rats when they were introduced into the conditioning environment (Fig. 2.2A, gray bars vs. their corresponding white/black bars). As expected, cocaine (15 mg/kg)-induced a significant increase in locomotor activity that was evident in both treatment groups (saline vs cocaine, ** p < 0.01 and D-serine/saline vs. D-serine/cocaine, * p < 0.05, one-way ANOVA/Holm-Sidak).

3.1.2 D-serine pretreatment does not affect the development of cocaine-induced place preference.

Cocaine-induced CPP following a total of four such conditioning protocol days was assessed on protocol day 7 by allowing the animal free choice between the cocaine/saline conditioning environments while in a drug-free state (Fig. 2.2B). Under these conditions, cocaine-conditioned rats (middle pair of bars) exhibited the expected significant shift in time spent in the drug-paired compartment (* p < 0.01, one-way ANOVA/Holm-Sidak), whereas saline-conditioning (left pair of bars) had no significant effect. Rats pretreated with D-serine prior to each conditioning session (protocol days 3-6) also exhibited a significant shift in time spent in the cocaine-paired environment during the post conditioning test (right pair of bars, * p < 0.05, one-way ANOVA/Holm-Sidak).
3.1.3 D-serine pretreatment does not affect the development of cocaine-induced locomotor sensitization.

The effects of D-serine pretreatment on the acquisition and development of locomotor sensitization were assessed on protocol day 13, one-week following the last cocaine conditioning session. At this time, the cocaine-induced enhancement of locomotor activity was further increased in the cocaine-conditioned, saline pretreatment rats compared with the initial response to cocaine from the protocol day 2 activity test (Fig. 2.3A vs. 2.3B). Similarly, the cocaine-induced enhancement of locomotor activity was further increased in the cocaine-conditioned D-serine pretreated rats compared with the initial response to cocaine from the protocol day 2 activity test (Fig. 2.3A vs. 203C). A summary analysis from the Challenge day test groups (Fig. 2.3D) indicates there was no significant difference between the cocaine-induced sensitization of the locomotor response observed with either saline or D-serine pretreatment during conditioning.

3.2 Experiment 2 (Fig. 2.1B) The influence of D-serine on the effectiveness of extinction and cocaine-primed reinstatement of CPP.

3.2.1 Test groups exhibited similar conditioning chamber preference (Pretest) and response following cocaine conditioning (Post Test).

Rats were placed into the CPP chamber for the first time and time spend in each compartment was measured (Fig. 2.4, Pretest). After four days of conditioning with cocaine, time spend in the drug-paired compartment significantly increased (* Post Test, one-way RM ANOVA F (1, 59) = 187.846, * p < 0.01). The animals were then split into four treatment groups; Control (n=10), D-serine 10 mg/kg (n=6), D-serine 30 mg/kg (n=7), and D-serine 100 mg/kg (n=14) for extinction and reinstatement studies. In our previous reports describing the actions of D-serine to enhance the effectiveness of extinction training to reduce drug-primed
reinstatement in self-administering rats, it was important to employ a modest extinction protocol that allowed the effects of D-serine to be evident [11, 12]. In the current saline group, one-way RM ANOVA analysis revealed that the cocaine-induced CPP was maintained throughout extinction training (Extinction Test, * p <0.01, one-way RM ANOVA/Holm-Sidak) as well as following drug-primed reinstatement (Reinstatement Test, p <0.01, one-way RM ANOVA/Holm-Sidak).

3.2.2 D-serine dose-dependently enhanced extinction training, reducing the shift in preference for the cocaine-paired compartment (Extinction Test).

Rats were treated each day during the extinction phase of the protocol (days 7-10) with either saline or D-serine immediately before being placed into the former cocaine-paired compartment of the conditioning chamber. A dose-dependent decrease in time spent in the drug-paired compartment was evident that persisted during reinstatement testing (Fig. 2.4). In order to facilitate comparisons between treatment groups, the results were expressed as the cocaine-induced shift in time spent in the drug-paired compartment (Fig. 2.5). Following extinction training, a significant shift in preference was observed in the 30 mg/kg D-serine group (* p < 0.01, one-way ANOVA/Holm-Sidak, Fig. 2.5C), and this effect was also significant in comparison to the saline group extinction results († p < 0.01, one-way ANOVA/Holm-Sidak, Fig. 2.5A vs. 2.5C). Similarly, a significant shift in preference was observed following extinction training in the 100 mg/kg D-serine group (* p < 0.05, one-way ANOVA/Holm-Sidak, Fig. 2.5D), and this effect was significant in comparison to the saline group extinction results († p <0.01, one-way ANOVA/Holm-Sidak, Fig. 2.5A vs. 2.5D). In both the saline group and the 10 mg/kg D-serine group there was not a significant shift in preference after extinction training (e.g. Fig. 2.5A & 2.5B). Taken together, these results suggest that under conditions in which
extinction alone is insufficient to reduce cocaine seeking, D-serine treatment was able to enhance extinction training in a dose-dependent manner.

3.2.3 Cocaine-primed reinstatement was reduced in animals treated with either 30 mg/kg or 100 mg/kg of D-serine (Reinstatement Test).

A drug-primed Reinstatement Test (protocol day 12) was administered to assess the effects of D-serine treatment. Following cocaine-priming (10 mg/kg), a significant shift in preference was observed in the 30 mg/kg D-serine group (* p < 0.01, one-way ANOVA/Holm-Sidak, Fig. 2.5C), and this effect was also significant in comparison to the saline group reinstatement results († p < 0.01, one-way ANOVA/Holm-Sidak, Fig. 2.5A vs. 2.5C). Once again, a significant shift in preference was observed following reinstatement in the 100 mg/kg D-serine group (* p < 0.05, one-way ANOVA/Holm-Sidak, Fig. 2.5D), and this effect was significant in comparison to the saline group reinstatement results († p < 0.05, one-way ANOVA/Holm-Sidak, Fig. 2.5A vs. 2.5D). In both the saline group and the 10 mg/kg D-serine group there was not a significant shift in preference (e.g. Fig. 2.5A & 2.5B). Altogether, D-serine treatment (either 30 mg/kg or 100 mg/kg) during extinction training was able to subsequently reduce drug-primed reinstatement.

3.3 Experiment 3 (Fig 2.1C) The effects of D-serine treatment 4 weeks after final cocaine exposure.

3.3.1 D-serine’s effectiveness in reducing drug-primed reinstatement is persistent, lasting more than 4 weeks.

In a subset of animals, a second cocaine-primed reinstatement test was given approximately 4 weeks after the first reinstatement test (protocol day 12) on protocol day 43. During the abstinent period the rats remained in their home cage environment. In order to
increase the overall statistical power, we collapsed the four treatment groups into two groups for analysis. As the saline and 10 mg/kg D-serine groups exhibited similar results in Experiment 2 (c.f. Fig. 2.5A & 2.5B), we collapsed these two groups into one analysis group (saline & 10 mg/kg D-serine, n=11). Similarly, as both the 30 mg/kg and the 100 mg/kg D-serine data were also comparable (c.f. Fig. 2.5C & 2.5D), we combined the 4 week reinstatement results of these two groups into a “30 & 100 mg/kg” analysis group (n=16).

As expected from the results depicted in Fig. 4, cocaine-induce CPP for the drug-paired compartment was evident in both the saline & 10 mg/kg D-serine combined analysis group as well as the 30 &100 mg/kg D-serine combined analysis group after cocaine-priming (* p<0.05, Pretest vs. Post Test, one-way RM ANOVA/Holm-Sidak, respectively). For the saline & 10 mg/kg D-serine group, this significant shift in preference was maintained following the abstinent period in the 4 wk Reinstatement Test (* p < 0.05, one-way RM ANOVA/Holm-Sidak). In contrast, the combined 30 & 100 mg/kg D-serine group CPP was no longer maintained to a significant extent at the 4 wk Reinstatement Test. Furthermore, the time spent in the drug-paired compartment was significantly decreased in this analysis group as compared to that of the saline & 10 mg/kg D-serine group († p<0.05, one-way ANOVA/Holm Sidak). Together these findings indicate that the effects of D-serine treatment during extinction training to reduce drug-primed reinstatement can persist more than 4 weeks following the last extinction session or previous cocaine exposure.

4. Discussion
The primary result of the present study is that systemic administration of D-serine immediately prior to extinction sessions can subsequently facilitate the effects of such training to decrease drug-seeking during a cocaine-primed reinstatement test. D-serine exhibited dose-
dependent effects, with the effective D-serine doses of 30 mg/kg and 100 mg/kg being quite modest relative to those used in other behavioral studies [26, 27]. The extinction protocol used in our experiments was insufficient to significantly reduce cocaine-seeking, however in combination with 30 mg/kg or 100 mg/kg of D-serine the effects of the behavioral training were enhanced and a persisting reduction in drug-primed reinstatement was observed. The potential importance of these findings with respect to the therapeutic potential of D-serine is discussed below.

Mechanisms involved in learning and memory of extinction training and the mechanisms involved in reinstatement have been extensively studied in paradigms such as fear conditioning, conditioned taste aversion, and inhibitory avoidance [28]. For example, the partial NMDA receptor agonist D-cycloserine has been found to facilitate conditioned fear responses [8, 29, 30]. D-cycloserine has also been used in CPP models of cocaine-seeking to enhance extinction training [15, 16]. Contrary to these studies, other published work notes that in cocaine-conditioned rats D-cycloserine given 30 minutes prior to extinction training slowed the rate of extinction [31]; infusion of D-cycloserine in the basolateral amygdala increased cue-induced relapse in cocaine-conditioned rat [32]; in cocaine-conditioned mice a dose of 30 mg/kg D-cycloserine resulted in renewed CPP at 2 weeks [15] and in morphine-conditioned rats treated with D-cycloserine at doses of 7.5 mg/kg, 15, mg/kg and 30 mg/kg had no effects on extinction or the expression of morphine-induced CPP [33]. The use of D-cycloserine as an adjunctive treatment for addiction has been tested in several clinical trials producing a wide range of conclusions including that D-cycloserine facilitates extinction in nicotine-dependent smokers [34, 35]; D-cycloserine increases craving in cocaine-dependent individuals [36-38] as well as in heavy alcohol drinkers [37] and D-cycloserine has no effect on cue-reactivity in alcohol-
dependent individuals [39]. Thus, the use of D-cycloserine has yielded mixed results with respect to its potential use for the treatment of addiction.

D-serine is different from D-cycloserine in that it acts as a full agonist at the glycine modulatory site of the NMDA receptor, whereas D-cycloserine is a partial agonist at this site [40]. Krystal et al [41] found that when D-cycloserine was given at low doses to healthy individuals in the presence of increased levels of glycine, it produced effects similar to those of NMDA receptor antagonists at [42]. It is possible that in an individual with a high basal level of glycine or D-serine activity, D-cycloserine may produce a competitively antagonistic effect rather than facilitating the activity of the NMDA receptor. In addition, D-serine is an endogenously produced amino acid and the brain contains mechanisms for degradation [43]. As a practical consideration for the therapeutic use of D-serine, these endogenous synthetic and degradative mechanisms and their regulation may help to protect against toxicity. For example, a high-dose D-serine regime of 60 mg/kg in a recent human trial has been described as being well tolerated [44]. In the current study, we found that pretreatment with 100 mg/kg D-serine does not affect locomotor activity during cocaine conditioning, and it does not interfere with the acquisition or development of either cocaine-induced sensitization or CPP. Therefore, the described effects of D-serine in the current studies are not consistent with any direct interaction with cocaine or its behavioral consequences, and are instead consistent with its activity as an agonist at the glycine modulatory site and the enhancement of NMDA receptor-dependent learning during extinction training.

When tested in cocaine self-administration reinstatement protocols, D-serine treatment along with extinction training results in a significant reduction of drug-primed reinstatement in rats with a history of either short or long access to drug [12, 45]. An important feature of these
studies was that the effect of extinction training was suboptimal, meaning that a significant reduction in drug-primed reinstatement was not observed with extinction alone. This was also the circumstance in the current report, as extinction training in the saline-treated rats was not effective in significantly diminishing the cocaine-induced CPP. However, when D-serine was combined with such training, CPP was completely eliminated in either the 30 mg/kg or 100 mg/kg treatment doses. This demonstrates an important feature of this adjuvant pharmacotherapy-the capacity to enhance an otherwise weak or ineffective behavioral protocol without increasing the number of training sessions or their duration. This is a potentially significant therapeutic advantage, as it may not be practical for treatment seeking individuals to attend sessions at greater frequency or of longer duration. Thus promising cognitive behavioral therapy protocols may achieve clinical efficacy when combined with D-serine treatment.

Importantly, we found that D-serine pretreatment before extinction training not only facilitated extinction and reduced cocaine-primed reinstatement tested 2 days later, but it also exhibited much longer effects in reducing drug-primed reinstatement up to 4 weeks. These results are in general agreement with the conclusions of Paolone et al [46], as they found D-cycloserine reduced drug-primed reinstatement assessed 25 days after the last extinction training session. These observations highlight another feature of practical importance regarding the potential use of D-serine along with behavioral therapy, that the interval between the “booster” sessions required for the ongoing maintenance of the extinguished condition can be lengthened. For example, monthly treatments involving a day or two are more likely to be amenable for the typical patient than would ongoing weekly or multiple days/week session schedule.
5. Conclusion

D-serine, a full agonist of the NMDA receptor at the glycine co-agonist site, facilitated extinction and reduced drug-primed reinstatement of cocaine-induced CPP in rats following systemic administration. The effects of D-serine treatment during extinction resulted in a persisting reduction of cocaine-primed reinstatement for more than 4 wks. These effects were dose-dependent and the amount of D-serine effective in these studies has already been tested and well tolerated in humans for use as an antipsychotic agent. These results and observations demonstrate that D-serine could be an effective adjunct treatment along with cognitive behavioral therapy for cocaine addiction by reducing the number of initial therapy sessions and/or by increasing the interval between future maintenance therapy sessions.
References


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Figure 2.1 Experimental designs employed in this study.

See Materials and Methods for description and further details.
A

Total Horizontal Counts

- saline
- cocaine
- D-serine/saline
- D-serine/cocaine

B

Time in drug-paired compartment (sec)

- saline
- cocaine
- D-serine/cocaine

Pretest Post Pretest Post Pretest Post
Figure 2.2 D-serine pre-treatment does not affect the acquisition of cocaine-induced CPP.

A) On the first day of cocaine conditioning, pretreatment with D-serine (100 mg/kg) does not affect either the baseline locomotor behaviour in the saline-paired compartment or the cocaine-induced increase in locomotor behaviour. Cocaine significantly increased activity in both saline and D-serine groups (** p<0.01 and * p<0.05, one-way ANOVA/Holm-Sidak). B) Following the completion of the conditioning protocol, a significant increase in the time spent in the cocaine-paired compartment was evident during the CPP Post test as compared to the Pretest. Pretreatment with D-serine prior to the conditioning sessions did not significantly affect the acquisition of CPP expressed during the Post test (* p<0.05, one-way RM ANOVA/Holm-Sidak).
Figure 2.3 D-serine pre-treatment does not affect the acquisition of cocaine-induced locomotor sensitization.

A) Locomotor activity was increased following i.p. administration of cocaine (10 mg/kg) compared with saline injection at t=30 min of the 90 minute open field Activity session. B) Following four days of conditioning, saline pretreated rats exhibited the expected increase in locomotor response to cocaine when retested one week post conditioning during the Challenge session. C) The response to cocaine was similarly enhanced in the D-serine (100 mg/kg) group that was pretreated prior to each of the four condition sessions. D) Summary data from the
Challenge open field test in which the first 30 minutes post injection locomotor activity is depicted from four test groups of rats: The sal/coc group was conditioned with saline and received cocaine for the first time during the Challenge test. Cocaine induced a significant increase in activity as compared with the saline injected control group (** p<0.01, one-way ANOVA/Holm-Sidak). Both of the cocaine conditioned groups exhibited a significant increase in activity as compared with the saline injected control group (** p<0.01, one-way ANOVA/Holm-Sidak) that was also increased as compared to the sal/coc group († p<0.05, one-way ANOVA/Holm-Sidak).
Figure 2.4 Illustration of time spent in drug-paired compartment between treatment groups and across tests.

Cocaine-conditioning increased time spent in the drug-paired compartment in all groups (* p<0.05 one-way RM ANOVA/Holm Sidak). The cocaine-induced CPP was maintained following extinction and during cocaine-primed reinstatement testing the in the saline group, demonstrating the sub optimal nature of the extinction training protocol alone.
Figure 2.5 D-serine decreases preference shift in a dose-dependent manner.

D-serine, when given at doses 30 mg/kg and 100 mg/kg significantly reduced the animals preference for their drug-paired compartment both after extinction training and following cocaine-primed reinstatement when compared to the preference test (* p<0.05, one-way ANOVA/Holm Sidak). Comparisons to the respective saline Extinction and Reinstatement group results were also significant († p<0.05, one-way ANOVA/Holm Sidak).
Cocaine-induce CPP for the drug-paired compartment was evident in both the saline & 10 mg/kg D-serine combined group as well as the 30 & 100 mg/kg D-serine combined group (* p<0.05, Pretest vs. Post Test, one-way RM ANOVA/Holm-Sidak, respectively). This significant increase in time spent in the drug-paired compartment was maintained following the 4 week abstinent period in the saline & 10 mg/kg D-serine group as compared to the Pretest (* p < 0.05, one-way RM ANOVA/Holm-Sidak). In contrast, the increase in time spent of the combined 30 & 100 mg/kg D-serine group was no longer significant at the 4 wk Reinstatement Test, and the time spent in the drug-paired compartment was significantly decreased as compared to that of the saline & 10 mg/kg D-serine group († p<0.05, one-way ANOVA/Holm Sidak).
CHAPTER 3

THE CONTEXT DEPENDENCY OF EXTINCTION NEGATES THE EFFECTIVENESS OF COGNITIVE ENHANCEMENT TO REDUCE COCAINE-PRIMED REINSTATEMENT

THE CONTEXT DEPENDENCY OF EXTINCTION NEGATES THE EFFECTIVENESS OF COGNITIVE ENHANCEMENT TO REDUCE COCAINE-PRIMED REINSTATEMENT

Abstract

With respect to the treatment of addiction, the objective of extinction training is to decrease drug-seeking behavior by repeatedly exposing the patient to cues in the absence of unconditioned reinforcement. Such exposure therapy typically takes place in a novel (clinical) environment. This is potentially problematic, as the effects of extinction training include a context dependent component and therefore diminished efficacy is expected upon the patient’s return to former drug-seeking/taking environments. We have reported that treatment with the NMDAR coagonist D-serine is effective in facilitating the effects of extinction in reducing cocaine-primed reinstatement. The present study assesses D-serine’s effectiveness in reducing drug-primed reinstatement under conditions in which the context of extinction training is altered in rats self-administering cocaine. After 22 days of cocaine self-administration (0.5 mg/kg) in context “A”, animals underwent 5 extinction training sessions in context “B”. Immediately after each extinction session in “B”, animals received either saline or D-serine (60 mg/kg) treatment. Our results indicate that D-serine treatment following extinction in “B” had no effect on either IV or IP cocaine-primed reinstatement conducted in “A”. These results stand in contrast to our previous findings where extinction occurred in “A”, indicating that D-serine’s effectiveness in facilitating extinction training to reduce drug-primed reinstatement is not transferable to a novel
extinction environment. This inability of D-serine treatment to reduce the context specificity of extinction training may explain the inconsistent effects observed in clinical studies published to date in which adjunctive cognitive enhancement treatment has been combined with behavioral therapy without significant benefit.

Key Words: D-serine, cocaine, self-administration, context, extinction, reinstatement.
1. Introduction

The environment an individual frequents to use addictive substances can play an important role in resumption of drug-taking, even after prolonged periods of abstinence. These contextual stimuli, along with stress, drug-associated cues, and the addictive drug itself are major impediments for maintaining abstinence during the treatment of cocaine addiction and have been observed to reinstate drug-seeking behavior in rodents [1]. Cocaine addiction can be defined as a psychological disease characterized by uncontrolled, compulsive drug-seeking and drug use despite negative health and social consequences [2].

To reduce the efficacy of drug predictive environmental stimuli to induce reinstatement of drug-seeking, extinction training or exposure therapy is often employed. The objective of extinction training is to decrease drug-seeking behavior by repeatedly exposing the individual to cues in the absence of the unconditioned reinforcer. Such exposure therapy typically takes place in a novel context (e.g. a rehabilitation center). This is potentially problematic because the effects of extinction therapy are context dependent and therefore diminished effectiveness of such therapy is expected upon the patient’s return to former drug-seeking/taking environments [3]. Most preclinical extinction/reinstatement studies do not take these environmental differences into account, as the extinction experience occurs in the same context as the acquisition of drug-taking [4].

Since the establishment of the involvement of N-Methyl-D-aspartate (NMDA) receptors during extinction learning in fear conditioning studies and reports of D-cycloserine facilitating conditioned fear extinction learning, interest in the use of NMDA receptor co-agonists during extinction training for the treatment of addiction has increased substantially [5]. Recent attention has been given to D-cycloserine, a partial agonist at the co-agonist site on the NMDA receptor, for the treatment of cocaine addiction [6]. Various studies have revealed the efficacy of D-
cycloserine to facilitate extinction and reduce drug-seeking using both the conditioned place preference (CPP) and the self-administration behavioral paradigms [7]. However, clinical research results to date have not been consistent with these promising preclinical reports. For example, clinical studies that have used D-cycloserine as an adjunctive treatment for addiction have revealed no effect on cue reactivity of heavy smokers [8]. D-cycloserine had no effect on cue reactivity or attentional tasks in heavy alcohol drinkers [9] and D-cycloserine administration increases craving in alcohol-dependent individuals [10] and cocaine-dependent participants [11, 12]. Thus, despite promising results from preclinical studies, those obtained from clinical trials have generally not been positive.

Previously, we have reported that treatment with the endogenous NMDA receptor co-agonist D-serine, is effective in facilitating the effectiveness of extinction in reducing cocaine-primed reinstatement using both the CPP [13] and the self-administration [14, 15] behavioral models in which extinction occurs in the same context as that of drug-seeking/taking. In the present study we assessed the effectiveness of D-serine in reducing the context specificity of extinction training to reduce cocaine-seeking behavior in rats with a history of extended access to cocaine self-administration.

2. Materials and Methods

Sprague–Dawley male rats (Harlan, Indianapolis, IN, USA) were singly housed in clear plastic cages. Rats were maintained on a 12 h light/dark cycle (0700 hr/1900 hr) with food and water available ad libitum. These studies were approved by the University of Georgia Institutional Animal Care and Use Committee and conducted in accordance with the Guide for the Care and Use of Laboratory Animals. Behavioral testing was conducted in self-
administration operant chambers, the “A” environment (Med associates Inc., St. Albans, VT) previously described by Kelamangalath and Wagner [14]. For extinction training the “B” environment, “B”, was a modified version of the previously describe self-administration chamber. To create the novel “B” environment mesh grid flooring was substituted for the rod flooring, a black and white striped paper was attached to the rear wall, and the chamber was illuminated with the inactive lever light.

Before self-administration began an indwelling catheter was surgically placed into the right jugular vein of all animals, a detailed description of the jugular vein catheterization is described elsewhere [15]. After one week of recovery from surgery, animals were placed into the operant chambers to lever press for cocaine. Animals learned that lever pressing resulted in one infusion of cocaine (0.5 mg/kg) after which a 30 second timeout period began. During this timeout period, responses on the active lever had no consequences. Self-administration was divided into three phases: 1) short access, ShA (90 min) on FR1 (days 1-10), 2) ShA on FR3 (days 11-15) and 3) long access, LgA (6 hr) on FR3 (days 16-22, Fig 3.1A).

For extinction training (days 23-27) animals were split into two groups, “B” saline and “B” D-serine. During the 90 minutes extinction training sessions, rats were placed into “B”, where responses on the active lever and inactive lever resulted in no programmed consequences. Immediately after each extinction session, animals were given an IP injection of their respective treatment saline (0.9 %) or D-serine (60 mg/kg). Although the dose of D-serine used is this study was comparably low in relation to other published work [16, 17], we recently published work supporting the use of 60 mg/kg of D-serine for enhancing the effectiveness of extinction to reduce drug-primed reinstatement [13].
Following extinction training rats were tested for cocaine-primed reinstatement. On day 28, animals with patent catheters were placed into the original drug-taking environment, “A” and responding on the active and inactive levers were measured for the first 60 minutes under extinction conditions to account for context-induced reinstatement. At 60 minutes rats received a single noncontingent IV cocaine-prime (0.5 mg/kg) and the lever responding for the next 30 minutes was assessed. This IV reinstatement protocol was repeated on day 29 using a saline-prime. On subsequent reinstatement test days, animals received either an IP injection of cocaine (10 mg/kg, day 30) or saline (day 31) immediately prior to being placed back into the operant chamber.

Statistics were done using SigmaStat software, version 3.1. (Chicago, IL, USA) A one-way analysis of variance (ANOVA) was applied for all data analysis. A value of P≤0.05 was taken as significant, being determined from the Holm-Sidak post hoc test method.

3. Results

Self-administration behavior between the subsequently divided saline (n=19) and D-serine (n=9) treatment groups was comparable throughout the acquisition phase (Fig 3.1B), demonstrating that they entered the extinction phase with similar prior drug-taking experience. Regarding the effects of transitioning to extended access to cocaine, when comparing the first 60 minutes of lever pressing during the last three days of ShA (25.4 ± 1.0 presses) to lever pressing during the last three days of LgA (29.2 ± 1.4 presses) a significant increase is observed (* p <0.05, one-way ANOVA/ Holm-Sidak; Fig 3.1B, inset.). The observation of escalation of drug intake after transitioning to extended access is in concert with our own and other published work [14, 18].
After 22 days of cocaine self-administration two groups of rats were subjected to extinction training in a novel context, “B”, for five days. In these treatment groups, either saline or D-serine (60mg/kg, i.p.) was administered i.p. immediately after each extinction training session and in the other group, animals were treated with immediately after each extinction training session. Regardless of treatment, rats extinguished operant responding at a similar rate (Fig 3.2). We have previously published work that is consistent with these results; D-serine did not consistently accelerate the extinction drug-seeking in either the CPP or self-administration behavioral paradigm when extinction was conducted in the acquisition environment, “A” [13-15].

Following extinction training in “B”, all animals were tested in “A” for drug-seeking behavior using two types of noncontingent cocaine-priming tests; one administrated via the intravenous (IV) route and another administered via the IP route. On day 28 animal, for the first 10 min, lever pressing was evaluated for context-induced reinstatement. Operant responding between the saline treatment group (16.4 ±3.2 presses) and the D-serine treatment group (18.7±5.4) were comparable. Moreover, an evaluation the rats activity (day 28) for 30 min before (preprime) and for 30 min after (post prime) a cocaine IV prime demonstrates a significant increase in active lever pressing within both the saline (* p <0.05, one-way ANOVA/Holm-Sidak) and D-serine (*p <0.05, one-way ANOVA/ Holm-Sidak) treatment groups (Fig, 3.2A). For the saline treatment group, comparing the day 28 cocaine post prime response (24.0 ± 7.0 presses) with the saline post prime response (5.5 ± 1.6 presses) on day 29, a difference in active bar pressing was evident († p <0.05, one-way ANOVA/ Holm-Sidak). Similarly, in comparing the D-serine treatment group’s cocaine post prime responses (27.7 ± 8.8 presses) from day 28 with the saline post prime response (2.0 ± 0.7 presses) on day 29, there was again a
significant difference in active lever pressing († p <0.05, one-way ANOVA/ Holm-Sidak). Note that active lever pressing in the D-serine treatment group 30 minutes after a cocaine IV priming (27.7 ± 8.9 presses), was quite comparable in magnitude to that observed in the saline treatment group (24.0 ± 7.0 presses). Two animals from the saline treatment group and two animals from the D-serine treatment group catheters were blocked at the time of the IV cocaine challenge and data from these animals were therefore excluded from the IV cocaine-prime data.

Evaluation of the IP reinstatement data showed that for the saline treatment group, when comparing the cocaine IP prime response (65.9 ± 15.9 presses) from day 30 to the saline IP prime (6.5 ± 1.1 presses) on day 31, there was a significant increase in active lever pressing (** p <0.01, one-way ANOVA/ Holm-Sidak; Fig. 3.2B). Similarly, in comparing the D-serine treatment group’s cocaine post prime response (52.3 ± 17.6 presses) from day 30 with the saline post prime response (12.2 ± 3.6 presses) from day 31, there was again a significant increase in lever pressing (* p <0.05, one-way ANOVA/ Holm-Sidak). Note again that active lever pressing in the D-serine treatment group 30 minutes after a cocaine IP priming (52.3 ± 17.6 presses), did not significantly differ from that observed in the saline treatment group (65.9 ± 15.9 presses). Thus, in concurrence with the IV priming results, the cocaine IP prime reinstatement test indicated no effect of D-serine treatment during extinction in “B” as compared to saline treatment. Together, these IV and IP drug-priming results indicate the effectiveness of D-serine in facilitating extinction training to reduce drug-primed reinstatement is not transferable when extinction training occurs in a novel context relative to that of drug self-administration.
4. Discussion

The primary result of the current report is systemic administration of D-serine does not provide the beneficial effect of reducing cocaine-induced drug-seeking when extinction training occurs in a novel context (i.e. “ABA” protocol). We have previously shown that systemic administration of D-serine after each extinction training session is effective at reducing drug-primed reinstatement in rats when extinction is conducted in the original drug-taking environment (i.e. “AAA” protocol) [14, 15]. However, the results of the present study are in concert with several other clinical studies [8-12] in which cognitive enhancement has not consistently resulted in decreased measures of craving or cue reactivity. The relation of these studies to the current results is discussed below.

We have considered D-serine, a full agonist at the strychnine-insensitive glycine modulatory site of the NMDA receptor, as a cognitive enhancer for the treatment of cocaine addiction. Previously, we have reported that when extinction training conditions are effective in reducing drug-primed reinstatement, an NMDA receptor antagonist can disrupt the ability of extinction training to decrease cocaine-induced drug-seeking [19]. In a complimentary fashion, we have also published work revealing the effectiveness of D-serine in reducing cocaine-primed reinstatement using ShA and LgA self-administration models [14, 15]. Despite this successful characterization of the effectiveness of D-serine in reducing cocaine-primed reinstatement tested in the “A” environment, the effectiveness of this treatment on drug-primed reinstatement when extinction training is conducted in a novel “B” environment has not been evaluated. This is of particular significance considering that a successful treatment for cocaine addiction with extinction training has yet to be established, likely due to the context specificity of extinction training [20, 21].
Our results revealed that after extended access to cocaine there was a significant escalation in drug intake over time. This result has also been observed in cocaine-dependent humans, as increases in frequency of drug intake and increases in the amount of drug use over time are hallmark characteristics of cocaine addiction [22]. In order to evaluate the effects of D-serine on the context dependency of extinction training, we modified the acquisition environment to create a novel context. Our results showed significantly increased lever pressing behavior upon the return to the acquisition environment after extinction training, as well as the animals in “A” lever pressed significantly more than animals extinguished in “B”, indicating that rats were able to differentiate between the two distinctive contexts (supplementary Figs.S3.1&S3.3). Finally, our results demonstrate that lever pressing after either an IV or an IP cocaine-prime was comparable between both treatment groups. Thus the context specificity of extinction training counteracted the positive effect of D-serine’s ability to reduce cocaine-primed reinstatement.

To date, there has been one other preclinical study that has assessed the effectiveness of an NMDA receptor co-agonist on the context dependency of extinction training to reduce drug-seeking behavior. In contrast to our current results, the published work from Torregrossa et al. [23] supports the use of cognitive enhancers for reducing the context dependency of extinction training. However, there are several noteworthy differences between their study and ours. For example, we have chosen to incorporate extended access into our rodent self-administration protocol, as it is thought to model some aspects of the state of addiction in humans [24]. A prominent characteristic of cocaine addiction is an increased frequency and an increase in the amount of drug ingested over time; the extended access protocol allows for the emulation of these traits. Additionally, we chose to examine the effects of D-serine on both IV and IP
noncontingent drug-priming, an aspect of reinstatement to drug-seeking that is sensitive to extended access/LgA [25] as opposed to the cue-primed reinstatement tested by Torregrossa et al. [23]. Finally, another noteworthy difference is the specific cognitive enhancing agent that was administered. D-serine is an endogenous full agonist, whereas D-cycloserine is a partial agonist at the glycine modulatory site of the NMDA receptor [26]. It remains unclear as to which, if any, of these differences may underlie the disparate results observed between the current work and that of Torregrossa et al. [23].

Although most preclinical studies have shown that D-cycloserine can reduce drug-seeking/taking behavior, one report has shown that potentiation of the reconsolidation of cocaine-associated memories occurs when D-cycloserine is infused directly into the Basolateral Amygdala [27]. Eisenberg et al. [28] published work that reveals that the strength of conditioning and the strength of extinction training may determine whether cognitive enhancing treatment facilitates the original drug-associated memories or effects learning that occurs during extinction. During extinction newly formed memories compete for control of behavior with the original drug-associated memories [29]. Thus, if the original training is robust and extinction training is weak (i.e. fewer trials), reconsolidation of drug-associated memories could acquire control of behavior promoting drug-seeking or vice versa. In addition to promoting reconsolidation, another possible explanation for the discrepancies seen with D-cycloserine may be because it acts as a partial agonist at the glycine site of the NMDA receptor. D-cycloserine, when given to healthy individuals in the presence of increased levels of glycine, produced effects similar to those of NMDA receptor antagonists at low doses [30]. Therefore, it is possible that in an individual with high endogenous levels of glycine or D-serine, D-cycloserine may act in an antagonistic manner rather than as an agonist. At this point, it is clear that the
various discrepancies among reports regarding the potential usefulness of D-cycloserine for the treatment of addiction may be explained by multiple possibilities.

Despite the accumulating clinical studies that have reported the relative ineffectiveness of D-cycloserine treatment for addiction [4], several recent reviews have recommended further investigation of the use of cognitive enhancers as treatment for cocaine addiction [31-33]. However, Kennedy et al. 2012 [34] have recently published a study in which the authors concluded that D-cycloserine treatment does not offer any additional advantages as compared to the placebo treatment group. Considering such results, we suggest that although treatments such as D-cycloserine and D-serine have been effective in treating fear-related conditions and decreasing the reinstatement drug-seeking behavior in rodents, they may not be effective in diminishing relapse in humans due to the interactions between context dependency and competition between consolidation and reconsolidation processes.

6. Conclusion

In summary, the efficacy of D-serine, a full agonist of the NMDA receptor at the glycine modulatory site in facilitating the effects of extinction training to reduce drug-primed reinstatement was not transferable to a novel context. This is an important observation when taken into consideration along with recent clinical work that has found D-cycloserine to be generally ineffective as an adjunctive agent for the treatment of addiction.
References:


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A

Day 1

“ShA”

15

“LgA”

22

Extinction

27

Reinstatement

31

D-serine or saline

IV

IV

IP

IP

COC

SAL

COC

SAL

B

Number of infusions (ShA & LgA)

Day of Self-Administration

Active Lever Presses (90 min)

Extinction Responding

Active Lever Presses (90 min)

"B" day 23

"B" day 24

"B" day 25

"B" day 26

"B" day 27

"B" day 28

"B" day 29

"B" day 30
**Figure 3.1 Experimental design and extinction results for this report.**

A) For the acquisition phase, context A (“A”) is the acquisition environment of cocaine self-administration. For the extinction phase, context B (“B”) is the novel environment of extinction training. Arrows represent one day. B) The saline and D-serine treatment groups were similar in their acquisition of cocaine self-administration (days1-22) before extinction training and treatments began. On day 16, cocaine self-administration sessions were increased from 90 min to 6 hrs (LgA). The bar graph inset indicates significant escalation of drug intake after animals were switched to the LgA sessions, there was significantly increased drug-taking within the first 60 minutes of the LgA session as compared to the ShA sessions (* p<0.05, one-way ANOVA/Holm-Sidak). C) Over the course of five days of extinction training, there was a similar decline in active lever responding for both the saline and D-serine groups treated post session across days 23-27.
The beneficial effects of cognitive enhancement following extinction training to reduce drug-primed reinstatement are not apparent using the “ABA” reinstatement protocol. A) Using the IV route, comparing 30 min before and after cocaine priming in either the saline (n=16) or D-serine (n=7) treatment groups, there was a significant difference in lever pressing after a drug
prime (PrePrime vs., Post Prime; * p<0.05, one-way ANOVA/Holm-Sidak). In addition, post prime active lever pressing on day 28 was significantly increased as compared to that following a saline IV prime test performed on day 29 († p<0.05, one-way ANOVA/Holm-Sidak). B) Using the IP route, cocaine (10mg/kg) significantly increased active lever pressing in both the saline treatment group (n=19, ** p<0.01, one-way ANOVA/Holm-Sidak) and the D-serine treatment group (n=9, * p<0.05, one-way ANOVA/Holm-Sidak) on day 30 as compared to a saline IP priming test on day 31. There was no significant difference in drug-seeking after a cocaine IP prime between the saline and D-serine treatment groups.
SUPPLEMENTARY RESULTS

Evaluation of rats on the first day of extinction training (day 23) in either the novel “B” environment of the current study (24.8 ± 4.1 presses) or the drug-taking “A” environment of a former study (51.0 ± 4.6 presses, [1]) were both significantly reduced as compared to activity lever pressing on the final day 22 of self-administration (112 ± 13.3 presses, ** p < 0.01, one-way ANOVA/ Holm-Sidak; Fig. S3.1). In addition, comparison between groups on day 23 of extinction training in the “B” vs. “A” environments exhibited a significant difference in the amount of drug-seeking († p < 0.01, one-way ANOVA/ Holm-Sidak). This latter result indicates that the context in which extinction sessions are held can impact responding from the first day of training, suggesting that the rats could discriminate between the “A” & “B” context.

As additional evidence that animals identified the “B” extinction environment as being distinct from the acquisition environment (“A”), the first 10 min of active lever responding during the initial extinction session (“B”, day 23) was compared with the first 10 min of active lever responding during both the final day of extinction (“B”, day 27) as well as the initial reinstatement session (“A”, day 28) in which reintroduction to the drug-taking environment occurred. For both the saline and D-serine treatment groups a significant increase in active lever responding on day 28 was evident in “A”, (** 13.7 ± 2.3 or 16.6 ± 4.5 presses, respectively, p < 0.01, one-way ANOVA/ Holm-Sidak) when compared to responding on day 23 in “B”, (6.9 ± 1.3 presses, Fig. S3.2). In addition, a comparison between the last day of extinction in “B” (day 27) and initial reintroduction to “A” (day 28) was made for each treatment group. For the saline group, a significant increase in lever pressing was evident when rats were place back in “A” († p <0.05, one-way ANOVA/ Holm-Sidak; Fig S3.2). Taking together, these results demonstrate
significant differences in extinction responding that suggest the animals were able to differentiate between the acquisition context of “A” and the novel extinction context of “B”.
Figure S3.1 Extinction responding in the “A” and “B” environment.

Active lever pressing over the 90 min session on the first day of extinction training (day 23) as compared to the last day of self-administration (day 22) was significantly reduced in a novel environment “B” (* p<0.01, one-way ANOVA/Holm-Sidak). For comparison, rats with self-administration in environment “A” (“A” data from Kelamangalath and Wagner 2010) show a decrease in lever pressing when compared to day 22 and rats that were extinguished in “B” showed a further reduction compared with animals extinguished in “A”
Figure S3.2 Evidence that modifications of the “B” context were perceptible to animals

As an indication that rats could distinguish between the “A” and “B” environments, lever pressing during the initial exposure to “B” (day 23) and re-exposure to “A” (day 28) were quantified. On day 28, both the saline treatment group (** p<0.01, one-way ANOVA/Holm-Sidak) and the D-serine treatment (** p<0.01, one-way ANOVA/Holm-Sidak) group significantly increased lever pressing upon reintroduction to “A”, as compared to the initial exposure to “B” on day 23. Additionally, when comparing the last day of “B” extinction training (day 27) with re-exposure to “A” (day 28), the saline treatment group († p<0.05, one-way ANOVA/Holm-Sidak) group significantly increased lever pressing.
CHAPTER 4

FOS EXPRESSION ASSOCIATED WITH CONTEXT-INDUCED REINSTATEMENT
IN RATS WITH A HISTORY OF EXTENDED ACCESS TO COCAINE

1 Hammond S, Freeman K, Gaylen E, Wagner JJ. To be submitted to Neuroscience.
FOS EXPRESSION ASSOCIATED WITH CONTEXT-INDUCED REINSTATEMENT IN RATS WITH A HISTORY OF EXTENDED ACCESS TO COCAINE

Abstract

One impediment in treating addiction is the susceptibility to relapse which can persist several years despite prolonged periods of abstinence. Craving, the strong desire to take drug, is associated with relapse and persists during abstinence. Contextual cues previously associated with cocaine acquisition play a major role in craving and relapse in addicts, thereby contributing to development and maintenance of cocaine addiction. The motivational impact of drug-associated stimuli is reinforced through Pavlovian conditioning resulting in the strengthening drug-related memories, and can thereby exaggerate the importance of stimuli and context that are related to drug seeking and taking. Fos is a transiently induced protein that is thought to be a marker for stimulus-elicited brain activity. Increases in Fos protein expression have been observed in rodents with acute cocaine intoxication, exposure to cocaine-paired environment and cocaine associated cues. The aim of the current report was to assess the effects of exposure to the cocaine self-administration environment on drug-seeking behavior and on Fos expression in the hippocampal formation. Thirteen rats self-administered either saline (n=5) or cocaine for 15 ShA days and 7 LgA days. Before being test for context-induced reinstatement, all animals underwent 5 consecutive days of extinction training. Exposure to the cocaine environment significantly increases Fos protein expression only in the ventral subiculum. However, there was a trend toward a significant effect in the ventral CA1 pyramidal cell layer. Although more work
must be done to add to the clarity of the current results, these result suggest that the ventral subiculum, and perhaps, the ventral CA1 pyramidal cell layer, plays a critical role in context-induced drug-seeking behavior.

Keywords: cocaine, context reinstatement, Fos expression, extended access, hippocampus
1. Introduction

One impediment in treating addiction is the susceptibility to relapse which can persist several years despite extended periods of abstinence [1]. Craving, the strong desire to take drug, is associated with relapse and persist during abstinence [2]. Contextual cues previously associated with cocaine acquisition play a major role in craving and relapse in addicts by contributing to development and maintenance of cocaine addiction [3]. The motivational impact of drug-associated stimuli is reinforced through Pavlovian conditioning resulting the strengthening drug-related memories, and can thereby exaggerating the importance of stimuli and context that are related to drug seeking and taking [4]. Consequently, the drug-taking environment is capable of reinstating drug seeking and taking behaviors. Therefore, understanding the neurobiological mechanisms of context-induced relapse will be pivotal for developing a successful treatment for cocaine addiction.

Human imaging studies have implicated several brain areas involved in learning and memory processes. Among which, the hippocampus was found to be activated during presentations of cocaine-associated stimuli and during acute cocaine intoxication. Additionally, a preclinical study by Meyers et al [5] revealed that pharmacological inactivation of the hippocampus during conditioning attenuated place preference on test day. Furthermore, Fuchs and colleagues [6] reported that inactivation of the hippocampus abolished context-induced reinstatement in rats allowed to self-administer cocaine. Taken together these reports implicate the hippocampus in drug-seeking/taking and more specifically, context-induced drug-seeking.

The hippocampus and subicular regions, considered together, make up the area defined as the hippocampal formation (HF) [7]. Generally, the HF is viewed to be important in contextual conditioning more specifically, in the processing of contextual cues by which memories can be
accessed and retrieved. Moreover Pavlovian and instrumental conditioned memories are thought to be controlled and stored by glutamate-dopamine interactions in a circuitry that involves the hippocampus [8]. The hippocampus receives dopaminergic input from the ventral tegmental area and has been shown to significantly innervate the nucleus accumbens. Therefore, by its innervation alone the hippocampus is well positioned within the reward circuitry and may play a role in mediating several of the neurocognitive aspects of addiction-related behaviors. Consistent with this, the dorsal hippocampus (dH) has been implicated in contextual reinstatement of cocaine seeking [6] while the vH plays a key role in cue-induced reinstatement and cocaine-primed reinstatement [9]. However, the vH has also been seen to be important in contextual reinstatement [10, 11]. Few studies have been published that investigate the hippocampus with respect to its dorsal-ventral axis and it involvement in contextual cue reinstatement. Thus, the focus of the experiments described in this report is on the HF with respect to is dorsal-ventral axis and its role in mediating the association between cocaine and the cocaine-paired environment

The neural circuitry involved in the incentive motivational effects of cocaine-associate stimuli has been investigated previously using Fos protein expression which is thought to be a marker for stimulus-elicited brain activity. Fos expression is transiently increased by cocaine [12], exposure to cocaine-paired environment [13] or cocaine-predictive stimuli [14] Previous reports have utilized Fos protein expression to implicated the hippocampus in reinstatement. Zhou, L et al [15] used a cocaine self-administration/reinstatement model to examine neuronal activation, as determined by Fos expression, following cue-induced reinstatement of cocaine seeking in male and female rats and they found no differences in expression in the hippocampus in their male rats. Additionally, Neisewander et al.[16] found in animals that
underwent extinction training there was no significant difference in Fos protein expression in the hippocampus as compared to control. Thus several previous reports on this subject have not been successful in observing significant changes in the HF; however none of these studies have employed the extended access self-administration model. In the current study, we have chosen to incorporate extended access into our rodent self-administration protocol, as it is thought to be more analogous to the state of addiction in humans [17]. A prominent characteristic of cocaine addiction is an increased frequency and an increase in the amount of drug ingested over time; the extended access protocol allows for the emulation of these traits [18, 19]. Additionally, as stated previously, there is a growing appreciation for the functional and anatomical connectivity differences of the hippocampal formation along its septotemporal (dorsal-ventral) axis [20, 21].

In the present study we focused solely on the HF in an effort to delve deeper into its role of environmental cue-induced reinstatement. We hypothesis that by investigating the dH and the VH as separate structures and by considering hippocampal subdivisions (CA1, CA3, and DG) and their laminar layers we can elucidate the gap in the Fos expression studies regarding the hippocampal neural activation during contextual reinstatement. Our current report is the first to examine Fos expression in rats with a history of extended access to cocaine and to investigate the laminar division of HF.
2. Material Methods

2.1 Drugs

Cocaine hydrochloride was obtained from NIDA (RTI International, NC, USA).

2.2 Animal Maintenance

Sprague–Dawley male rats (Harlan, Indianapolis, IN, USA) were singly housed in clear plastic cages. Rats were maintained on a 12 h light/dark cycle (0700 hr/1900 hr) with food and water available ad libitum. Animals were allowed to acclimate to their home cages for one week and were habituated to handling for 3 days prior to surgery. Sessions were conducted daily between 0900hr and 1500hr. These studies were approved by the University of Georgia Institutional Animal Care and Use Committee and conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

2.3 Self-administration Chambers

The self-administration chambers (Med associates Inc., St. Albans, VT) were housed in sound attenuating cubicles, each with a ventilation fan. Operant chambers were equipped with a house light, a rod floor and a speaker/tone generator (2.9 kHz, 10 dB above ambient). Each chamber had two levers (one active and one inactive) accompanied by lever lights positioned above each lever. Syringe pumps were located outside the sound attenuating cubicles. For drug delivery, a modified 22 gauge cannula mounted on the rat’s skull was connected to the liquid swivel with PE-50 tubing protected by a metal spring. Attached to the liquid swivel was Tygon tubing connected to the syringe mounted in the syringe pump. Syringes contained cocaine hydrochloride dissolved in PBS at 4 mg/kg of solution. Each infusion delivered a dose of 0.5 mg/kg/infusion, dispensing a volume of 0.125 ml/kg/infusion. Med Associates software recorded all active lever presses, inactive lever presses, and the number of infusions.
2.4 Jugular Catheterization

The procedure used for the jugular vein catheterization is described elsewhere [22]. Briefly, rats were anesthetized using a Ketamine (70 mg/kg) and Xylazine (10 mg/kg) cocktail. Once animals were fully anesthetized, a silastic catheter was passed subcutaneously and exteriorized at the base of the skull. To position the cannula atop the skull, animals were placed in a stereotaxic frame (Stoelting, Wood Dale, IL). A right-angle cannula (Plastic One Roanoke, VA) was mounted to the skull using four metal screws and dental cement. Immediately following surgery and for 4 consecutive days, rats were treated with 5 mg/kg of Gentamicin. During a recovery week and every day after each self-administration session catheters were flushed with 0.5 ml heparin (16 USP/ml). Catheter patency was verified by the infusion of Ketamine randomly throughout the experiment.

2.5 Experimental Design

After one week of recovery from surgery, animals were placed into the operant chambers to lever press for cocaine or saline. Animals learned that lever pressing resulted in one infusion of cocaine (0.5 mg/kg) or saline after which a 30 second timeout period began. During this timeout period, responses on the active lever had no consequences. Self-administration was divided into three phases: phase 1, short access, ShA (90 min) on FR1 (days 1-10), phase 2, ShA on FR3 (days 11-15) and phase 3, long access, LgA (6 hr) on FR3 (days 16-22) (Fig 1).

For extinction training (days 23-27) animals underwent 5 consecutive 90 minutes extinction training sessions, rats were placed back into the acquisition environment where responses on the active lever and inactive lever resulted in no programmed consequences. Following extinction training rats were tested for environment cue-induced reinstatement (day
Animals were placed into the original acquisition environment. Responding on the active and inactive lever was observed for the 90 minutes to account for context-induced reinstatement.

2.5 Immunohistochemistry

Immediately following behavioral testing, animals were deeply anesthetized. Rats were anesthetized with pentobarbital (80 mg/kg, i.p.) and perfused transcardially with ice-cold 0.1 M PBS, followed by 4% paraformaldehyde (in 0.1 M PBS). The brains were removed and kept in paraformaldehyde for an additional 2 h to achieve proper tissue fixation. After 48 h of incubation in 20% sucrose solution (in 0.1 M PBS) at 4 °C, samples were frozen at −20 °C until further processing.

Coronal sections (30 μm) were cut in a cryostat and tissue was collected at the level of -2.56 and -5.6 mm from bregma. The sections were washed twice with the 0.1 M PBS and immersed in 0.3% H2O2 (1 h). Sections were rinsed in 0.1 M PBS (for 10 min, three times) and incubated in 0.1 M PBS containing 5% normal goat serum (NGS) for 2h. Sections were then incubated for 48 hr at 4°C in 0.1 M PBS containing anti-Fos rabbit polyclonal antibody (1:20,000; Oncogene Science, Cambridge, MA) and 0.1% Triton X-100. Afterward, sections were then rinsed in 0.1 M PBS (for 20 min, three times) and incubated in 0.1 M PBS containing biotinylated goat anti-rabbit IgG (1:200; Vector Laboratories) and 1% NGS for 1 hr. The sections were rinsed again using and 0.1% Triton X-100 (for 20 min, three times) and incubated with avidin-biotinylated peroxidase complex (ABC Elite kit; Vector Laboratories) for 1 hr. The reaction was terminated by rinsing the tissue first for 20 min in 0.1% Triton X-100, followed by 2 20 min washed in 0.1 M PBS. The immunoreaction was visualized with peroxidase substrate
(DAB Substrate Kit). After staining sections were mounted onto gelatin-coated slides and dried before coverslipping. Fos immunoreactivity was quantified using an Zeiss microscope (20x magnification). Fos positive nuclei, brown labelling in the nucleus, were counted by an observer blinded to group’s assignments. Anatomical locations and boundaries of each region use determined using Paxinos and Watson rat brain atlas (Fig. 2, 1998).

2.6 Statistical Analysis

Statistics were done using SigmaStat software, version 3.1. (Chicago, IL, USA) A one-way repeated measures analysis of variance (ANOVA) was applied for all behavioral analysis except context-induced reinstatement analysis. To analyze reinstatement data, a one-way ANOVA was performed. A one-way ANOVA was applied for Fos protein expression analysis. A value of $P \leq 0.05$ was taken as significant, being determined from the Holm-Sidak post hoc test method.

3. Results

3.1 Cocaine-seeking behavior

Two groups of animals, saline group (n=5) and cocaine group (n=8) underwent 22 days of self-administration (Fig. 4.1a). The saline group self-administered saline, while the cocaine group self-administered cocaine. As expected the animals in the cocaine self-administration group increased operant responding and intake of cocaine over time. They received significantly more infusion than the saline group on all self-administration days except the first day (* $p < 0.01$, one-way RM ANOVA/ Holm-Sidak; Fig 4.1b). After 22 days of self-administration the rats were subjected to extinction training in for five constitutive days. Evaluation of rats lever pressing on the first day of extinction training was significantly reduced in the cocaine group as compared to activity lever pressing on the final day 22 of self-administration ($\dagger p < 0.01$, one-
way RM ANOVA / Holm-Sidak; Fig. 4. 2a). A two-way ANOVA analysis revealed that rats in the cocaine group exhibited more operant responding than controls during extinction training days 1 - 4 (* p <0.05 one-way RM ANOVA/Holm-Sidak). However by the fifth day of extinction this significance was no longer evident (Fig. 4.2a). This latter comparison indicates that the saline and cocaine groups exhibited similar levels of operant responding before a context-induced reinstatement test. Examination of lever pressing during the first 10 min of the reinstatement test day showed a significant increase in drug-seeking behavior in the cocaine group compared to the saline group (*p < 0.01, one-way ANOVA / Holm-Sidak; Fig. 4.2b).
<table>
<thead>
<tr>
<th>Region of Interest</th>
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<th>Cocaine</th>
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<tr>
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<td></td>
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<tr>
<td>CA1</td>
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<tr>
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<td>22.7 ± 1.7</td>
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<tr>
<td>radiatum</td>
<td>5.9 ± 2.2</td>
<td>5.5 ± 1.5</td>
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<tr>
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</tr>
<tr>
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</tr>
<tr>
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</tr>
</tbody>
</table>

**Table 4.1 Results of Fos protein expression**

Mean ± SEM of Fos positive nuclei / 0.1 mm² in all regions sampled (* p <0.05, one-way ANOVA/ Holm-Sidak).
3.2 Fos protein expression

Figure 4.3 illustrates the regions of the hippocampus that were analyzed for the current study and Table 1 summarizes the results for each region and group. There was no effect of context-induced reinstatement in the pyramidal cell layer of the dorsal CA1 region (Fig. 4.5a). However, there was a trend toward a significant effect in the ventral CA1 pyramidal cell layer (Figs. 4.4a &b and 4.5b). No effect was evident in the dorsal and ventral CA3 pyramidal (Fig. 4.5c &d) cell layer or in the dorsal or ventral DG (Fig. 4.5e & f). There was a significant enhancement of Fos protein expression in the ventral subiculum (* p<0.05, one-way ANOVA/Holm-Sidak; Figs. 4.4c & d and 4.6). In addition to recording Fos expression in the pyramidal cell layer, we also examined the stratum radiatum layer of the CA1 and CA3 regions. There was not significant increase in Fos expression in the cocaine group compared to the saline group (Table 4.1). Environmental reinstatement increased Fos protein expression only in the ventral subiculum in rats that have a history of extended access to cocaine.

4. Discussion

The primary result of the current report is that following extended access to cocaine, context-induced reinstatement increased Fos protein expression in the ventral subiculum of the HF. Additionally, there was a trend toward a significant effect in the ventral CA1 region. Although lever pressing activity by the fifth day of extinction training animals with cocaine self-administration history was similar to animals with a saline self-administration history, reintroduction into the cocaine-taking environment resulted in a renewal of drug seeking that in the cocaine group. Therefore our extinction training protocol was not robust enough to normalize Fos protein expression in the ventral subiculum and perhaps the ventral CA1 region and thereby suggesting the increase in Fos protein expression in these regions is due to drug-
seeking resulting from a contextual cue priming event. Thus, suggesting that these HF regions may play a role in cocaine-seeking behavior elicited by the cocaine-paired environment.

Although, it is widely accepted that the dH plays a major role in contextual reinstatement of drug-seeking behaviors [6], there is emerging evidence that the vH may also be associated the environment reinstatement. Conditioning studies have revealed that the vH and the ventral subiculum are essential for the retrieval of context-fear memories and the acquisition of context-cocaine associations, respectively [11, 23]. Lasseter and colleagues [10] reported that pharmacologically inactivating the vH, excluding the ventral DG, attenuated context-induced drug-seeking, without altering novelty response or operant responding during extinction training. In present study we found a trend toward significance in the ventral CA1 region and significance in the ventral subiculum, these result are consistent with previous findings. As recently stated, we observed changes in the ventral subiculum in rats with a history of extended access to cocaine and exhibited drug-seeking behavior induced by environmental cues. The ventral subiculum is the primary output from the hippocampus and are the major source of hippocampal innervation to the nucleus accumbens [24]. This connection has been shown to be crucial for successful spatial information processing [25]. Vorel and colleagues [26] reported that stimulation of the ventral subiculum reinstated drug-seeking behavior in rats that self-administered cocaine. Additionally, it has been demonstrated that inactivation of this region of the hippocampus attenuates context-induced reinstatement in rats conditioned to cocaine [5]. Therefore, our results are in line with these reports indicating the ventral subiculum has a role in motivated behavior. Although we did not find any significant result in the dH, other reports have implicated this region in context-induced drug-seeking. Therefore, more work is necessary
elucidate this region’s involvement in context-induced reinstatement in animals with extended access to cocaine.

Contrary to our results, Neisewander and colleagues [16] did not find significant changes in Fos expression in rats with either a contextual prime or a cocaine-prime, a result also reported by Weiss et al. [27]. There are a few noteworthy differences between our work and that of Neisewander et al. In Neisewander et al. report, animals acquired cocaine for a total 15 days, all of which were under the ShA schedule. In the current report animals acquired cocaine for an additional 7 days under an extended access protocol. Moreover, our extinction protocol only included 5 days of training as compared to 21 days in Neisewander et al. reports. Another difference that might be of significance is that our animals were left in the drug-taking environment 30 minutes longer that the Neisewander et al. animals. It remains unclear as to which of these may underlie the different results observed between two reports.

In summary, drug-seeking behavior elicited by exposure to the cocaine self-administration environment increased Fos protein expression in the ventral subiculum and ventral CA1 region of the HF. Further research is needed to better understand the role of hippocampal subregions in environmental cue-induced drug-seeking behavior. Understanding the neural mechanisms involved in motivation for cocaine may help develop and evaluate treatments for cocaine addiction.
References


Figure 4.1 Experimental design and self-administration results

(A) The experimental design employed in the study. (B) Self-administration was divided into three phases: phase 1, short access, ShA (90 min) on FR1 (days 1-10), phase 2, ShA on FR3 (days 11-15) and phase 3, long access, LgA (6 hr) on FR3 (days 16-22). Followed by five days of extinction (days 23-27) and one reinstatement test day (days 28). Animals self-administering cocaine received significantly more infusions than animals self-administering saline on all self-administration day (p<0.05, one-way RM ANOVA/Holm-Sidak).
Figure 4.2 Extinction training is not robust enough to attenuate context-induced reinstatement in the cocaine group

(A) Lever pressing during extinction training. The active lever on first day of extinction training in the cocaine group was significantly reduced as compared to active lever pressing on the final day 22 of self-administration († p < 0.01, one-way RM ANOVA / Holm-Sidak; **
p<0.001 one-way ANOVA/ Holm-Sidak SA day 22 saline vs cocaine). Throughout extinction training the cocaine group lever pressed significantly more than the saline group during extinction training days 1 -4 (* p <0.05 one-way RM ANOVA / Holm-Sidak). This significance was no longer evident by the fifth day of extinction. (B) Context-induced reinstatement significantly increased lever pressing in the cocaine group (* p < 0.01, one-way ANOVA/ Holm-Sidak).
Figure 4.3 Brain regions analyzed

Schematic representations of the dorsal and ventral hippocampus of the rat taken at -2.56 and -5.8 mm from bregma (Paxinos and Watson, 1998). Numbers represent regions analyzed: (1) dorsal CA1 (pyramidal and radium layers), (2) dorsal CA3 (pyramidal and radium layers), (3) dorsal dentate gyrus, DG, (4) ventral CA1 (pyramidal and radium layers), (5) ventral CA3 (pyramidal and radium layers), (6) ventral DG (7) ventral subiculum.
Figure 4.4 Photomicrographs of Fos protein expression in the ventral CA1 and subiculum regions

Representative photomicrographs of Fos protein expression, dark ovals (highlighted by arrows, 10x). Fos expression in the ventral CA1 saline group (A) and cocaine group (B), trending toward significance (p=0.07). Photomicrographs depicts the significant difference in
Fos protein expression between the saline group (C) and cocaine group (D) the subiculum (p<0.01, one-way ANOVA/Holm-Sidak; see Fig. 6).
pyramidal Fos Positive Nuclei / 0.1 mm²

Dorsal CA1

Ventral CA1

Dorsal CA3

Ventral CA3

Dorsal DG

Ventral DG

Saline SA

Cocaine SA

Fos Positive Nuclei / 0.1 mm²
Figure 4.5 Fos protein expression results

Number of Fos positive nuclei/mm$^2$ in the pyramidal cell layer of the dorsal and ventral CA1 (A and B, respectively), CA3 (C and D, respectively) and DG (E and F, respectively).
Figure 4.6 Increase Fos protein expression in the ventral subiculum

Number of Fos positive nuclei/mm$^2$ in the subiculum in the saline and cocaine groups.

*Represents a significance between groups (*p<0.01, one-way ANOVA/Holm-Sidak).
CHAPTER 5
SUMMARY AND CONCLUSION

Although cocaine addiction is an individual struggle, it is also a battle that is felt globally. In 2012, the United Nations Office on Drugs and Crime (UNODC) reported that the annual cocaine users in 2010 ranged from 13.3 million to 19.7 million equally about, 0.3-0.4% of the global adult population. The UNODC report, “The negative health consequences of cocaine use therefore remain undiminished and violence related to cocaine trafficking continues to be an important contributory factor in the affected subregions, some of which are currently experiencing the highest homicide rates in the world.” At a national level, the National Survey on Drug Use and Health reported, that cocaine was the third most used illicit substance in the United State with an estimated 1.6 million cocaine users. Although the overall use of cocaine has declined between the years of 2002 and 2009, cocaine use among individuals 12 years and older remains to number in the millions, with estimates of 617,000 new users in 2009, approximately 1,700 initiates per day. To date the treatment for cocaine addiction has proven to be problematic and currently there are no FDA approved treatments available. As a result of the continued use and ineffective treatment for cocaine addiction, many researchers have dedicated their time and resources to develop treatment for this addiction. Cocaine effects are wide ranging, affecting not only the cocaine addicts themselves but also families, the communities, and the world, thus constituting a major biomedical and public health concern. Several health issues may arise as a result of the use and abuse of cocaine, including cardiovascular disease, neurological impairment, psychiatric disorders, respiratory disorders and death; resulting in an
increase in emergency room visits, burdening our healthcare system both financially and physically. Furthermore, drug abuse promotes risky sexual behavior leading to the transmission of sexual diseases. Additionally loved ones are often devastated and family life destroyed as a consequence cocaine use. Drug abuse during pregnancy may result in premature babies with low birth weight and these newborn babies are also at increased risk for attention deficit hyperactivity disorder, behavioral problems and childhood obesity. Child and spousal abuse are common in households where one parent abuses cocaine. On a larger scale, cocaine use affects the community by contributing to homelessness, to the spreading of HIV/AIDS via the sharing of needles, and to crime and violence such as theft, homicide, and assault. Moreover, babies born with prenatal cocaine exposure are often special needs children leading to increased educational costs. Adults under the influence of cocaine frequently fail to show for work resulting in unemployment, decreasing/losing the income to care for family, to support basic needs and in turn to fund their drug habit. Cocaine addiction, whether directly or indirectly, effects the lives of a major portion of our society.

Despite continued use, there is currently no successful treatment for cocaine addiction and there are no approved FDA treatments either. As recreational use of cocaine turns to chronic cocaine exposure, neuroadaptations occur that leads to tolerance, sensitization, dependence and addiction. As a result many researchers have devoted their time and resources to developing an effective treatment for cocaine addiction. Recently, considerable attention has been given to the use of cognitive enhancers for the treatment of cocaine addiction. Treatments involving cognitive enhancers have proven to be very successful in the treatment of fear conditions. Although currently, cognitive enhancement treatment seem to be ineffective in treating
addiction, perhaps additional work is need to elucidate the difficulties with treating addiction with cognitive enhancers, hence my first two experiments.

As cocaine addiction continues to be a major socio economic problem in United States, the aim of the current report was three-fold. In the first series of experiments, I assessed the D-serine effect on the acquisition and development of cocaine-induced locomotor sensitization and conditioned place preference. Additionally, I evaluated D-serine’s effects on extinction training and cocaine-primed reinstatement in the CPP animal behavioral model. In the next set of experiments, we investigated D-serine’s ability to reduce drug-primed reinstatement in an altered extinction training environment. Finally, my last experiment was designed to observe the effects of exposure to the cocaine self-administration environment on drug-seeking behavior and Fos protein expression in the hippocampal formation.

To summarize my first group of experiments (Chapter 2), D-serine, a full agonist of the NMDA receptor at the glycine modulatory site, pretreatment before extinction training not only facilitated extinction and reduced cocaine-primed reinstatement tested 2 days later, but it also exhibited much longer effects in reducing drug-primed reinstatement up to 4 weeks. These observations highlight another feature of practical importance regarding the potential use of D-serine along with behavioral therapy, that the interval between the “booster” sessions required for the ongoing maintenance of the extinguished condition can be lengthened. For example, monthly treatments involving a day or two are more likely to be amenable for the typical patient than would ongoing weekly or multiple days/week session schedule.

The primary result of my second groups of experiments (Chapter 3) is that the efficacy of systemic administration of D-serine in facilitating the effects of extinction training to reduce drug-primed reinstatement does not provide the beneficial effect of reducing cocaine-induced
drug-seeking when extinction training occurs in a novel context. We speculate that context-dependent learning is enhanced as a result of hippocampal neurons being persistently activated sufficient drug exposure. Thereby, creating a problem when an ABA change in context occurs as seen in my second set of experiments. From this study and recent clinical work, I concluded that cognitive enhancement may be generally ineffective as an adjunctive agent for the treatment of addiction.

As treatment with D-serine prove to be ineffective in reducing drug-primed reinstatement in an “ABA” self-administration protocol, I decided to take an immunohistological approach to increase my understanding of the neural mechanisms associated with cocaine addiction in an effort for develop more successful treatments. Cue-drug memories are persistent, triggering relapse after years of abstinence. Thus, drug-associated stimuli can invoke memories of the drug, and induce craving during periods of abstinence in addicts, and induce relapse hence, the idea of addiction as a learning and memory disease state. As stated previously, imaging studies have implicated several brain areas involved in learning and memory processes. Among which, the hippocampus was found to be activated during presentations of cocaine-associated stimuli and during acute cocaine intoxication. Additionally, it has been reported that inactivation of the hippocampus abolished context-induced reinstatement in rats allowed to self-administer cocaine. Therefore, the objective of my final study was to assess the involvement of the hippocampal subregions following extended access to cocaine in context-induced reinstatement. The primary result of the current report is that context-induced reinstatement increased Fos protein expression in the ventral subiculum and, perhaps (with additional work) the ventral CA1 region of the HF. While the current results of this study are interesting and consistent with other literature, more work on this subject is needed and currently being conducted.
In conclusion, cognitive enhancers like D-cycloserine and D-serine, although successful in treating fear disorders, are not effective in reducing drug-seeking prime reinstatement in a preclinical model of drugs-seeking when environmental context is altered. Since addiction patients are typically treated in a distinct environment from that in which they seek and take drugs, the context dependency of the efficacy of cognitive enhancement is a major limitation.